Identification of botanical origin of Chinese unifloral honeys by free amino acid profiles and chemometric methods

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Abstract

The amino acid contents of five floral sources Chinese honeys (jujube, rape, chaste, acacia, and lungan) were measured using reversed phase high-performance liquid chromatography (RP-HPLC). The results showed that proline was the main amino acid in most of the analyzed samples. Phenylalanine presents at the highest content in chaste honey samples, and the total amino acid contents of chaste honeys were also significantly higher than those of other honey samples. Based on the amino acid contents, honey samples were classified using chemometric methods (cluster analysis (CA), principal component analysis (PCA), and discriminant analysis (DA)). According to the CA results, chaste honeys could be separated from other honeys, while the remaining samples were correctly grouped together when the chaste honey data were excluded. By using DA, the overall correct classification rate reached 100%. The results revealed that amino acid contents could potentially be used as indicators to identify the botanical origin of unifloral honeys.

1. Introduction

Honeys that come from different botanical origins vary widely in composition, nutritional contribution, and market price [1]. Thus, finding accurate ways to determine the origin of honey has become an important issue. Kaškonienė and Venskutonis [2] reviewed the chemical markers in honey, and indicated that volatile compounds [3], phenolic compounds [4,5], carbohydrates [6], nitrogen-containing compounds [7], pollen [8], and other minor components might distinguish certain types of honey from others. However, there is no unambiguous and unique indicator that accurately identifies the botanical origin of honey. Therefore, in order to establish methods for differentiating honey types, research on chemical markers requires further development.

As characteristic small compounds found in honey, amino acids can provide some information about botanical origin. The amino acids present in honey mainly arise from the pollen of the plant, so they are derived directly from the plant source. Honey contains about 1% amino acids, of which proline is the dominant amino acid accounting for 50%–85% of total amino acids [9]. This is because proline is the main amino acid in pollen [10]. Furthermore, proline is used as a quality parameter of honey according to the laws in many countries, such as the AOAC [11]. There are many other amino acids in honey, such as glycine, alanine, leucine, and phenylalanine. It has been previously reported that amino acid content can serve as an useful indicator for estimating the origin of honey [12,13]. However, the classification of Chinese honey using amino acids data has not been described so far.

As an important honey importer and exporter, China is very rich in bee resources. Jujube, acacia, rape, lungan and chaste have the largest production capacity; especially jujube, acacia and chaste honey belong to the four most famous honey types. Jujube trees are grown originally in China, and jujube honey is mainly produced in Shaanxi, Shanxi, and Henan provinces of China and it is a specially Chinese honey [14]. Acacia, chaste and rape honeys are light color honey; the colors of these honey are from transparent to light yellow to light amber. In contrast, the color of jujube and lungan honeys is amber, more or less dark. Lungan honey is a top-grade spirit, and acacia, chaste and jujube honeys also have better quality. These four types of honeys are easily adulterated for their higher price. The price of rape honey is significantly lower than that of other honeys, so it is often used to adulterate.

Chemometrics can extract useful information from numerous data and make complicated analysis simple. Its combination with high-
performance liquid chromatography (HPLC) has been used in the
determination of certain chemical fingerprints of natural products
including honey [4, 15]. The cluster analysis (CA), principal component
analysis (PCA) and discriminant analysis (DA) are common to handle
data [16]. By calculating the data of different parameters, such as
physicochemical indicators, phenolic acids and polyphenols, proteins,
voltiles, as well as free amino acids, these methods could analyze,
identify and classify honeys [17].

The aim of the present study was to assess whether it is a useful way to
identify the botanical origin of Chinese unifloral honeys by analyzing
amino acid profiles. Five different Chinese honey types were investigated
by analyzing their amino acid profiles using reversed phase high-
performance liquid chromatography (RP-HPLC). In addition, PCA was
performed to reduce dimensionality, and CA and DA were employed to
distinguish and classify the botanical origins of Chinese unifloral honeys.

2. Materials and methods

2.1. Samples

A total of 75 honey samples (jujube, 15; rape, 15; chaste, 15; acacia,
15; and lungan, 15) were analyzed. All samples were collected from local
beekeepers in China in 2013 or 2014, centrifuged and unpasteurized. All
samples were stored at 4 °C until analysis. The botanical origins and
geographical regions of the samples are presented in Table 1.

