JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Authentication of Monofloral Yemeni Sidr Honey Using Ultraviolet Spectroscopy and Chemometric Analysis

Abdul-Rahman A. Roshan,[†] Haidy A. Gad,[†] Sherweit H. El-Ahmady,*[,†] Mohamed S. Khanbash,[#] Mohamed I. Abou-Shoer,[§] and Mohamed M. Al-Azizi[†]

[†]Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo, Egypt

[§]Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

[#]Honey and Date Center, Hadramout University of Science and Technology, Hadramout, Yemen

Supporting Information

ABSTRACT: This work describes a simple model developed for the authentication of monofloral Yemeni Sidr honey using UV spectroscopy together with chemometric techniques of hierarchical cluster analysis (HCA), principal component analysis (PCA), and soft independent modeling of class analogy (SIMCA). The model was constructed using 13 genuine Sidr honey samples and challenged with 25 honey samples of different botanical origins. HCA and PCA were successfully able to present a preliminary clustering pattern to segregate the genuine Sidr samples from the lower priced local polyfloral and non-Sidr samples. The SIMCA model presented a clear demarcation of the samples and was used to identify genuine Sidr honey samples as well as detect admixture with lower priced polyfloral honey by detection limits >10%. The constructed model presents a simple and efficient method of analysis and may serve as a basis for the authentication of other honey types worldwide.

KEYWORDS: *Ziziphus spina-christi, Sidr, honey, UV spectroscopy, chemometrics, multivariate analysis*

INTRODUCTION

Honey is defined as "the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants, or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature".[1] Bees forage different plants, and due to different proportions of the possible sources of nectar, honey is always a mixture of different sources.[2] Monofloral honey is produced from nectar that mainly originates from a single plant species and possesses distinctive organoleptic characteristics. These honey types of distinct botanical origin are often traded at a higher price than honeys from mixed botanical origins and can thus be considered premium products.[3]

Honey production and beekeeping are very old professions in Yemen, dating back to the 10th century B.C.E.[4] Yemen produces, annually, up to 5600 tons of several natural varieties, of which the most famous is the Sidr or Elb honey (*Ziziphus spina-christi* L. Desf. Rhamnaceae), which is mainly exported to the Gulf States with annual total gross revenues of U.S. $40 million.[5] The natural specifications of the Sidr honey produced from Dawan and Gerdan valleys in Hadramout and Shabwa governorates, respectively, are well preserved because the bees build their hives without any human interference, making this particular honey one of the finest and best quality monofloral honeys worldwide.[6,7] Similar to many other well-regarded honey types, limited availability and high pricing of Sidr honey are probably the biggest temptations for its adulteration or admixture with other local polyfloral honey types. Hence, identification of authenticity is important for financial reasons in addition to consumer and producer protection. The 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.[8]

Many different techniques are employed in authenticity testing of honey including pollen analysis (melissopalynology), which was used as the traditional method to determine the honey's botanical origin.[9] In the past few decades, new analytical techniques were implemented for the determination of honey's botanical origin in an effort to find alternative methods for honey authentication. These methods were based on statistical evaluation of their physicochemical data[10,11] or the determination of certain chemical constituents to be used as biomarkers by applying various chromatographic and spectroscopic techniques.[12−14] Recently, analytical techniques in conjunction with multivariate analysis and chemometrics have been widely implemented in the quality control of various foods and herbal drugs.[15,16] Reports on the authentication of honey samples using this approach have also received wide recognition where various honey characteristics were associated with chemometrics for botanical and geographical classification. Analytical methods used included the measurement of physicochemical parameters of honey,[17−19] application of GC-MS for the characterization of volatile compounds,[20,21] and quantitation and characterization of sugars using Raman spectroscopy and HPLC[22] and of flavonoids using LC-DAD-ESI/MS.[23] In addition, a commonly used spectroscopic method

Received: January 24, 2013
Revised: July 4, 2013
Accepted: July 9, 2013
Published: July 9, 2013

was near-infrared (NIR) spectroscopy, which was used to classify floral origins,[24] to detect honey adulteration by the addition of fructose, glucose, and corn syrup,[25,26] and to develop calibrations for the quantitative prediction of physicochemical measurements of honey as protein, fat, and moisture.[9] Fourier transform mid-infrared (FT-MIR) spectroscopy was established for analyzing physicochemical parameters of honey[27,28] whereas MIR-ATR spectroscopy was shown to offer a valuable approach to honey authentication.[29,30]

The application of UV spectroscopy in the analysis of food products has increased during the past decade, probably due to its advantage of being simple, quick, nondestructive, and relatively inexpensive to carry out.[31−34] Although there has been a steady growth in applications of honey authentication using IR spectroscopy as well as other spectroscopic methods, little research has been carried out using ultraviolet (UV) spectroscopy for this purpose despite its many advantages of simplicity, rapidity, and low cost.[35] A study using size exclusion chromatography (SEC) coupled to UV diode array detection (UV-DAD) for botanical and geographical classification[36] and another applying front face fluorescence for the botanical classification of honey samples in Switzerland,[37] both combined with chemometrics, were the closest to the presented approach.

The work presented in this study aims at developing and establishing a simple model, based on chemometric analysis of UV spectroscopic data, to confirm any botanical claim made on Yemeni Sidr honey. The designed model will allow for the detection of adulteration resulting from admixture with honeys of lower quality and price as well as the authentication of genuine Sidr honey. This model may also serve as a basis for implementing this technique to other honey types worldwide.

