Untargeted headspace gas chromatography – ion mobility spectrometry analysis for detection of adulterated honey

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Abstract

The recognized properties of honey together with its price have, almost inevitably, led to economically motivated adulteration. In this work, headspace gas chromatography coupled to ion mobility spectrometry (HS-GC-IMS) is proposed for the differentiation of honey according to its purity and the level of adulteration by sugar cane or corn syrups. An easy and rapid sample treatment, consisting of incubating 1 g of honey at 100 °C for 15 min and then injecting 750 µL of the sample headspace into the GC-IMS system, is proposed. A 3-dimensional data map is obtained in 32 min. The proposed method was used for the analysis of 198 honey samples (56 pure honeys of different botanical origins, 71 honeys adulterated with sugar cane syrup and 71 adulterated with corn syrup. The influence of the adulterant on variations in the honey sample spectrum was studied. In order to obtain chemometric models for the detection of adulterated honey samples, the data obtained by HS-GC-IMS were processed selecting the significant markers of the spectrum fingerprint. OPLS-DA models were constructed using 80% of the samples, and the remaining 20% were used for method validation. The differentiation between pure and adulterated honeys had a validation success of 97.4%, and the assessment of adulterant content was obtained with a 93.8% validation success rate for both adulterant

agents assayed. Nine commercial honey samples were analyzed using the proposed methodology, and seven of them were classified as adulterated.

Keywords: ion mobility spectrometry, headspace; gas chromatography; honey adulteration; untargeted analysis; chemometric models.

1. Introduction

Honey is considered a natural high-quality product much appreciated for its nutritional and medicinal properties [1,2].The exact chemical composition of honey is complex since it depends on the source of nectar, the plant species and the environmental conditions. Aldehydes of from one to five carbon atoms, alcohols and esters, such as methyl and ethyl formate, as well as phenylacetic esters, are the main volatile components [3]. Its worldwide use, makes it a target for fraudsters, and, indeed, it is one of the most adulterated foods in the market [4]. Since the price of honey depends mainly on its floral origin, mislabelling of the floral and geographical origin, as well as the addition of cheaper sweeteners or water are the most common economic frauds suffered by this product. Although human health is not generally affected by adulteration, purity of honey is obviously a priority for producers and regulatory authorities, which need to guarantee the quality of their products.

The most commonly used sweeteners added to honey are starch (rice or corn) and inverted syrups, due to their high fructose levels, wide availability and low cost [5,6]. The food can be directly or indirectly adulterated depending on whether the sugar syrups are added to the final product or used to feed bee colonies during the main nectar period.

Different methods have been developed to detect the adulteration of honey by sugar syrups [1,5]. For example, fingerprint profiles of honey carbohydrates have been obtained using chromatographic methods such as high performance liquid chromatography (HPLC), with refractive index [7,8], quadrupole time flight mass spectrometry (Q-TOF-MS) (Q-TOF-MS) [9], or pulsed amperometric detection [10,11]. Gas chromatography (GC), with flame ionization detection (FID) [10] or mass spectrometry (MS) [12,13] have also been used for the same purpose, the carbohydrates in this case being determined as their corresponding trimethylsilylated derivatives. Liquid chromatographic monitoring of other adulteration markers such as 2-acetylfuran-3-glucopyranoside [14] and phenolic acids [15] has also been applied to detect the fraudulent addition of sugar to honey, using diode array and electrochemical detection, respectively.

For its part, elemental analyzer-isotope ratio mass spectrometry (EA-IRMS), which allows an accurate stable carbon isotope ratio analysis, is useful for checking honey

authenticity, since the isotopic ratio of $^{13}C/^{12}C$ differs in monocotyledonous (C4 plants, such as cane and corn) and dicotyledonous species (C3 plants, such as flowering plants that provide nectar for bees). The methodology has been applied to detect both direct [16,17,18] and indirect [19] honey adulteration. Nevertheless, some adulterants such as rice syrup are difficult to detect by IRMS because they are also produced by C3 plants [5]. Another disadvantage of IRMS is the need for expensive instrumentation and specialized personnel [19]. Other techniques that have been used for the same purpose are near-infrared spectroscopy [20,21], Fourier transform infrared spectroscopy [22,23], electrical impedance spectroscopy [22], Raman spectroscopy [24] and nuclear magnetic resonance (NMR) [25]. Of interest also are the recently published procedures that propose the use of an electronic tongue based on potential multistep pulse voltammetry [26] and conventional and real-time polymerase chain reaction [27].