2.2. Reagents and standards

Acetonitrile of HPLC grade and 18 amino acid standards, including
glycine (Gly), alanine (Ala), serine (Ser), cysteine (Cys), threonine (Thr),
valine (Val), leucine (Leu), isoleucine (Iso), methionine (Met), phenylala-
nine (Phe), tryptophan (Try), tyrosine (Tyr), aspartic acid (Asp),
 glutamic acid (Glu), glutamine (Gln), arginine (Arg), and histidine (His), were purchased from Sigma-Aldrich (St. Louis, MO, USA). The derivatizing reagent (kit Waters AccQ·Fluor™) and eluent A were supplied by Waters (Massachusetts, USA). Waters AccQ·Fluor™ kit consists of Waters AccQ·Fluor borate buffer, Waters AccQ-Fluor reagent powder, and Waters AccQ-Fluor reagent diluent. HPLC grade water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Cellulose acetate filters (0.45 µm) were supplied by Anpel (Shanghai, China).

2.3. Sample pretreatment

Three grams of honey was accurately weighed and diluted with
25 mL of ultrapure water. The solution was sonicated for 5 min, and filtered through a 0.45 µm cellulose acetate filter.

![Fig. 1. The RP-HPLC chromatogram of 18 amino acid standards.](image-url)
The method of derivatization was as follows: aliquot of 10 μL sample was placed in a derivative tube; 70 μL AccQ-Fluor borate buffer was added to monitor pH in the range of 8.2–10.0; then approximately 20 μL AccQ-Fluor reagent was added, and the mixture was heated in an oven at 55 °C for 10 min [18].

Amino acids were simultaneously separated in an HPLC system using Dionex UltiMate 3000 with a diode array detector and a Waters AccQ-Tag amino acid analysis column (3.9 mm × 150 mm, 4 μm). The column temperature was 37 °C, the injection volume was 10 μL, and the flow rate was 1 mL/min [17]. The mobile phase consisted of 10% aqueous eluent A (ultrapure water, B), and acetonitrile (C). The elution gradients were as follows: 100%–99% A and 0%–1% C from 0 to 0.5 min; 99%–95% A and 1%–5% C from 0.5 to 18 min; 95%–91% A and 5%–9% C from 18 to 19 min; 91%–83% A and 9%–17% C from 19 to 29 min; 83%–0% A, 17%–60% C, and 0%–40% B from 29 to 38 min; 40% B and 60% C for 30 min; 100% A for 30 min; finally 40% B and 60% C to wash the column.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 19 software. The chemometric analysis methods, CA, PCA, and DA, were used to discriminate Chinese honeys according to their botanical origin. CA is an unsupervised classification model. Combining unsupervised clustering with supervised information could ensure whether these data are appropriate to determine the botanical origin of honey. PCA is a technique for linearly compressing multidimensional data into lower dimensions with minimal loss of information [19]. DA is a multivariate probabilistic classification method based on a multivariate probability distribution with the hypothesis of a normal distribution with the same variance–covariance matrix in all the considered classes [20].

### 3. Results and discussion

#### 3.1. Method validation

Eighteen amino acids were determined in the present work. Fig. 1 shows a typical chromatogram of standards, using the proposed methodology. The calibration curves were established in Table 2 based on the corresponding relationship between the peak area and concentration. All of 18 amino acids had good linearity (R² > 0.997). The limit of detection (LOD) was less than 0.56 μg/mL and the limit of quantitation (LOQ) was less than 1.68 μg/mL.

