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Contribution of pyrolysis-mass spectrometry (Py-MS) to authenticity testing of honey

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Abstract

Pyrolysis Mass Spectrometry (Py-MS) was used to investigate discrimination of honey samples from different floral (ten) and geographical (seven) origins. The data were analysed statistically (using Principal Component and Discriminant Factor Analysis) in order to check the possibility of the use of these profiles for characterising the botanical source of honey. Py-MS showed as a very useful tool for the rapid discrimination of honeys from different botanical origins. Separation of the geographical origin of honey was unsuccessful and this is likely to be due to the very large and varied botanical origins of honey located within a single geographical region. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Pyrolysis; Mass spectrometry; Honey; Authenticity

1. Introduction

The composition and the manufacture of honey within the European Union (EU) are regulated by Community Directive 74/409/EEC [1]. Driven by the needs to harmonise the common European market, the European Commission has adopted a proposal to amend this directive. Finally, the name 'honey' thus has now to be supplemented by information referring to the product's floral and geographical origin. Traditionally, the determination of the floral and geographical origin of honey [2,3] is achieved by analysis of the pollen (mellisopalynology) present in

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honey. This method is based on the identification of pollen by microscopic examination. It requires a very experienced analyst, it is very time consuming and dependent on expert's ability and judgment [4]. Other methods that could be more widely used for characterising honeys have been sought for many years [5].

Pyrolysis-mass spectrometry (Py-MS) is a very fast and sensitive fingerprinting technique. Using this method, the sample is placed in high vacuum and heated under controlled conditions. The organic material undergoes rapid decomposition and the low molecular weight products (called pyrolysate) enter into a mass spectrometric device, where the pyrolysate is quantified.

Py-MS was shown to be a fast and sensitive technique for classification of food according to its chemical composition. The latter can be correlated mainly to geographical and botanical origin and processing techniques. Examples for the application of Py-MS to prove authenticity of or detect frauds include olive oil [6–8] and other vegetable fats [9,10], whisky [11–13], wine [14], vinegar [15,16], orange juice [17,18], tea [19] and milk products [20,21].

The aim of the present study was to investigate the extent to which Py-MS allowed the classification of honey according to its botanical origin. An influence of two different ways of statistical data treatment on this classification was examined as well as the possibility of reducing the number of mass ions used in the analysis. Finally, an attempt of multivariate data analysis of obtained Py-MS spectra for the determination of geographical origin of honey was made.

2. Experimental

2.1. Samples

Honey samples (47) were obtained from various hive sites in seven different EU member states. Standard pollen analysis [22] was performed on all honey samples in order to confirm their floral authenticity. The variety of unifloral samples was limited by the collection scheme. Ten unifloral types were provided by 47 samples. These were: acacia (seven samples), chestnut (nine samples), eucalyptus (four samples), heather (nine samples), lime (four samples), rapeseed (four samples), sunflower (four samples), and citrus, lavender and rosemary (two samples in each group).

2.2. Pyrolysis-mass spectrometry (Py-MS)

All sample analysis were performed on a RAPyD-400 (SS Scientific Limited, East Sussex, UK) based on a quadrupole mass analyser and employing Curie-point pyrolysis. A thin layer of honey was put onto clean iron–nickel foils (SS Scientific Limited). The pyrolysis temperature (530°C) was held for 3 s and the resulting pyrolysate was ionised at 25 eV. The mass range was scanned between m/z 51 and 250. Samples were analysed in triplicate. This allows the reproducibility of the resulting data to be assessed, and also any instrumental abnormalities could be

Table 1
Samples of the training sets

Number of samples	Botanical origin	Number of samples	Geographical origin
4	Acacia	2	England
9	Chestnut	6	France
2	Eucalyptus	7	Germany
7	Heather	11	Italy
4	Lime	4	Netherlands
3	Rapeseed	3	Portugal
4	Sunflower	2	Spain
2	Citrus		
2	Lavender		
2	Rosemary		

identified. The IBM-compatible PC used to control RAPyD-400 was also programmed (using software provided by the manufacturers) to record spectral information on ion count for the individual masses scanned and the total ion count for each sample analysed.

Prior to analysis the mass spectrometer was calibrated using the chemical standard perfluorokerosene (Aldrich).

2.3. Data handling

The Py-MS data are displayed as quantitative pyrolysis-mass spectra in which the abscissa represents the m/z ratio and the ordinate gives information on the ion count for any particular m/z value ranging from 51 to 250. Data were normalised to a total ion count of to remove the effect of sample size differences.

The data were divided into a training and a test set. The test set was chosen randomly to represent the different botanical origins of honeys. Details are given in Tables 1 and 2.

For the analysis either the full mass range from 51 to 250 m/z (200 mass ions) was used or a reduced mass range from 51 to 130 m/z (80 mass ions) was employed. The latter was chosen because on inspection of the mean plot of all the training data (Fig. 1) it can be seen that the majority of the information is found with $m/z < 130$.