MATERIALS AND METHODS

Sample Collection. A total of 38 honey samples were used in this study including 13 samples that represented genuine monofloral Sidr honey (*Ziziphus spina-christi*) samples (SG1−SG13), 14 samples marketed and claimed to be Sidr honey (SM1−SM14), including 3 imported samples (2 from Kashmir, India, and 1 sample from the Kingdom of Saudi Arabia (KSA)), 5 polyfloral samples (PF1−PF5), and 6 non-Sidr samples of different botanical origins (NS1−NS6). All samples were collected from different regions of the Republic of Yemen. Genuine Sidr honey and marketed sample codes, botanical and geographical origins, price, and collection season are presented in Tables 1 and 2, respectively. A map of Yemen showing collection sites is presented in Figure 1. With regard to the genuine Sidr samples, a thorough field survey was conducted prior to collection in areas abundant with *Ziziphus* trees, and the honey was collected directly from the hives (by the help of the beekeepers) in Dawan valley of Hadramout governorate, during the flowering season of *Ziziphus* trees (October−November, 2010) to ensure the authenticity of the samples. These samples were certified as genuine Sidr honey samples by Professor M. Khanbash, an expert on Sidr honey at the Honey and Date Research Center in Hadramout, Yemen; sensory and physicochemical analyses (refractive index, water content, pH, free acid content, and electrical conductivity) were conducted. The remaining 25 samples were collected from the market, and their botanical origins were identified by sensory as well as physicochemical analyses. An overview of the physicochemical measurements for all samples (unpublished data) is presented in Table 1 of the Supporting Information. Accordingly, the physicochemical data obtained from most of the marketed samples claimed to be Sidr samples were within the genuine Sidr criteria except for five samples: SM1, SM4, SM8, SM9, and SM13.

Sample Preparation. Each honey sample (2.50 g) was dissolved in 20 mL of 80% ethanol solution (Carlo Erba anhydrous HPLC grade ethanol diluted with distilled water), which provided a solvent of expanded polarity range to dissolve most of the honey components. The samples were then filtered (using grade 1 Whatman filter paper), transferred into a 25 mL volumetric flask, and completed to volume with 80% ethanol to be kept as stock solution. For the preparation of admixed samples, two sets of samples representing admixture with two polyfloral honey samples, PF3 for the first set and PF4 for the second set, were prepared. The admixed samples were prepared by diluting four different genuine Sidr honey samples (SG2−SG4 and SG12), chosen at random with different concentrations (10, 20, 30, and 40%) of each of PF3 for the first set and PF4 for the second set to amount to 16 adulterated samples in each set.

Table 1. Genuine Sidr (*Z. spina-christi*) Honey Samples

sample code	geographical origin	price/kg (YR)[a]	collection season in 2010
SG1	Dawan valley	15000	December
SG2	Dawan valley	15000	December
SG3	Dawan valley	15000	November
SG4	Dawan valley	17000	November
SG5	Dawan valley altitude	16000	November
SG6	Dawan valley altitude	15000	November
SG7	Dawan valley	15000	November
SG8	Hagr valley	17000	November
SG9	Hagr valley	15000	November
SG10	Dawan valley	20000	November
SG11	Dawan valley altitude	16000	November
SG12	Dawan valley	15000	December
SG13	Dawan valley	15000	November

[a]Exchange rate for Yemeni riyal (YR) in January 2013: U.S. $1 = 215 YR.

Ultraviolet Spectroscopy. A dilution of 1 mL was taken from the stock solution for each sample into a 10 mL volumetric flask and completed with 80% ethanol. All samples were analyzed by UV spectroscopy using a UV-1601 PC UV−visible spectrophotometer (Shimadzu, Japan) equipped with a quartz cell with an optical path of 1 cm and spectral resolution of 1 nm in the range 200−400 nm. The obtained absorption readings over the spectral points of all the samples were converted into a data matrix using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) with the spectral points as variables represented by columns and their corresponding spectral absorption measurements of different samples represented by rows. For each of the 13 genuine Sidr samples, 20 replicates (to be used as the training set in the model construction) and 5 replicates for each of the 25 marketed samples were prepared.

Multivariate Data Analysis. The data measured were represented in a matrix consisting of the total number of samples and their replicates multiplied by 200 variables in MS Excel and exported to the appropriate software for chemometric analysis of the spectra. The UV spectral data were subjected to unsupervised recognition techniques of data analyses performed by applying hierarchical cluster analysis (HCA) using Hierarchical Clustering Explorer 3.5 (Human computer interaction laboratory, University of Maryland, College Park, MD, USA) and principal component analysis (PCA) using Unscrambler 9.7 (CAMO SA, Oslo, Norway). The UV data matrix preprocessing was performed prior to data analysis by mean centering of the raw spectral data matrix of all the samples, a default option in the software. Both HCA and PCA methods aim to reduce the multivariate space in which objects (samples) are distributed but are complementary in their ability to present results. HCA was used to sort the sample into groups using average linkage method for cluster building, and the distance between clusters was computed by Pearson's correlation method as a measure of similarity. PCA was utilized as a data reduction technique to generate a visual plot of the samples and their distribution on a score plot often showing trends that, despite having to be interpreted and explained, constitute a first step in subsequent modeling for samples classification. In the model construction, HCA and PCA were followed by the supervised pattern recognition technique of soft