#### 2.4. Free amino acid analysis

Analyte | Regression equation | R² | Linear range (μg/mL) | LOD (μg/mL) | LOQ (μg/mL) |
|----------|---------------------|----|----------------------|-------------|-------------|
| Asp | y=14.811x+0.133 | 0.9991 | 1.23–1.86×10³ | 0.31 | 0.94 |
| Ser | y=21.547x+0.293 | 0.9976 | 3.05–1.04×10² | 0.25 | 1.03 |
| Glu | y=14.622x+0.218 | 0.9995 | 2.53–1.50×10¹ | 0.32 | 1.04 |
| Gly | y=29.244x+0.167 | 0.9992 | 1.02–1.10×10⁵ | 0.27 | 0.82 |
| His | y=12.107x+0.062 | 0.9993 | 3.25–4.10×10⁴ | 0.56 | 1.68 |
| Arg | y=12.836x–0.051 | 0.9998 | 0.95–9.50 | 0.19 | 0.53 |
| Thr | y=21.929x+0.030 | 0.9998 | 0.85–7.50×10¹ | 0.23 | 0.67 |
| Ala | y=27.332x+0.2070 | 0.9994 | 4.05×10⁴–3.00×10⁴ | 0.46 | 1.38 |
| Trp | y=9.189x+0.067 | 0.9999 | 0.55–1.05×10² | 0.12 | 0.42 |

#### Table 3

The peak area (PA) and retention time (RT) of precision, stability, and repeatability, and recovery rate.

| Analyte | Precision (n=6, % RSD) | Stability (n=12, % RSD) | Repeatability (n=6) | Recovery rate (n=3) |
|---------|------------------------|-------------------------|---------------------|---------------------|
| Asp | 1.03 | 1.23 | 1.93 | 1.78 | 2.49 | 2.34 | 98.32 | 1.07 |
| Ser | 2.34 | 2.13 | 2.35 | 2.41 | 1.35 | 1.23 | 99.32 | 1.65 |
| Glu | 1.58 | 1.34 | 1.45 | 1.39 | 1.93 | 1.53 | 97.54 | 1.98 |
| Gly | 1.32 | 1.45 | 2.12 | 2.30 | 2.54 | 1.93 | 98.78 | 0.78 |
| His | 2.13 | 2.01 | 1.67 | 1.52 | 2.19 | 1.83 | 100.92 | 2.13 |
| Arg | 1.93 | 1.78 | 1.86 | 1.72 | 1.67 | 1.52 | 97.29 | 1.39 |
| Thr | 1.29 | 1.32 | 1.35 | 1.42 | 1.39 | 1.09 | 102.72 | 2.07 |
| Ala | 1.34 | 1.54 | 1.37 | 1.29 | 1.73 | 1.34 | 103.25 | 1.94 |
| Pro | 1.73 | 1.93 | 2.09 | 1.97 | 1.82 | 1.32 | 100.83 | 1.85 |
| Cys | 2.01 | 1.78 | 2.21 | 2.05 | 2.23 | 1.92 | 96.57 | 2.79 |
| Tyr | 2.29 | 2.13 | 1.02 | 1.15 | 2.52 | 2.04 | 98.27 | 1.47 |
| Val | 1.92 | 2.13 | 1.35 | 2.14 | 1.59 | 1.34 | 99.35 | 1.98 |
| Met | 1.28 | 1.09 | 1.37 | 1.47 | 1.52 | 1.28 | 101.45 | 0.74 |
| Lys | 1.45 | 1.56 | 2.05 | 2.13 | 1.25 | 1.09 | 103.71 | 1.95 |
| Ile | 2.39 | 2.54 | 1.56 | 1.47 | 0.92 | 1.32 | 96.32 | 2.17 |
| Leu | 1.59 | 1.62 | 1.78 | 1.39 | 0.73 | 1.19 | 97.34 | 1.48 |
| Phe | 1.94 | 2.14 | 1.94 | 1.68 | 1.83 | 1.43 | 100.54 | 0.74 |
| Trp | 2.01 | 1.94 | 1.52 | 1.43 | 1.25 | 1.43 | 97.69 | 1.58 |
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Spanish honey [22], and Pro, Phe, Tyr, and Lys accounted for the (69.61% in chaste honey samples), respectively. Compared with these

The total content of amino acids in chaste type was 1527.9 mg/100 g, which was considerably higher than that in other honey types. The mean value of Phe was 1094.9 mg/100 g, and Phe was the dominant amino acid in all analyzed chaste honey samples. The same observation for Phe was also found in lavender honey [22], in which Phe content was 1152.8 mg/100 g. Meanwhile, the mean values of other amino acids in chaste type were higher than those in other types of honey: Ala (97.2 mg/100 g), and Tyr (50.6 mg/100 g).