Ion mobility spectrometry (IMS) has demonstrated its efficiency for the characterization and analysis of volatile compounds in very different matrices, including food samples [28]. The IMS is characterized by a good sensitivity, minimal sample treatment, in addition to the advantages of rapidity and low cost of analysis compared with other analytical techniques. The gas phase ions separation in the IMS instrument is based on their different mobility inside a drift tube submitted to a constant electric field maintained at atmospheric pressure. When used as a detector for GC, a two-dimensional separation of the volatile compounds in a given sample can be achieved due to the combination of the GC retention time and IMS drift time. The potential of GC-IMS coupling to improve sensitivity and selectivity has recently been reviewed [29]. The high number of data provided by GC-IMS analyses requires chemometric processing. Orthogonal partial least squares-discriminant analysis (OPLS-DA) was here used as chemometric tool. In this work, fingerprint profiles due to untargeted volatile compounds are obtained by headspace-gas chromatography coupled to ion mobility spectrometry (HS-GC-IMS) and are used to detect honey adulteration by sugar cane or corn syrups. The potential of HS-GC-IMS for honey characterization based on their floral origin [30,31] has been demonstrated. Very recently, the adulteration with corn molasses has been also described [32]. However, to the best of our knowledge, this is the first time that IMS is used as an analytical tool for the simultaneous detection of several adulterants in honeys of different floral origins, and the assessment of adulterant content.

2. Materials and methods

2.1. Honey samples

A total of 56 honey samples from different botanical origin (orange blossom, multifloral, rosemary, melon, lemon/orange blossom, lavender, broom and thyme honeys) were used. These were supplied by Murcia beekeepers. Two different syrups were used as adulterants (sugar cane and corn syrup) and were purchased in local markets.

Each variety of honey was adulterated with sugar cane and corn syrups at different percentages of adulterant (10, 20, 30, 40, 50, 60, 70, 80 and 90%). Consequently, 200 samples of honey were analysed (56 pure and 144 adulterated). Adulterated samples were prepared by mixing 10 g of pure honey with the corresponding proportions of adulterant.

In addition, 5 honey samples were purchased in local markets from Murcia and analysed in order to show the applicability of the method for detecting possible frauds.

2.2. HS-GC-IMS method

For HS-GC-IMS analysis, an Agilent Technologies 6890N (N.05.05 version) gas chromatograph (Agilent, Waldbronn, Germany) equipped with a headspace sampling unit and a 2.5 mL syringe (Gerstel GmbH, Mühlheim, Germany) was coupled to an IMS module from G.A.S (Gesellschaft für Analytische Sensorsysteme mbH, Dortmund, Germany).

The experiments were carried out using 1 g of sample that was transferred into a 20 mL vial, and incubated at 100 °C for 15 min with speed agitation of 750 rpm. Then, a 750 µL aliquot of the headspace was injected by a heated syringe (100 °C) into the heated injector (100 °C) in splitless mode. Nitrogen of 99.99% purity (supplied by Air Liquide, Madrid) was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The analytes eluted in a non-polar GC column HP-5MS UI (Agilent J&W GC Column) with 0.25 µm of film thickness, 30 m length and 0.25 mm internal diameter (19091S-433UI, USA). The oven program was set as follows: initial temperature of 50 °C held 3 min, which was increased from 50 °C to 130 °C at 10 °C min^{-1} and held 130 °C for 6 min (total run 17 min). Analytes were driven to the IMS module and ionized by a Tritium source at atmospheric pressure

in a positive ion mode. Nitrogen was also used as drift gas at a constant flow of 150 mL min^{-1}. Once ions are formed in the ionisation chamber, they were placed into a 98 mm length drift tube operated with a constant voltage (500 V cm^{-1}) at 80 °C. Each spectrum had an average of 32 scans, obtained using a repetition rate of 30 ms, a grid pulse width of 150 µs and a drift and blocking voltages of 241 and 80 V, respectively.

2.3.Multivariate processing

The chemometric strategy is based on the method described by Contreras et al. [33]. Every spot of topographic maps is observed as a 3D-signal characterized by a drift time in milliseconds and a retention time in seconds, due to the double separation carried out in the drift tube and in the chromatographic column, and intensity value (V), represented by a colour scale. To perform the multivariate analysis, markers were selected by visual exploration of the topographic plots of all the samples analysed, and all those that could be distinguished from the baseline were selected. In this way, a total of 130 markers were used and the intensity above the baseline of each marker was selected as the analytical signal, which was obtained using LAV software.