Table 2. Marketed Honey Samples

sample code	claimed origin		market	price/kg (YR)	collection season in 2010
	botanical	geographical			
SM1	Sidr	Nakhal valley[a]	Dawan	14000	November
SM2	*Ziziphus spina-christi*	Hareeb Shabwa gov	Aden	13000	November
SM3		Geradan Shabwa gov	Aden	14000	November
SM4		Hareeb Shabwa gov	Aden	10000	November
SM5		Saewn[a]	Saewn	15000	July
SM6		Dawan[a]	Dawan	15000	July
SM7		Ghyl binyamen[a]	Saewn	16000	July
SM8		Hadramout gov[a]	Aden	10000	August
SM9		Hadramout gov[a]	Aden	12000	August
SM10		Dawan[a]	Aden	18000	August
SM11		Kashmir, India	Aden	4000	November
SM12		Kashmir, India	Aden	4000	October
SM13		Saudi Arabia (KSA)	KSA	12000	October
SM14		Hareeb Shabwa gov	Aden	14000	November
PF1	polyfloral with *Acacia tortilis*	Hadramout gov[a]	Aden	5000	August
PF2	polyfloral with *Ziziphus spina-christi*	Dawan[a]	Dawan	4500	November
PF3	polyfloral	Hadramout gov[a]	Aden	2500	November
PF4	polyfloral	Hadramout gov[a]	Aden	3000	September
PF5	polyfloral	Hadramout gov[a]	Aden	3000	September
NS1	Sumar *Acacia tortilis*	Hadramout gov[a]	Aden	5000	November
NS2	Sumar *Acacia tortilis*	Dawan[a]	Dawan	5000	November
NS3	Marow	Abyan gov	Aden	5000	November
NS4	polyfloral with *Dracaena*	Suqatra Island	Aden	14000	December
NS5	Dhaba *Acacia mellefera*	Taez gov	Aden	4000	December
NS6	Sal *Euphorbia* sp.	Taez gov	Aden	8000	November

[a]Geographically located in Hadramout governorate.

Figure 1. Map of Yemen showing collection sites.

independent modeling of class analogy (SIMCA) using Unscrambler 9.7, which is considered a key chemometric approach for classification. This technique allows for the classification of the samples into an already existing group, assigning new objects to the class to which they show the largest similarity. SIMCA is strongly based on PCA, because each class is defined by an independent PCA. In our study, both PCA models (genuine Sidr and polyfloral) were developed using full cross validation, and the number of PCs used in each model was automatically selected by the software using the predicted residual error sum-of-squares (PRESS) function. The choice of the SIMCA technique in contrast to other supervised pattern recognition techniques is based on the modeling properties of SIMCA, which provides approaches more versatile than those obtained using discriminant techniques.[38]

RESULTS AND DISCUSSION

The UV absorption bands of the presented samples are usually associated with the presence of different chromophores exemplified in various components as phenolics, flavonoids, and conjugated systems as well as other UV-absorbing systems,[39] and recently these compounds have been used as markers for the determination of the botanical origin of honey.[23] The UV spectrum of each of the studied honey samples was recorded (20 replicates for the 13 genuine Sidr samples and 5 replicates for each of the 25 marketed samples) versus 200 variables representing the absorbencies in the region between 200 and 400 nm. The UV spectra for all of the samples are presented as Figure 1 of the Supporting Information.

Hierarchical Cluster Analysis. The search for natural groupings among the samples is one preliminary way to study data sets. Because of its unsupervised character, HCA was used to perform a preliminary data scan and to uncover the structure residing in the data. The dendogram in Figure 2a shows the clustering pattern of the data set constituting the 13 genuine

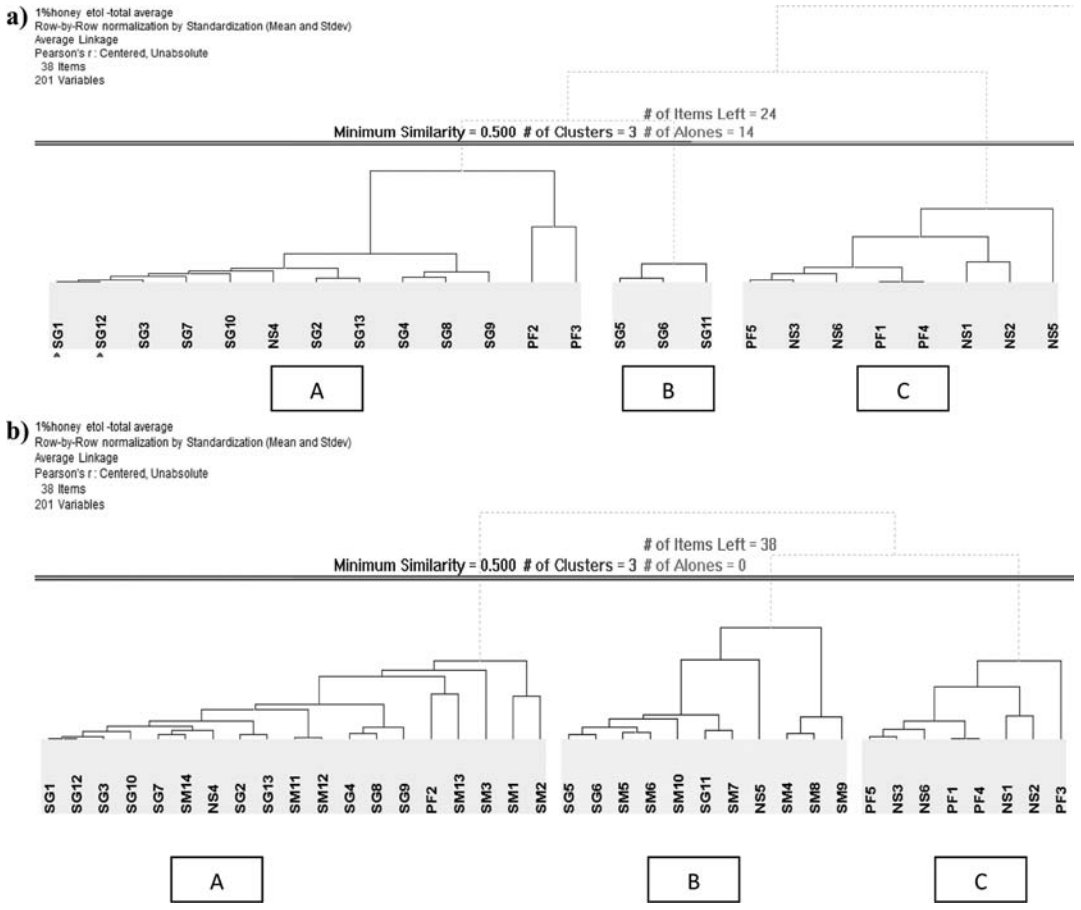

Figure 2. (a) Clustering dendogram of genuine Sidr, polyfloral, and non-Sidr honeys; (b) clustering dendogram of the total honey samples from (a) + 14 marketed samples. A = genuine Sidr samples from lowland of Dawan valley; B = genuine Sidr samples collected from mountainous areas of Dawan valley; C = polyfloral and non-Sidr samples.