Jujube honey is a special honey originating in China, and it is popular and well recognized by consumers because of its attractive taste and aroma. The contents of amino acids in jujube honey display special characteristics. The total amount of amino acids in the jujube honey was 719.1 mg/100 g, higher than that in the other three honey types. The mean value of Pro was 457.5 mg/100 g, and Pro was the dominant amino acid in jujube honey. Meanwhile Ala, Tyr, and Glu were the next most abundant amino acids in jujube honey.

For the rest analyzed honey samples, the total amount of amino acids ranged from 397.4 mg/100 g (rape honey) to 455.4 mg/100 g (acacia honey). Pro was the predominant amino acid in these honey samples, and its percent contents ranged between 44.33% (rape honey) and 63.93% (acacia honey). The contents of Ile and Leu were lower but also important in these honey samples. Trp is an important amino acid, but it was not detected in rape samples. Meanwhile, as compared to other honey types, rape honey displayed significant differences in percentages of Ser, His, and Lys (9.99%, 9.02%, and 8.84%). Acacia honey samples contained all detected amino acids except Met and Cys. The contents of Asp, Gly, Lys, Leu and Phe were very low in acacia honey samples. Arg, Ala, and Lys were comprised the minority amino acids in lungan honey, whereas the contents of Glu, His, Val, Phe, and Trp were higher in the other tested honey samples.

3.2. Amino acid profile

We studied systematically the amino acid profiles of five types of Chinese honey. Amino acid data obtained from RP-HPLC are shown in Table 4. Most of the amino acids were detected in all analyzed samples; however, the sulfur-containing amino acids (Cys and Met) were not found. This is in agreement with the results reported by Rebane et al. [21] involving Cys and Met content of Estonian honeys. That does not, however, mean that no honey samples contain Cys and Met. These amino acids had been found in honeys from some areas, such as certain French and Spanish honeys [22,23]. Therefore, sulfur-containing amino acids could be potential geographical markers. Proline was found to have the highest concentration in most of samples, approxi-

Table 4

| Analyte | Rape | Jujube | Acacia | Lungan | Chaste |
|---------|------|--------|--------|--------|--------|
| Asp (mg/100 g) | 6.3 ± 3.1 | 1.59 | 15.6 ± 10.7 | 2.17 | 8.9 ± 4.1 | 1.96 |
| Ser (mg/100 g) | 39.4 ± 2.4 | 9.99 | 20.0 ± 16.8 | 2.78 | 16.9 ± 5.5 | 3.71 |
| Glu (mg/100 g) | 9.5 ± 2.7 | 2.42 | 20.2 ± 6.5 | 2.81 | 11.7 ± 4.7 | 2.58 |
| Gly (mg/100 g) | 16.2 ± 8.4 | 4.11 | 12.2 ± 8.6 | 1.70 | 6.6 ± 3.6 | 1.44 |
| His (mg/100 g) | 35.6 ± 19.1 | 9.02 | 26.5 ± 19.4 | 3.69 | 15.3 ± 12.2 | 3.37 |
| Arg (mg/100 g) | 16.2 ± 4.0 | 4.11 | 14.4 ± 9.3 | 2.00 | 10.5 ± 6.5 | 2.30 |
| Thr (mg/100 g) | 6.1 ± 1.6 | 1.54 | 7.5 ± 2.6 | 1.04 | 9.3 ± 4.8 | 2.05 |
| Ala (mg/100 g) | 5.3 ± 1.6 | 1.35 | 31.8 ± 12.8 | 4.42 | 15.7 ± 9.4 | 3.44 |
| Pro (mg/100 g) | 175.0 ± 29.9 | 44.33 | 457.5 ± 50.8 | 63.62 | 291.1 ± 25.5 | 63.93 |
| Met (mg/100 g) | nd | nd | nd | nd | nd |
| Tyr | 18.3 ± 5.7 | 3.38 | 40.6 ± 28.8 | 5.65 | 18.5 ± 7.4 | 4.06 |
| Val | 13.4 ± 3.8 | 3.40 | 15.7 ± 3.3 | 2.18 | 14.3 ± 2.7 | 3.15 |
| Cys | nd | nd | nd | nd | nd |
| Lys | 34.9 ± 4.0 | 8.84 | 4.6 ± 2.0 | 0.64 | 7.1 ± 5.4 | 1.56 |
| Ile | 5.7 ± 1.5 | 1.44 | 6.0 ± 1.7 | 0.83 | 9.6 ± 6.2 | 2.11 |
| Leu | 4.8 ± 1.5 | 1.21 | 4.1 ± 1.1 | 0.64 | 4.5 ± 1.7 | 0.98 |
| Phe | 13.6 ± 4.7 | 3.29 | 17.5 ± 9.0 | 2.43 | 39.4 ± 4.4 | 0.86 |
| Trp | nd | nd | 24.6 ± 1.2 | 3.42 | 11.5 ± 1.2 | 2.51 |
| total AA | 394.7 ± 74.4 | 100.0 | 719.1 ± 69.8 | 100.0 | 455.4 ± 44.6 | 100.0 |