Topographic maps were previously aligned by overlaying the samples to a reference honey sample. Reactant ion peak (RIP) [34], which appears as constant red line in the topographic map, was used as internal standard, and data were also normalized with respect to RIP intensity.

Data were randomly split into two groups: calibration set (80% of the analysed samples) and validation set (20% of the analysed samples). Subsequently, orthogonal partial least squares-discriminant analyses (OPLS-DA) were performed with the calibration set using SIMCA software version 14.1 (Umetrics, Sartorius Stedim Biotech AS, Umea, Sweden), and validated using validation set. In addition, six different scaling (unit variance (UV), unit variance none (UVN), pareto (Par), pareto none (ParN), centering (Crt) and freeze) [35], as well as the logarithmic transformation of the data, were investigated.

Models were evaluated by means of success of validation, R2X(cum), R2Y(cum), and Q2(cum) [36]. R2Y(cum) and Q2(cum) are the cumulative percentage of the variation of the dependent variable explained by the model and a measure of the predictive ability,

respectively. These parameters are particularly relevant with regard to R2X(cum), the cumulative fraction of X variation modelled up to the specific component. All of them range between 0 and 1, being the values closer to one which indicates better fit of the model. Concerning the Q2 parameter, a significant threshold of 0.5 is generally acceptable, for that reason the model achieves a good predictive ability [37,38].

3. Results and discussion

3.1.Optimization of HS-GC-IMS parameters

The parameters of sample incubation time and temperature, oven program and drift tube temperature were investigated in order to optimize the HS-GC-IMS method. An orange blossom honey sample was used as reference matrix

The effect of the sample incubation temperature was studied in the 60-100 °C range. The release of volatile organic compounds with a high boiling point is further facilitated by an increase in incubation temperature. This causes an increase of the number of signals and their intensity. For this reason, the incubation temperature was set at 100 ºC (Supplemental Figure S1). The incubation temperature was not raised above 100 ºC to avoid condensation of the compounds in the drift tube, since it supports a maximum temperature of 100 ºC.

Then, sample incubation time was studied between 5 and 20 min (Supplemental Figure S2). By increasing the incubation time, the number of signals and their intensity increased. However, no significant differences were appreciated between 15 and 20 min. Therefore, 15 min was selected for further experiments.

The oven program started with an initial temperature of 50 ºC held for 3 min and then it was raised to 130 ºC. For this, the heating rate was studied in the 6-10 ºC min^{-1} range. When the heating rate was increased, topographic plot signals appeared before and also new signals appeared, which were previously despised (Supplemental Figure S3). For that reason, a 10 ºC min^{-1} rate was selected. A higher rate was not used because of the risk of a poor resolution among the more grouped signals.

Finally, the drift tube temperature was investigated between 60 and 90 ºC (Supplemental Figure S4). As can be seen, no significant differences were obtained, and so a temperature of 80 ºC was selected.

3.2.Method characterization and precision study

The optimized analytical method was characterized by means of a precision study in terms of repeatability and intermediate precision. In order to study the precision, octan-2-one, heptan-2-one, hexan-2-one, pentan-2-one and butan-2-one compounds were selected as standards, as they showed very different drift and retention time values in the GC column [34], consequently precision could be studied at different sections of topographic plot.