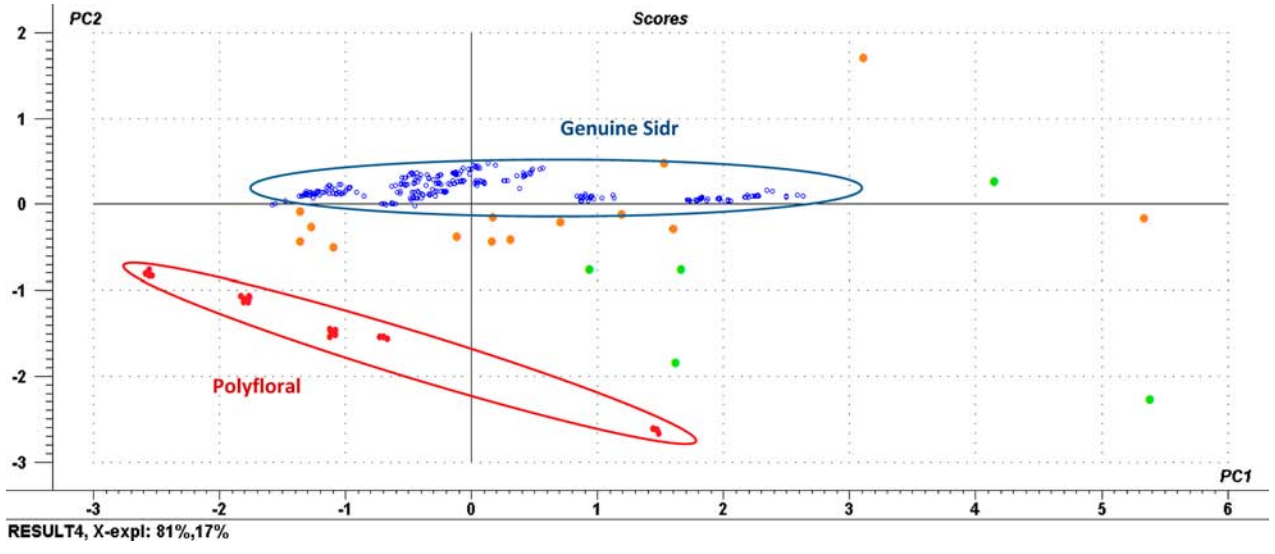

Figure 3. PCA of total honey samples: (open blue circles) genuine Sidr honey; (solid red circles) polyfloral honey; (solid orange circles) claimed to be Sidr honey; (solid green circles) non-Sidr honey. It should be noted that ellipses do not denote statistical significance but are rather for better visibility of clusters discussed. Sample NS4 was off scale and was excluded from the PCA model due to its high leverage effect.

Sidr, 5 polyfloral, and 6 non-Sidr honey samples. The model revealed a rational segregation of the samples into three clusters; clusters A and B representing genuine Sidr and cluster C including most of the non-Sidr samples in addition to three polyfloral samples. The reason behind the splitting of genuine Sidr samples into two clusters may be related to the difference in collection altitude, because the samples in cluster B were collected from the mountainous area of Dawan valley, whereas those in cluster A were collected from the lowland of the valley as indicated in Table 1. The chemical compositions of honeys