Values within the same row follow by the same small letter are not significantly different at the level of 5%.

nd: Not detected.
results in Fig. 2B were based on data with the chaste honey samples data removed. We can see that samples of different botanical origins gathered together in Fig. 2B. There were no samples assigned to the wrong group, and an ideal result was obtained. This result demonstrated that the amino acid contents could serve as indicators to authenticate the botanical origin of Chinese honeys.

3.4. PCA

Honey is a kind of natural product with a complex matrix, so it is difficult to reach a conclusion by analyzing the data of all classes of compounds. PCA is a chemometric analytical method that reduces data dimensions to make it possible to use fewer data to represent most of the variables and achieve the intended goal [19]. Data on all amino acids were analyzed by PCA and three principal components were extracted whose eigenvalues exceeded 1. Table 5 shows eigenvalue, variance and cumulative variance of three principal components. The first principal component (PC1) was strongly associated with the contents of Ser, Arg, Ala, Pro, Tyr, Lys, Leu, and Phe, representing 50.334% of variance. The second principal component (PC2) represented 14.595% of variance, and was mainly associated with Gly, His, and Thr. The third principal component (PC3) was mainly associated with the contents of Asp, Glu, and Trp, and represented 12.305% of variance. The cumulative variance accounted for by these principal

| Amino acid | Principal component |
|------------|---------------------|
|            | PC1     | PC2     | PC3     |
| Asp        | 0.295   | 0.059   | 0.576   |
| Ser        | 0.959   | 0.165   | 0.087   |
| Glu        | 0.341   | -0.032  | 0.752   |
| Gly        | 0.179   | 0.902   | -0.095  |
| His        | 0.592   | 0.733   | 0.066   |
| Arg        | 0.840   | 0.210   | 0.156   |
| Thr        | 0.056   | 0.842   | 0.112   |
| Ala        | 0.864   | 0.110   | 0.055   |
| Pro        | 0.913   | 0.087   | 0.175   |
| Tyr        | 0.854   | 0.161   | 0.205   |
| Val        | 0.671   | 0.103   | 0.470   |
| Lys        | 0.862   | 0.101   | -0.229  |
| Ile        | 0.721   | 0.169   | 0.270   |
| Leu        | 0.900   | 0.256   | 0.186   |
| Phe        | 0.890   | 0.194   | 0.163   |
| Trp        | -0.462  | 0.017   | 0.728   |
| Variance (%) | 50.334  | 14.595  | 12.305  |
| Cumulative variance (%) | 50.334  | 64.929  | 77.234  |

PC: Principal component.

Fig. 2. Dendrogram of the cluster analysis of honey samples. (A) Included chaste honey samples; (B) excluded chaste honey samples.
components was 77.234%. This ratio was higher than that found in the case of Estonian (75.35%) [21] and Spanish (64%) [22] honeys.

To identity the relationship between the amino acid contents and the type of honey, a diplot was performed (Fig. 3A). This diplot was obtained from PC1 and PC2. The loading of PC1 and PC2 and the representative samples for which all amino acid contents had the mean value in this group are marked in this figure. The other analyzed samples were omitted to improve the graphical representation. It can be observed that all quantified amino acids are strongly related to some honeys in Fig. 3A. For example, the rape and acacia honeys are mainly associated with Glu and Trp. The same conclusion was proposed by Cometto et al. [26]. The first quadrant mainly includes chaste honey; jujube and acacia honeys are mostly distributed in the third quadrant; and lungan and rape honeys are in the fourth quadrant. Fig. 3B is a scatterplot of all samples with PC1 as the vertical axis and PC2 as the horizontal axis. PC1 of the chaste samples had positive values; some other samples had negative values. Most of the rape and acacia honey samples had negative values of PC1, and the lungan and jujube honeys had positive values. It can be seen that not all samples were correctly clustered together, with many samples distributed among other types of honey. This was not the case for chaste honeys analyzed.