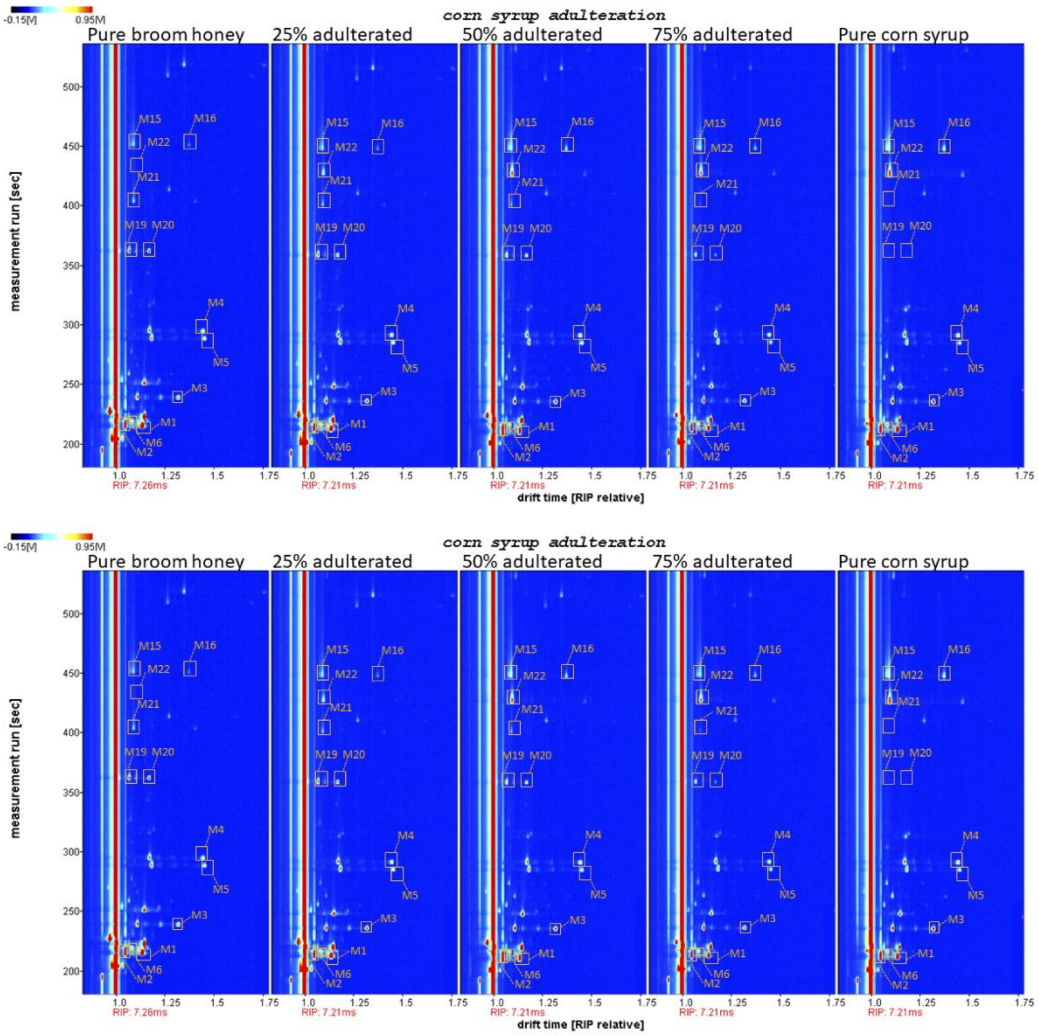
The GC-IMS method was applied repetitively to ten standard solutions of the ketones at 0.5 mg L^{-1}. Standard solutions were prepared using a non-aqueous matrix (refined oil), in order to avoid the evaporation of solvent when incubating at 100 °C. Each standard solution was injected before analyses of honey samples on the same day and repeatability was thus evaluated. Intermediate precision was assessed using ten standard samples prepared with the same procedure and analysed in ten different days. Supplemental Table S1 shows the results expressed as relative standard deviation (RSD) of intensity (absolute values without normalizing), drift time and retention time for the protonated monomer and proton-bound dimer peaks. As can be seen, the best values of RSDs, lower than 0.34 %, were obtained for drift time, whereas RSDs of retention time were lower than 0.42 %, therefore in all cases good precision was obtained. RSDs of peak intensity were always lower than 10 %.

3.3.Topographic plots comparison

Topographic plots of pure and adulterated samples obtained with the proposed methodology showed some visual differences.

Figure 1a and 1b show the variation of topographic plots when a broom honey sample was adulterated with sugar cane and corn syrups, respectively. The area between 200 and 300 s (retention time) was the richest in signals, and therefore this area collected most of the differences. Specifically, the intensity of markers M1 (t_R= 219.78 s, t_D= 8.295 ms, K_o=1.820 $cm^2\ V^{-1}\ s^{-1}$), M2 (t_R= 220.77 s, t_D= 7.672 ms, K_o= 1.968 $cm^2\ V^{-1}\ s^{-1}$), M3 (t_R=

241.56 s, t_D= 9.652 ms, K_o=1.564 cm^2 V^{-1} s^{-1}), M4 (t_R=297.99 s, t_D=10.541 ms, K_o= 1.432 cm^2 V^{-1} s^{-1}), M5 (t_R=292.050 s, t_D= 10.586 ms, K_o= 1.426 cm^2 V^{-1} s^{-1}) and M6 (t_R= 223.74 s, t_D= 7.939 ms, K_o= 1.902 cm^2 V^{-1} s^{-1}) increased as the content of sugar cane syrup increased. On the contrary, the intensity of these markers remained constant when the other adulterant (corn syrup) was added.