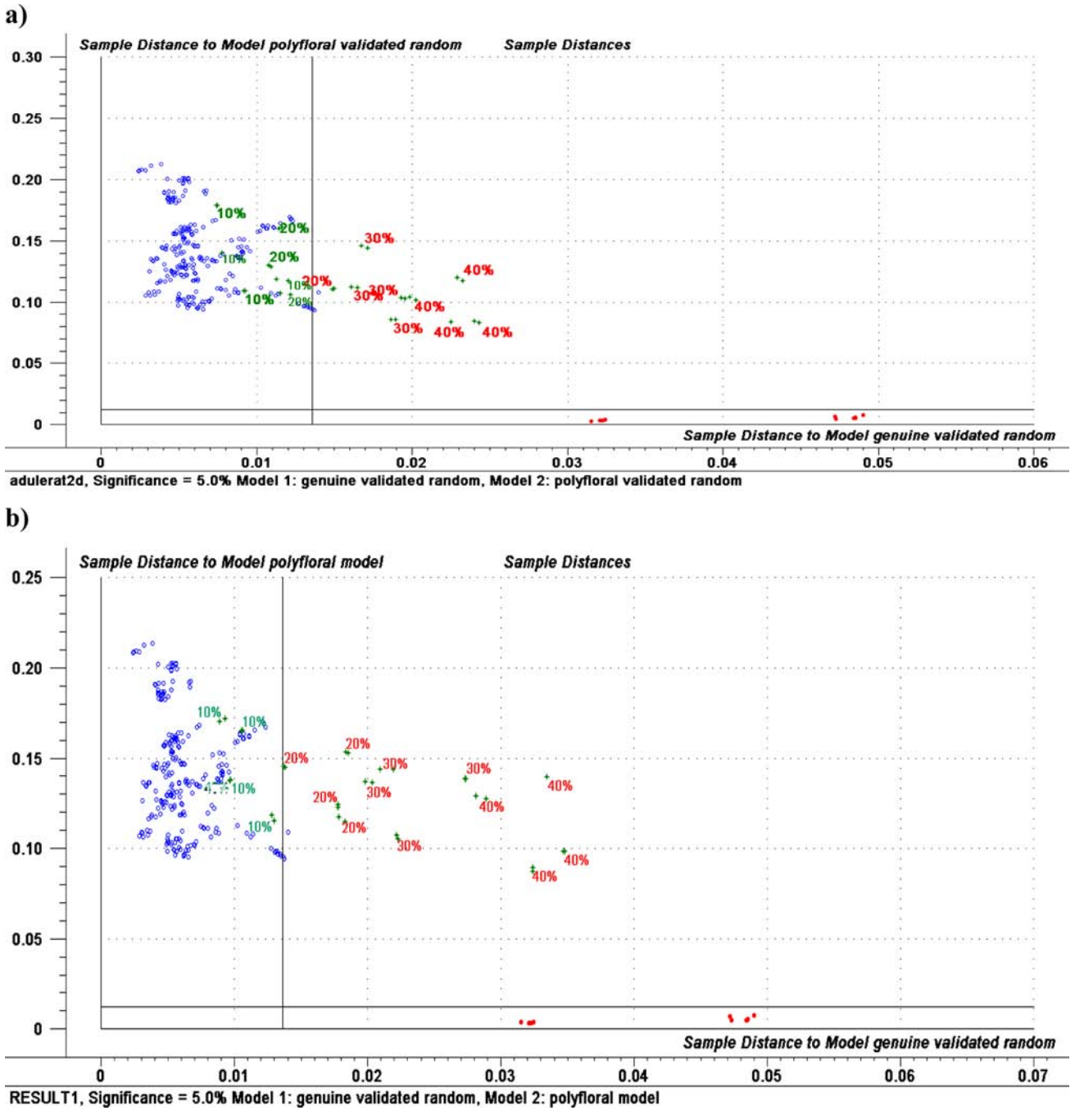

Figure 4. Identification of Sidr honey samples admixed with polyfloral honey: PF3 (a) and PF4 (b) using the SIMCA model.

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.[12] In the spring season, if it rains, a large number of plants, including *Ziziphus*, will flower in Dawan valley where honey production takes place and, hence, the constituents of the polyfloral honey produced may resemble that of genuine Sidr samples. In autumn (September−November), the flowering season of *Ziziphus* trees, only a few other plants will flower at the same time;[40] therefore, the honey produced is considered of premium quality (Bagheah Sidr honey), whereas that produced in spring is called Marbaee and is mostly polyfloral and hence of less quality. Accordingly, this can explain the resemblance of PF2 and PF3 to Sidr samples and their grouping in cluster A.

The only non-Sidr sample grouped with the Sidr samples in cluster A was NS4 collected from Suqatra Island. The UV absorbance spectrum of this sample was strikingly different from the rest of the samples, and it is worth mentioning that this sample showed a distinctive flavor in the sensory analysis and different physicochemical characters from any of the samples. The anomalous grouping of this sample in cluster A is, however, sometimes observed in HCA models and has been reported in similar studies.[41] The addition of the 14 marketed samples to the data set resulted in the same three clusters as shown in Figure 2b with minor but meaningful changes. Sample PF3 was regrouped with cluster C, whereas PF2 remained in

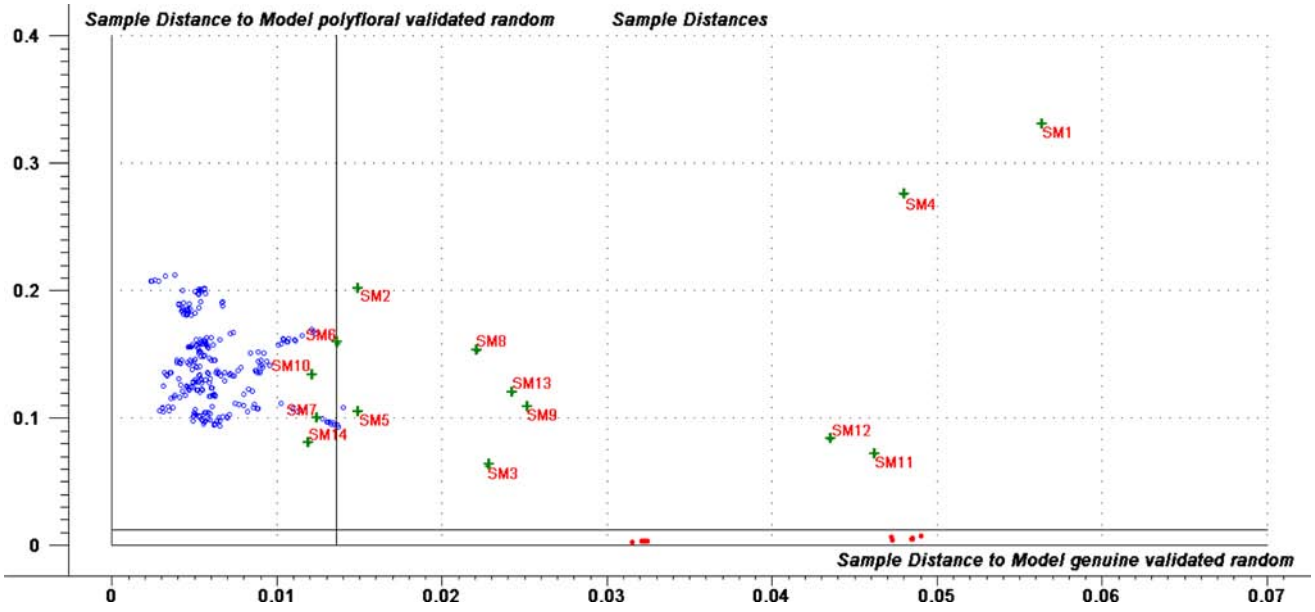

Figure 5. Authentication of marketed Sidr honey samples using the SIMCA model.

cluster A due to its geographic and somewhat similar botanical origin of the latter to Sidr samples as indicated in Table 2. Cluster A originally constituting Sidr samples collected from the valleys also grouped SM1 from the Nakhal valley in addition to all non-Hadramout samples including Kashmir and KSA marketed samples. Cluster B comprised seven marketed samples (SM4−10), all of which originated from the Hadramout governorate, except for SM4, in addition to the three Sidr samples collected from the mountainous area of Dawan valley.