3.5. DA

Based on the results of CA and PCA, we realized that amino acids in Chinese honey could provide information about botanical origin. However, there was no definitive way to determine the botanical origin of a honey sample only based on its amino acids data. So DA was performed to set up a model for honey classification. DA is a multivariate statistical method used to identify the respective type of a sample according to various values, under a defined classification system [20]. Amino acid data obtained from RP-HPLC were selected as dependent variables, and the botanical origins of the honey samples as independent variables. The Fisher stepwise DA was the selected classification method. Four discriminant functions were obtained by DA, and all functions were demonstrated to be significant for correct classification. The first function could account for 47.8% of the total variance, with Wilks’ Lambda=0.000, X²=442.746, df=64, and sig.=0.000. The second function could account for 43.1% of the total variance, with Wilks’ Lambda=0.002, X²=277.196, df=45, and sig.=0.000. The third and fourth functions accounted for 8.3% and 0.7% of the total variance. Together, all functions accounted for 100% of the total variance. The centers of each group of honey mass were as follows: rape honey (3.221, −9.490, 3.406, 0.643), jujube honey (−2.752, 0.427, −3.640, 1.076), acacia honey (−0.078, −5.375, −2.289, −1.223), lungan honey (−9.887, 4.489, 2.496, −0.239), and chaste honey (8.530, 6.959, 0.546, −0.118) in a four-dimensional coordinate plot.

Fig. 4 shows a sample distribution plot with the first function as the horizontal axis and the second function as the vertical one. In it, all samples are divided into five categories, and the samples of different botanical origin cluster together. The results of DA are superior, and the accuracy reached 100%. Other researchers have used DA processing of data to classify honeys according to botanical origin using phenolics, physicochemical parameters, and so on, but not all samples were grouped appropriately or there were overlapping areas between the different types of honey [27].

4. Conclusion

The amino acid profiles of five Chinese honey types demonstrated that the botanical origin of honey influences the amino acid contents. Although most of the amino acid contents were similar, some significant differences could be found. The content of Phe in chaste honey samples was apparently higher than that in other samples. And Trp,
Pro, Asp, Glu, Lys, and Ser exhibited differences according to honey botanical origin. Multivariate statistical analyses employing CA, PCA, and DA showed that samples could be classified correctly according to their botanical origin. Additionally, DA offered a more precise mode to determine the botanical origin of Chinese honey. In conclusion, our results support the hypothesis that amino acid profiles are adequate for the determination of Chinese honey botanical origin.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