v

The presence of the adulterant sugar cane syrup involves the appearance of the markers M7 (t_R=278.19 s, t_D=7.649 ms, K_o= 1.974 cm^2 V^{-1} s^{-1}), M8 (t_R=232.65 s, t_D=7.677 ms, K_o= 1.967 cm^2 V^{-1} s^{-1}), M9 (t_R=251.46 s, t_D=7.673 ms, K_o= 1.968 cm^2 V^{-1} s^{-1}), M10 (t_R=316.8 s, t_D=7.649 ms, K_o= 1.974 cm^2 V^{-1} s^{-1}), M11 (t_R=295.02 s, t_D=7.649 ms, K_o= 1.974 cm^2 V^{-1} s^{-1}) and M12 (t_R=270.27 s, t_D=8.495 ms, K_o= 1.777 cm^2 V^{-1} s^{-1}). These markers did not appear with the addition of the other adulterant and, therefore, could be

used as discriminatory signals between them. Something similar happens with markers M13 (t_R=298.98 s, t_D=9.461 ms, K_o= 1.593 $cm^2\ V^{-1}\ s^{-1}$) and M14 (t_R=292.05 s, t_D=9.506 ms, K_o= 1.582 $cm^2\ V^{-1}\ s^{-1}$), the presence of corn syrup in the honey sample implies the appearance of both markers, but in this case, their intensity does not increase with increasing concentration of adulterant.

After 300 s, the signals of the topographic plot are less abundant but there are also signals that allow to differentiate adulterated honeys from unadulterated. Specifically, the signal of markers M15 (t_R=455.400 s, t_D= 7.920 ms, K_o= 1.907 $cm^2\ V^{-1}\ s^{-1}$) and M16 (t_R=454.410 s, t_D= 10.050 ms, K_o= 1.502 $cm^2\ V^{-1}\ s^{-1}$) also increase by increasing the percentage of both adulterants, although in a subtler way. However, M17 (t_R= 320.76 s, t_D= 8.718 ms, K_o= 1.732 $cm^2\ V^{-1}\ s^{-1}$) and M18 (t_R= 320.76 s, t_D= 9.274 ms, K_o= 1.628 $cm^2\ V^{-1}\ s^{-1}$) increase as the adulterant sugar cane syrup content increases, but these markers do not appear when corn syrup is used as the adulterant.

In addition, the markers M19 (t_R= 363.33 s, t_D= 7.787 ms, K_o= 1.939 $cm^2\ V^{-1}\ s^{-1}$), M20 (t_R= 363.33 s, t_D= 8.541 ms, K_o= 1.768 $cm^2\ V^{-1}\ s^{-1}$) and M21 (t_R= 412.830 s, t_D= 8.031 ms, K_o= 1.880 $cm^2\ V^{-1}\ s^{-1}$) can be assigned to compounds present in pure honey, since their signals decrease when the adulterant content increases, being imperceptible in the spectrum of the pure adulterant. Conversely, M22 (t_R=444.510 s, t_D= 8.095 ms, K_o= 1.865 $cm^2\ V^{-1}\ s^{-1}$) is not present in pure honey and appears as soon as one of the two adulterants is present. Its intensity also increases with concentration.

The Supplemental Figures S5 and S6 collect the intensity of each of these markers in the different types of pure and adulterated honey. As can be seen, a large variability in the abundance of each marker was found, and the establishment of clear markers that allow the visual classification of adulterated honeys is practically impossible. In a general way, when honey was adulterated with sugar cane syrups, M3, M4, M5, M9, M10, M12, M13, M14, M15, M17, M18 and M22 showed a tendency to have more intensity at a higher concentration of adulterant, therefore, these markers could be associated with the adulteration with sugar cane syrup. However, only the M22 showed clearly the same tendency when the honey was adulterated with corn syrup. In addition, a clear decrease in the signal of the M21 was reached when the honey was adulterated with corn syrup.

3.4.Chemometric models and classification

As indicated in section 2.3., initially, topographic maps were aligned by overlaying the samples to a reference honey sample using LAV software (version 2.0.0) from G.A.S. Then, markers (defined by retention and drift times) were manually selected by visual exploration of the topographic plots. A total of 130 markers were selected after the exploration of all the analyzed samples (pure honeys of the different varieties and adulterated honeys). Two of the samples deliberately adulterated, suffered an accidental contamination during handling, so they were not finally used for this work. Therefore, the final data matrix had dimensions of 198 (samples) x 130 (markers). This matrix was used to create chemometrics models to differentiate between pure and adulterated honey samples and for the assessment of the percentage of adulterant.