Principal Component Analysis. PCA was applied to the matrix formed by the total spectral data corresponding to all of the different honey samples. The maximum number of PCs was set at six; however, the first two components explained almost all of the data variance. As shown in the score plot of the first two PCs in Figure 3, the genuine Sidr honey samples (SG1−SG13 × 20) were clustered together on the horizontal (PC1) axis and well separated from the polyfloral samples (PF1−PF5 × 5), which were clustered in the lower left quarter of the score plot. The remaining non-Sidr honey samples (NS1−NS6 × 5) were scattered in the lower right quarter of the score plot a distance away from the two former clusters. However, some of the marketed samples claimed to be Sidr honey were scattered at a distance closer to the genuine Sidr cluster, showing some similarity.

PCA is among the most versatile of all chemometric methods; it involves a mathematical procedure that reduces data dimensionality by performing a covariance analysis between factors and visualizes the hidden trends in a data matrix without much loss of information.[37] Even though the results obtained from the PCA revealed obvious clustering of the samples according to their botanical origin, which indicated differences in the honey samples composition, and was in fact mostly coherent with the HCA results, a more precise demarcation was required to be achieved between the genuine Sidr honey samples and its blends collected from the market. As a consequence, it was necessary to approach the problem with the SIMCA technique, which utilized principal component per category to further confirm the classification obtained by the PCA and HCA.

Soft Independent Modeling of Class Analogy. *Development of the Classification Model.* The SIMCA classification process consists of two stages, namely, the training stage, in which the individual models of the data classes are developed, and the testing or validation stage, in which new samples (not used in the training stage) are classified within the established class models to evaluate the model's efficiency. Classes corresponding to genuine Sidr honey and polyfloral honey were developed using independent principal component analysis. The training set for the class of genuine Sidr consisted of 10 samples, whereas the polyfloral training set was composed of 4 samples. External validation was carried out with samples that were not used for the model's development, which consisted of three genuine Sidr samples (SG1, SG6, and SG8), one polyfloral sample (PF1), and all non-Sidr samples except for NS4, which was excluded due to its high leverage effect apparent from the PCA score plot.

Validation of the Model. Cooman's plots were used to evaluate the results of classification where an object belonging to a certain class should fall within the membership limit, on the left of the vertical line or below the horizontal line. The validation samples corresponding to genuine Sidr and polyfloral honey were all within the limit of each membership, indicating the perfect predictability and sensitivity of the model (Figure 2 of Supporting Information). Also, the model showed good specificity as all non-Sidr honey samples were not classified into either class. It is worth noting that the developed SIMCA model provided interclass distances of about 380, and all variables showed a discrimination power higher than 3 and a modeling power higher than 0.8, which reflected the discrimination capability of the developed model to discriminate the spectral signals of genuine Sidr honey samples with respect to polyfloral samples, with 95% confidence limits. For the above reasons, the constructed SIMCA model was employed in the classification of formulated admixed samples and later for the authentication of genuine Sidr samples.

Challenging the Model Using Formulated Admixed Sidr Honey Samples. The results in Figure 4a show that the Sidr samples admixed with 10 and 20% polyfloral honey (PF3) fell within the class membership limit of the genuine Sidr

model, whereas samples adulterated with 30 and 40% did not. In the case of admixture with polyfloral sample PF4, only the 10% admixed samples were classified as genuine Sidr honey (Figure 4b). These results are quite promising because both polyfloral samples used to adulterate the genuine samples shared the same geographic location as that of genuine Sidr honey samples, which was confirmed by the HCA (Figure 2a), and in many cases such polyfloral honey may have originated from *Ziziphus* species and have constituents common to that of genuine Sidr honey as explained previously. The fact that the model was able to allow admixture of genuine Sidr with up to 20% of PF3 and only 10% of PF4 maybe was in coherence with HCA results showing a higher resemblance of PF3 to genuine Sidr samples than PF4.

Authentication of Marketed Sidr Honey Samples. The 14 marketed samples claimed to be Sidr honey were tested using the constructed and validated SIMCA model for their classification into Sidr or non-Sidr honey as shown in Figure 5. Samples SM6, SM7, SM10, and SM14 were classified as Sidr honey, which was in coherence with the HCA, which grouped the former three samples with cluster B and the latter with cluster A, all classified as genuine Sidr honey. These results were also in agreement with the data from the physicochemical analyses showing characters of genuine Sidr including higher pH and electrical conductivity as well as lower free acid content. In addition, these results were compatible with our expectations as these samples were collected from four highly reputable honey sellers in the honey market, and the prices of these samples ranked the topmost when compared to the other marketed samples as shown in Table 2. It is also worth noting that the two samples, SM2 and SM5, closest in distance on the plot to the membership limit for genuine Sidr had the source, physicochemical characteristics, and price criteria comparable to those of the samples classified as genuine Sidr honey. The claimed Sidr honey samples from Kashmir (SM11 and SM12) were placed at a further distance from the genuine Sidr class model, whereas the sample from KSA (SM13) projected within the Yemeni claimed Sidr honey near 0.2 distance to the Sidr model. These results may be explained by the similarity of the latter sample to the Yemeni samples due to the closer geographic location of the KSA southern border to Yemen and hence the increase in the similarity of botanical origin than that present in Kashmir. Table 3 presents the classification of the 14 marketed samples in accordance with the authentication results obtained.