[1] M.L. Al, D. Daniel, A. Moise, et al., Physico-chemical and bioactive properties of different floral origin honeys from Romania, Food Chem. 112 (2009) 863–867.
[2] V. Kaikonen, P.R. Venskonitis, Floral markers in honey of various botanical and geographic origins: a review, Compr. Rev. Food Sci. F 9 (2010) 620–634.
[3] K.A. Aliﬀeris, P.A. Tarantillas, P.C. Harizanis, et al., Botanical discrimination and classification of honey samples applying gas chromatography/mass spectrometry fingerprinting of headspace volatile compounds, Food Chem. 121 (2010) 856–862.
[4] J. Zhao, X. Du, N. Cheng, et al., Identiﬁcation of monofloral honeys using HPLC–ECD and chemometrics, Food Chem. 194 (2016) 167–174.
[5] J. Wang, X. Xue, X. Du, et al., Identiﬁcation of acacia honey adulteration with rape honey using liquid chromatography–electrochemical detection and chemometrics, Food Anal. Method. 7 (2014) 2003–2012.
[6] M. Megherbi, B. Herbreteau, R. Faure, et al., Polysaccharides as a marker for detection of corn sugar syrup addition in honey, J. Agr. Food Chem. 57 (2009) 2105–2111.
[7] J. Wang, M.M. Kliks, W. Qu, et al., Rapid determination of the geographical origin of honey based on protein ﬁngerprinting and barcoding using MALDI TOF MS, J. Agr. Food Chem. 57 (2009) 10881–10888.
[8] Z. Kaya, R. Binzet, N. Orcan, Pollen analyses of honeys from some regions in Turkey, Apiacta 40 (2005) 10–15.
[9] E. Anklam, A review of the analytical methods to determine the geographical and botanical origin of honey, Food Chem. 63 (1998) 549–562.
[10] D.W. Ball, The chemical composition of honey, J. Chem. Educ. 87 (2007) 1643–1646.
[11] AOAC, Method 979.20, Ofﬁcial methods of analysis of AOAC International, in: W. Horwitz (Ed.) Proline in Honey, AOAC International, Gaithersburg, Maryland, USA, 2005: 25–37.
[12] M.T. Iglesias, C. de Lorenzo, M.D.C. Polo, et al., Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area, J. Agr. Food Chem. 52 (2004) 84–89.
[13] H.Z. Senyva, J. Gilbert, S. Silici, et al., Proﬁling Turkish honeys to determine authenticity using physical and chemical characteristics, J. Agr. Food Chem. 57 (2009) 3911–3919.
[14] J. Zhou, Z. Sun, P. Zhao, et al., Jujihe honey from China: physicochemical characteristics and mineral contents, J. Food Sci. 78 (2013) 387–394.
[15] Y. Wang, Q. Li, Q. Wang, et al., Simultaneous determination of seven bioactive components in Oolong tea Camellia sinensis: quality control by chemical composition and HPLC ﬁngerprints, Food Chem. 60 (2011) 256–260.
[16] S.N. Deming, Y. Michotte, D.L. Massart, et al., Chemometrics: A Textbook 2, Elsevier, Holland, 1988.
[17] I.K. Karabagias, M.V. Vavoura, C. Nikolau, et al., Floral authentication of Greek unifloral honeys based on the combination of phenolic compounds, physicochemical parameters and chemometrics, Food Res. Int. 62 (2014) 753–760.
[18] M.J. Asilavat, D.M. Hashim, B. Jamilah, et al., Validation of a reverse-phase high-performance liquid chromatography method for the determination of amino acids in gelatins by application of 6-aminoquinolinyl-N-hydroxysuccinimidyl carbamate reagent, J. Chromatogr. A. 1353 (2014) 49–56.
[19] Y. Yücel, P. Sultanoğlu, Characterization of Hatay honeys according to their multi-element analysis using ICP-OES combined with chemometrics, Food Chem. 140 (2013) 231–237.
[20] I.K. Karabagias, A. Badeka, S. Kontakos, et al., Characterisation and classiﬁcation of Greek pine honeys according to their geographical origin based on volatile, physicochemical parameters and chemometrics, Food Chem. 146 (2014) 548–557.
[21] R. Rebane, K. Herdes, Evaluation of the botanical origin of estonian uni- and polyﬂoral honeys by amino acid content, J. Agr. Food Chem. 56 (2008) 10716–10720.
[22] I. Hermos, R.M. Chición, M.D. Cabreznio, Free amino acid composition and botanical food chemistroyorigin of honey, Food Chem. 83 (2003) 263–268.
[23] J.F. Cotte, H. Casabianca, B. Giroud, et al., Amino acids proﬁling of three light-coloured Italian honeys, Application to honeys from a small geographic area, J. Sci. Food Agr. 93 (2013) 3368–3376.
[24] S. Gok, M. Severcan, E. Goormaghtigh, et al., Differentiation of Anatolian honey samples from different botanical origins by ATR-FTIR spectroscopy using multivariate analysis, Food Chem. 170 (2015) 234–240.
[25] M.L. Al, D. Daniel, A. Moise, et al., Physico-chemical and bioactive properties of different floral origin honeys from Romania, Food Chem. 112 (2009) 863–867.
[26] P.M. Cometto, P.F. Faye, R.D. Di, et al., Comparison of free amino acids composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area, J. Agr. Food Chem. 52 (2004) 84–89.
[27] Z. Zhao, N. Cheng, X. Xue, et al., Chromatographic ECD ﬁngerprints combined with a chemometric method used for the identiﬁcation of three light-coloured unifloral honeys, Anal. Methods 7 (2015) 8393–8401.