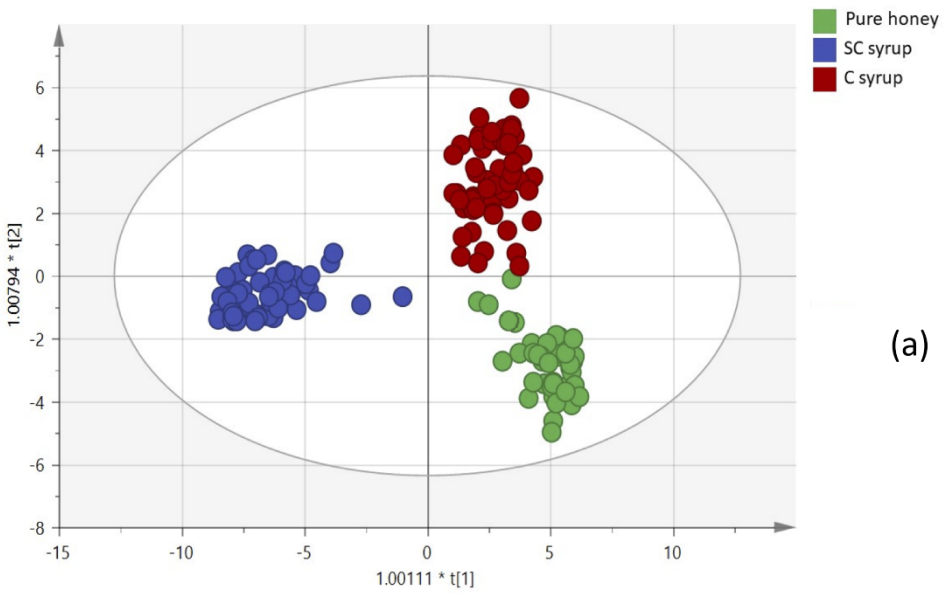
3.4.1. Differentiation between pure and adulterated honey samples

In order to create chemometrics models allowing pure and adulterated honey samples to be differentiated, data from 56 pure honey samples of 8 different varieties and 142 adulterated honey samples were used. The 142 adulterated samples were made by adding different concentration (10, 20, 30, 40, 50, 60, 70, 80 and 90%) of two adulterants (sugar cane and corn syrups).

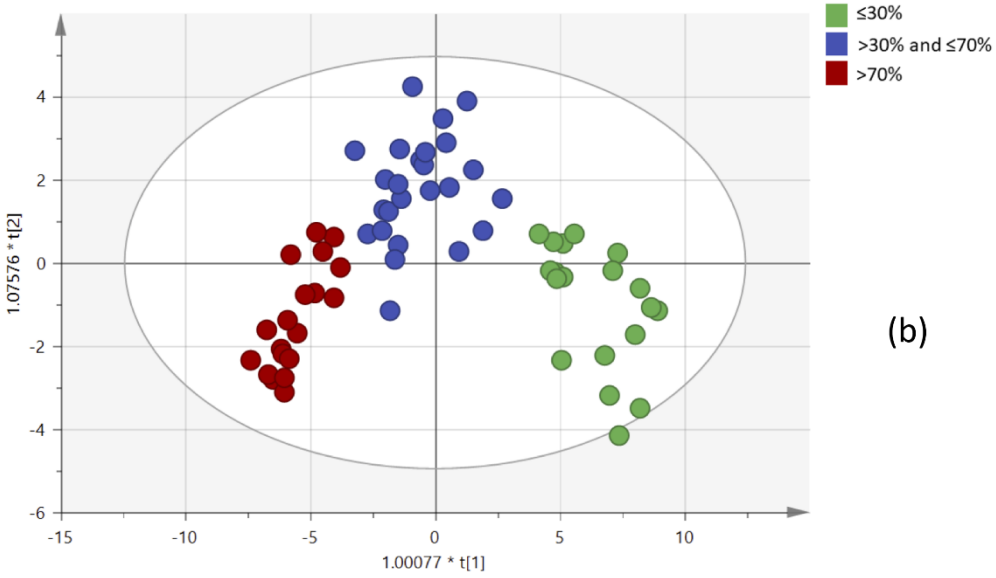
To obtain the best classification models, the need to correct the signals with an internal standard was investigated. In this case, following the suggestions indicated elsewhere [33], RIP was set as an internal standard, and the intensity of each marker was corrected with the intensity of RIP at the beginning of the analysis. Two different data matrices with the same dimensions (198 x 130) were obtained, one of them with the raw data and the other with the data normalized with respect to the RIP. Multivariate analysis was carried out in both matrices in parallel, in order to compare the results.