In conclusion, considering the many variables involved in the production of Yemeni Sidr honey such as geographic location, botanical origins, and collection season, the approach of using UV spectroscopy coupled with chemometric analysis methods was confidently able to segregate genuine Sidr samples from non-Sidr and relatively inferior polyfloral samples available in Yemen and even accomplish classification within the genuine Sidr samples. Applying HCA to the spectroscopic data resulted in the successful segregation of genuine Sidr honey from polyfloral and non-Sidr honey as well as the distinction of Sidr honey according to collection altitude. Both HCA and PCA showed obvious grouping patterns and high resemblance of most of the 14 marketed samples to the Sidr samples. SIMCA was successfully able to classify the authentic Sidr honey from non-Sidr honey samples with a clear demarcation. Also, the model was able to detect admixture of genuine Sidr honey with closely related polyfloral honey by detection limits of >10%, which potentially serves the trading of honey in Yemen. Finally, the model was capable of distinguishing genuine Sidr honey collected in Hadramout from that collected in other regions as far as KSA and India. Further studies on the under-investigated Yemeni Sidr honey involving the incorporation of considerably more samples, of different botanical and geographical origins, into the model as well as compositional analysis of the honey would definitely accentuate the model and is considered for future work. Finally, adopting the model for the authentication of other honey types worldwide is recommended as a simple and inexpensive approach.

Table 3. Classification of Marketed Claimed To Be Sidr Honey Samples

	contaminated with other nectars (impure)	
genuine or pure Yemeni Sidr	less contaminated	highly contaminated
SM2	SM3	SM1
SM5	SM8	SM4
SM6	SM9	SM11
SM7	SM13	SM12
SM10		
SM14		

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figure 1: UV absorbance spectra of all 38 samples. Figure 2: Cooman's plot for the validation of the SIMCA model. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(S. H. El-Ahmady) Postal address: Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt. E-mail: selahmady@pharma.asu.edu.eg. Phone: +2 0122 4604855. Fax: +202 2 405 1107.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) European Economic Community. EEC Council Directive of 20 December 2001 relating to honey. *Off. J. Eur. Communities* **2002**, *110*, 47−50.

(2) Oddo, L. P.; Bogdanov, S. Determination of honey botanical origin: problems and issues. *Apidologie* **2004**, *35*, S2−S3.

(3) Donarski, J. A.; Jones, S. A.; Harrison, M.; Driffield, M.; Charlton, A. J. Identification of botanical biomarkers found in Corsican honey. *Food Chem.* **2010**, *118*, 987−994.

(4) Khanbash, M. S. *Development of Beekeeping in Yemen*; Honeybee Center, Hadhramout University of Science and Technology, 2003.

(5) FAO. *Non-wood forest products in the near east: a regional and national overview*; http://www.fao.org/docrep/003/Y1797E/y1797e04.htm.

(6) Alzubier, A. A.; Okechukwu, P. N. Investigation of anti-inflammatory, antipyretic and analgesic effect of Yemeni Sidr honey. *World Acad. Sci. Eng. Technol.* **2011**, *80*, 47−52.

(7) Lush, J. *Bee-keeping in Yemen*; http://www.al-bab.com/bys/articles/lush01.htm.

(8) Cajka, T.; Hajslova, J.; Pudil, F.; Riddellova, K. Traceability of honey origin based on volatiles pattern processing by artificial neural networks. *J. Chromatogr., A* **2009**, *1216*, 1458−1462.

(9) Arvanitoyannis, I. S.; Chalhoub, C.; Gotsiou, P.; Lydakis-Simantiris, N.; Kefalas, P. Novel quality control methods in

conjunction with chemometrics (multivariate analysis) for detecting honey authenticity. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 193−203.
(10) Ruoff, K.; Luginbühl, W.; Kilchenmann, V.; Bosset, J. O.; von der Ohe, K.; von der Ohe, W.; Amadò, R. Authentication of the botanical origin of honey using profiles of classical measurands and discriminant analysis. *Apidologie* **2007**, *38*, 438−452.
(11) Marini, F.; Magri, A. L.; Balestrieri, F.; Fabretti, F.; Marini, D. Supervised pattern recognition applied to the discrimination of the floral origin of six types of Italian honey samples. *Anal. Chim. Acta* **2004**, *515*, 117−125.
(12) Anklam, E. A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chem.* **1998**, *63*, 549−562.
(13) Kaškonienė, V.; Venskutonis, P. R. Floral markers in honey of various botanical and geographic origins: a review. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 620−634.
(14) Yao, L.; Jiang, Y.; Singanusong, R.; Datta, N.; Raymont, K. Phenolic acids and abscisic acid in Australian eucalyptus honeys and their potential for floral authentication. *Food Chem.* **2004**, *86*, 169−177.
(15) Gad, H. A.; El-Ahmady, S. H.; Abou-Shoer, M. I.; Al-Azizi, M. M. Application of chemometrics in authentication of herbal medicines: a review. *Phytochem. Anal.* **2013**, *24*, 1−24.
(16) Tistaert, C.; Thierry, L.; Szandrach, A.; Dejaegher, B.; Fan, G.; Frédérich, M.; Vander Heyden, Y. Quality control of *Citri reticulatae* pericarpium: exploratory analysis and discrimination. *Anal. Chim. Acta* **2011**, *705*, 111−122.
(17) Baroni, M. V.; Arrua, C.; Nores, M. L.; Fayé, P.; Díaz, M. d. P.; Chiabrando, G. A.; Wunderlin, D. A. Composition of honey from Córdoba (Argentina): assessment of north/south provenance by chemometrics. *Food Chem.* **2009**, *114*, 727−733.
(18) Bentabol Manzanares, A.; García, Z. H.; Galdón, B. R.; Rodríguez, E. R.; Romero, C. D. Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. *Food Chem.* **2011**, *126*, 664−672.
(19) Lazarević, K. B.; Andrić, F.; Trifković, J.; Tešić, Ž.; Milojković-Opsenica, D. Characterisation of Serbian unifloral honeys according to their physicochemical parameters. *Food Chem.* **2012**, *132*, 2060−2064.
(20) Aliferis, K. A.; Tarantilis, P. A.; Harizanis, P. C.; Alissandrakis, E. Botanical discrimination and classification of honey samples applying gas chromatography/mass spectrometry fingerprinting of headspace volatile compounds. *Food Chem.* **2010**, *121*, 856−862.
(21) Stanimirova, I.; Üstün, B.; Cajka, T.; Riddelova, K.; Hajslova, J.; Buydens, L. M. C.; Walczak, B. Tracing the geographical origin of honeys based on volatile compounds profiles assessment using pattern recognition techniques. *Food Chem.* **2010**, *118*, 171−176.
(22) Özbalci, B.; Boyaci, İ. H.; Topcu, A.; Kadılar, C.; Tamer, U. Rapid analysis of sugars in honey by processing Raman spectrum using chemometric methods and artificial neural networks. *Food Chem.* **2013**, *136*, 1444−1452.
(23) Bertoncelj, J.; Polak, T.; Kropf, U.; Korošec, M.; Golob, T. LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. *Food Chem.* **2011**, *127*, 296−302.
(24) Corbella, E.; Cozzolino, D. The use of visible and near infrared spectroscopy to classify the floral origin of honey samples produced in Uruguay. *J. Near Infrared Spectrosc.* **2005**, *13*, 63−68.
(25) Zhu, X.; Li, S.; Shan, Y.; Zhang, Z.; Li, G.; Su, D.; Liu, F. Detection of adulterants such as sweeteners materials in honey using near-infrared spectroscopy and chemometrics. *J. Food Eng.* **2010**, *101*, 92−97.
(26) Chen, L.; Xue, X.; Ye, Z.; Zhou, J.; Chen, F.; Zhao, J. Determination of Chinese honey adulterated with high fructose corn syrup by near infrared spectroscopy. *Food Chem.* **2011**, *128*, 1110−1114.
(27) Pataca, L. C. M.; Neto, W. B.; Marcucci, M. C.; Poppi, R. J. Determination of apparent reducing sugars, moisture and acidity in honey by attenuated total reflectance-Fourier transform infrared spectrometry. *Talanta* **2007**, *71*, 1926−1931.
(28) Lichtenberg-Kraag, B.; Hedtke, C.; Bienefeld, K. Infrared spectroscopy in routine quality analysis of honey. *Apidologie* **2002**, *33*, 327−337.
(29) Ruoff, K.; Luginbühl, W.; Künzli, R.; Iglesias, M. T.; Bogdanov, S.; Bosset, J. O.; von der Ohe, K.; von der Ohe, W.; Amadò, R. Authentication of the botanical and geographical origin of honey by mid-infrared spectroscopy. *J. Agric. Food Chem.* **2006**, *54*, 6873−6880.
(30) Tewari, J. C.; Irudayaraj, J. M. K. Floral classification of honey using mid-infrared spectroscopy and surface acoustic wave based z-nose sensor. *J. Agric. Food Chem.* **2005**, *53*, 6955−6966.
(31) Souto, U. T. C. P.; Pontes, M. J. C.; Silva, E. C.; Galvão, R. K. H.; Araújo, M. C. U.; Sanches, F. A. C.; Cunha, F. A. S.; Oliveira, M. S. R. UV-vis spectrometric classification of coffees by SPA-LDA. *Food Chem.* **2010**, *119*, 368−371.
(32) Sârbu, C.; Naşcu-Briciu, R. D.; Kot-Wasik, A.; Gorinstein, S.; Wasik, A.; Namieśnik, J. Classification and fingerprinting of kiwi and pomelo fruits by multivariate analysis of chromatographic and spectroscopic data. *Food Chem.* **2012**, *130*, 994−1002.
(33) Cozzolino, D.; Cynkar, W. U.; Shah, N.; Smith, P. A. Can spectroscopy geographically classify Sauvignon Blanc wines from Australia and New Zealand? *Food Chem.* **2011**, *126*, 673−678.
(34) Gad, H. A.; El-Ahmady, S. H.; Abou-Shoer, M. I.; Al-Azizi, M. M. A modern approach to the authentication and quality assessment of thyme using UV spectroscopy and chemometric analysis. *Phytochem. Anal.* **2013**, DOI: 10.1002/pca.2426.
(35) Reid, L. M.; O'Donnell, C. P.; Downey, G. Recent technological advances for the determination of food authenticity. *Trends Food Sci. Technol.* **2006**, *17*, 344−353.
(36) Beretta, G.; Fermo, P.; Maffei Facino, R. Simple and rapid simultaneous profiling of minor components of honey by size exclusion chromatography (SEC) coupled to ultraviolet diode array detection (UV-DAD), combined with chemometric methods. *J. Pharm. Biomed. Anal.* **2012**, *58*, 193−199.
(37) Karoui, R.; Dufour, E.; Bosset, J. O.; De Baerdemaeker, J. The use of front face fluorescence spectroscopy to classify the botanical origin of honey samples produced in Switzerland. *Food Chem.* **2007**, *101*, 314−323.
(38) Esbensen, K. H. *Multivariate Data Analysis in Practice*; Camo: Oslo, Norway, 2002.
(39) Andersen, Ø. M.; Markham, K. R. *Flavonoids: Chemistry, Biochemistry, and Applications*; CRC, Taylor and Francis: Boca Raton, FL, 2006; pp 1237.
(40) Nuru, A.; Awad, M. A.; Al-Ghamdi, A. A.; Alqarni, A. S.; Radloff, S. E. Nectar of *Ziziphus spina-christi* (L.) Willd (Rhamnaceae): dynamics of secretion and potential for honey production. *J. Apic. Sci.* **2012**, *56*, 49−59.
(41) Tamayo, P.; Slonim, D.; Mesirov, J.; Zhu, Q.; Kitareewan, S.; Dmitrovsky, S.; Lander, E.; Golub, T. R. Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 2907−2912.