The 198 samples were divided into two groups, 80% of samples for training set (159 samples) and 20% of samples for validation set (39 samples), and different OPLS-DA were performed using six scaling (UV, UVN, Par, ParN, Crt and Freeze) and raw data or logarithmic transformation. Supplemental Table S2 summarizes the results of each chemometric model including the information of model (number of components, and

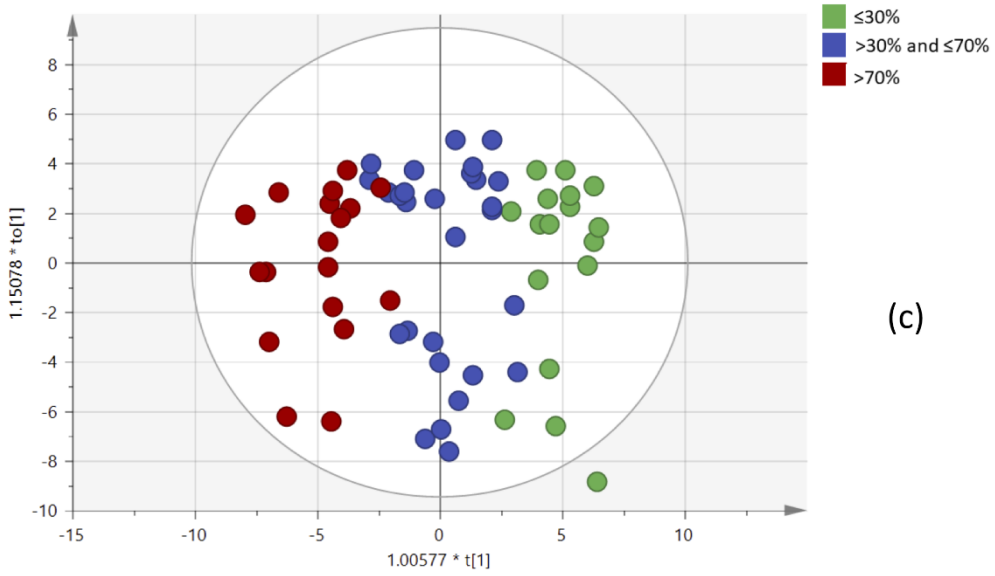
R2X(cum), R2Y(cum) and Q2(cum) parameters), classification rate obtained for calibration and validation set, and the percentage of success for each category in the validation set to demonstrate the sensitivity of the method, calculated as ∑ True positive / (∑True positive + ∑False negative) × 100. As can be seen, the best results were obtained with normalized data respect to RIP intensity, using UV scaling without logarithmic transformation. The OPLS-DA model is shown in Figure 2a. The classification rate was 96.4%, in accordance with previous investigations [32] and the validation rate was 97.4%, classifying correctly the 100% of adulterated samples, and therefore no false positives were obtained, and the 91.7% of pure honey samples, since only one pure honey was classified as honey adulterated with corn syrup (Table 1).


Pure honey
SC syrup
C syrup
1.00794 * t[2]
1.00111 * t[1]

(a)


≤30%
>30% and ≤70%
>70%
1.07576 * t[2]
1.00077 * t[1]

(b)


≤30%
>30% and ≤70%
>70%
1.15078 * to[1]
1.00577 * t[1]

(c)

3.4.2. Chemometric models for assessment the percentage of sugar cane syrup adulteration

In order to assess the concentration of sugar cane adulterant present in the honey samples, different OPLS-DA models were also investigated. Three intervals of adulterant concentration were stablished: honey adulterated with a percentage of sugar cane less than or equal to 30%, higher than 30% and less than or equal to 70% and higher than 70%. In this case, data matrix was composed for 79 samples, including 71 samples adulterated with sugar cane at different percentages (10, 20, 30, 40, 50, 60, 70, 80 and 90%) from the 8 varieties of honey and 8 pure sugar cane samples. As in the previous section, 80% of these samples (63 samples) were used to build the model and the remaining 20% (16 samples) to validate it. Supplemental Table S3 summarizes the results obtained for models built with different scales, data transformation, and correcting with the RIP and without correction. The best separation between groups and validation rate were obtained using UV scale with logarithmic transformation and normalized data respect to RIP intensity (Figure 2b).

In this case, good classification (95.2%) and validation (93.8%) rates were obtained. The 100% of adulterated samples at concentration higher than 30% were classified correctly, and only one sample with a concentration of adulterant less than 30% was classified in a higher concentration group (Table 2).

3.4.3. Chemometric models for assessment the percentage of corn syrup adulteration

In this case, the same concentration levels were stablished: honey adulterated with a percentage of corn syrup less than or equal to 30%, higher than 30% and less than or equal 70% and higher than 70%. The dimensions of the data matrix were also the same. It was composed for 79 samples, including 71 samples adulterated with corn syrup at different percentages from the 8 varieties of honey and 8 pure corn syrup samples, of which 63 built the training set and 16 the validation set. Supplemental Table S4 shows the results of applying different pre-treatment methods (normalization, scaling and transformation) and as can be seen, again the best results were obtained using normalized data respect to RIP intensity and UV scaling with logarithmic transformation (Figure 2c). Table 3 shows

the validation matrix of ternary OPLS-DA for the classification of adulterated honey samples at different concentration of corn syrup. As with the other adulterant, the 93.8% of samples were obtained correctly, only one sample at concentration less than or equal to 30%, was misclassified, whereas the 100% of samples with concentration higher than 30% were classified correctly.

3.5.Analysis of real samples

To check the applicability of the method, nine commercial samples of honey were purchased in local markers from Murcia and analyzed using the proposed HS-GC-IMS methodology.

Samples were analyzed in duplicate, and seven of them were classified as adulterated honey with corn syrup, which confirms the convenience for an analytical method to detect frauds in beekeeping. After applying the OPLS-DA models to determine the percentage of adulteration, a percentage ≤30% was detected for four samples, two samples were included in the group with a percentage of adulterant >30 and ≤70% and the other one was classified with a percentage of corn syrup >70%.

4. Conclusions

The results from this work demonstrated the potential of HS-GC-IMS for a reliable classification of honeys according to its purity and percentage of adulteration with sugar cane or corn syrups. The jointly use of HS-GC-IMS, UV scaling as pre-treatment and OPLS-DA models for classification allowed pure honey to be differentiated of honey adulterated with corn or sugar cane syrups, and the adulteration percentage to be calculated. In all the cases, data normalization respect to RIP intensity was necessary. None false negatives were obtained with this strategy, since all adulterated samples of the validation set were detected. Only one pure honey was classified as honey adulterated with corn syrup, obtaining validation rate of 97.4%. In addition, the chemometric models developed to determine the percentage of adulteration allowed the correct classification of 93.75% of samples. In this case, a logarithmic transformation of data was required.

The good results achieved with this methodology evidence that it could be used to detect label honey frauds. It could be a useful tool for quality control since no pre-treatment of

sample is necessary, the analysis time takes only 32 min and it has relative low cost of the experiment compared with other methodologies.

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Figure captions

Figure 1. (a) Comparison of topographic plots of pure broom honey, adulterated broom honey at different concentration of sugar cane syrup and pure sugar cane syrup. (b) Comparison of topographic plots of pure broom honey, adulterated broom honey at different concentration of corn syrup and pure corn syrup.

Figure 2. (a) Ternary OPLS-DA model using normalized data and UV scaling to discriminate between pure and adulterated honey samples. SC syrup: honey samples adulterated with sugar cane syrup; C syrup: honey samples adulterated with corn syrup. (b) Ternary OPLS-DA model using normalized data, UV scaling and logarithmic transformation to classify adulterated honey samples at different concentrations of sugar cane syrup. (c) Ternary OPLS-DA model using normalized data, UV scaling and logarithmic transformation to classify adulterated honey samples at different concentrations of corn syrup.

Table 1. Validation matrix of ternary OPLS-DA for the differentiation of pure honey and adulterated honeys with sugar cane (SC) and corn (C) syrups.

		Actual classes			
		Pure	SC Syrup	C Syrup	
Predicted classes	Pure	11	0	0	**Success 97.44%**
	SC Syrup	0	15	0	
	C Syrup	1	0	12	

Table 2**.** Validation matrix of ternary OPLS-DA for the classification of adulterated honey samples at different concentration of sugar cane syrup.

		Actual classes			
		≤30%	>30% and ≤70%	>70%	
Predicted classes	≤30%	4	0	0	**Success 93.75%**
	>30% and ≤70%	1	6	0	
	>70%	0	0	5	

Table 3. Validation matrix of ternary OPLS-DA for the classification of adulterated honey samples at different concentration of corn syrup.

		Actual classes			
		≤30%	>30% and ≤70%	>70%	
Predicted classes	≤30%	5	0	0	**Success 93.75%**
	>30% and ≤70%	1	4	0	
	>70%	0	0	6	