Table 2
Samples of the test sets

Number of samples	Botanical origin	Number of samples	Geographical origin
3	Acacia	2	England
2	Eucalyptus	3	France
2	Heather	3	Germany
1	Rapeseed	3	Italy
		1	Spain

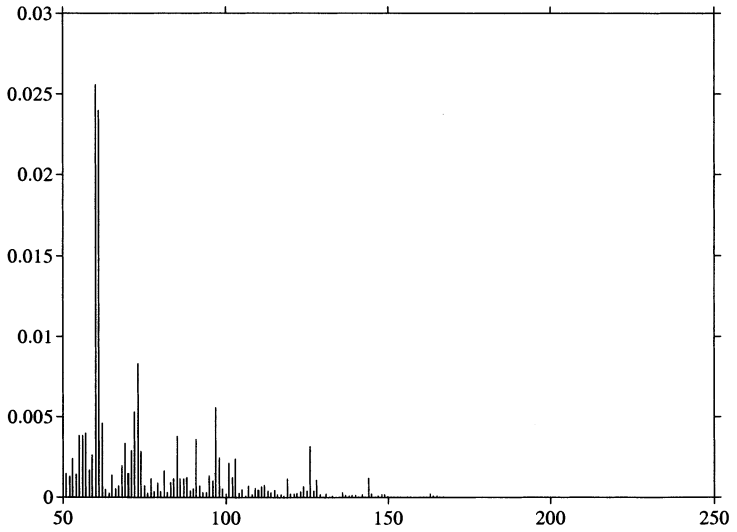

Fig. 1. The mean Py-MS spectrum calculated for all spectra in the training set.

The initial stage of the cluster analysis involved the reduction of the multidimensional Py-MS and FT-IR data by principal components analysis (PCA) [23]. PCA is a well-known technique for reducing the dimensionality of multivariate data whilst preserving most of the variance, and the Matlab program was employed to perform PCA according to the NIPALS algorithm [24]. Discriminant function analysis (DFA), also known as canonical variates analysis (CVA), then discriminated between groups on the basis of the retained principal components (PCs) and the a priori knowledge of which spectra belonged to which botanical (Tables 1 and 2) or geographical class of honey [25]. The number of PC scores used by the DFA algorithm was optimised by running between five and 50 PCs and observing each individual PC-DFA plot. It was found that 22 PCs gave the best separation in terms of known botanical origin for the training set.

To identify the botanical origin of the honeys after PC-DFA was performed on the training set (Table 1), the 'unknown' test data (Table 2) were projected into this PC-DFA space. Two data analyses regimes were adopted:

2.3.1. Method 1

Both the training and test data were used in the PCA analysis, DFA was performed on the PC scores from only the training set, and then the test set PC scores were projected into this DFA space.

2.3.2. Method 2

PCA and DFA were carried out on only the training set, the test set spectra were first projected into the PCA space and then the resultant PCs projected into the DFA space.

Data analysis was performed using Matlab version 5.0.0.4069 (The Math Works, Inc., 24 Prime Par Way, Natick, MA, USA), which runs under Microsoft Windows NT on an IBM-compatible PC.

3. Results and discussion

As is typical of Py-MS applied to the analysis of food and other biological materials, simple visual inspection of the spectra (data not shown) identified no prominent peaks that could be used for classification of the obtained spectra. Nevertheless, whilst there was very little qualitative difference between these spectra, on closer inspection, quantitative differences could be observed. These spectra can be considered as quantitative fingerprints of the organic components of the honey samples, and these data may be used for comparisons between honey samples using multivariate-based pattern recognition procedures.

Careful examination of the mean mass spectrum of all the data in the training set (Fig. 1) showed that there was very little information above m/z 130. To ascertain whether omitting mass ions with intensities >130 m/z could influence the chemometric data processing. Both the full mass spectrum from m/z 51 to 250 (containing 200 mass ions) and a reduced set from m/z 51 to 130 (containing only 80 mass ions) were analysed as described above.

The next stage was to perform PC-DFA as described above. The first analysis was performed on the full mass spectrum, using both training and test data in the PCA, and 22 PCs were used by the DFA algorithm on only the training data with the a priori knowledge of the botanical class of the honeys. Table 1 shows that there were ten classes used, and DFA was performed to group the ten different botanical origins of honey into different clusters. It can be seen in Fig. 2A that this was quite successful and that six groups can be distinguished when the first two discriminant function scores are plotted. Honey samples from sunflower (Su), heather (He), lime (Li) and chestnut (Ch) are clearly single member clusters, whilst lavender (La) and eucalyptus (Eu) are grouped together, finally, acacia (Ac), citrus (Ci), rapeseed (Ra) and rosemary (Ro) honeys form one large group, although some separation between the different honeys can be observed. Whilst perfect separation between these samples was possible if more then 40 PCs were used this was 'artificial' and due to chance correlation with noise in the spectra.

Projection of the triplicate test set data from eight honeys into this DFA space (Fig. 2A) showed that identification of the honey to the correct botanical origin was feasible. The two heather honeys both clustered with the heathers from the training set. The two eucalyptus honeys were in the middle of the eucalyptus/lavender group. The rapeseed honey was projected into the bottom of the large mixed cluster, and on close inspection this can be seen to be where the majority of rapeseed honey training data were located. Finally, the three acacia honeys were located near their correct botanical honeys in the large mixed cluster.

The above analysis used both the training and test sets in the calculations of PCA and so may be thought of as computational intense, since when a new honey

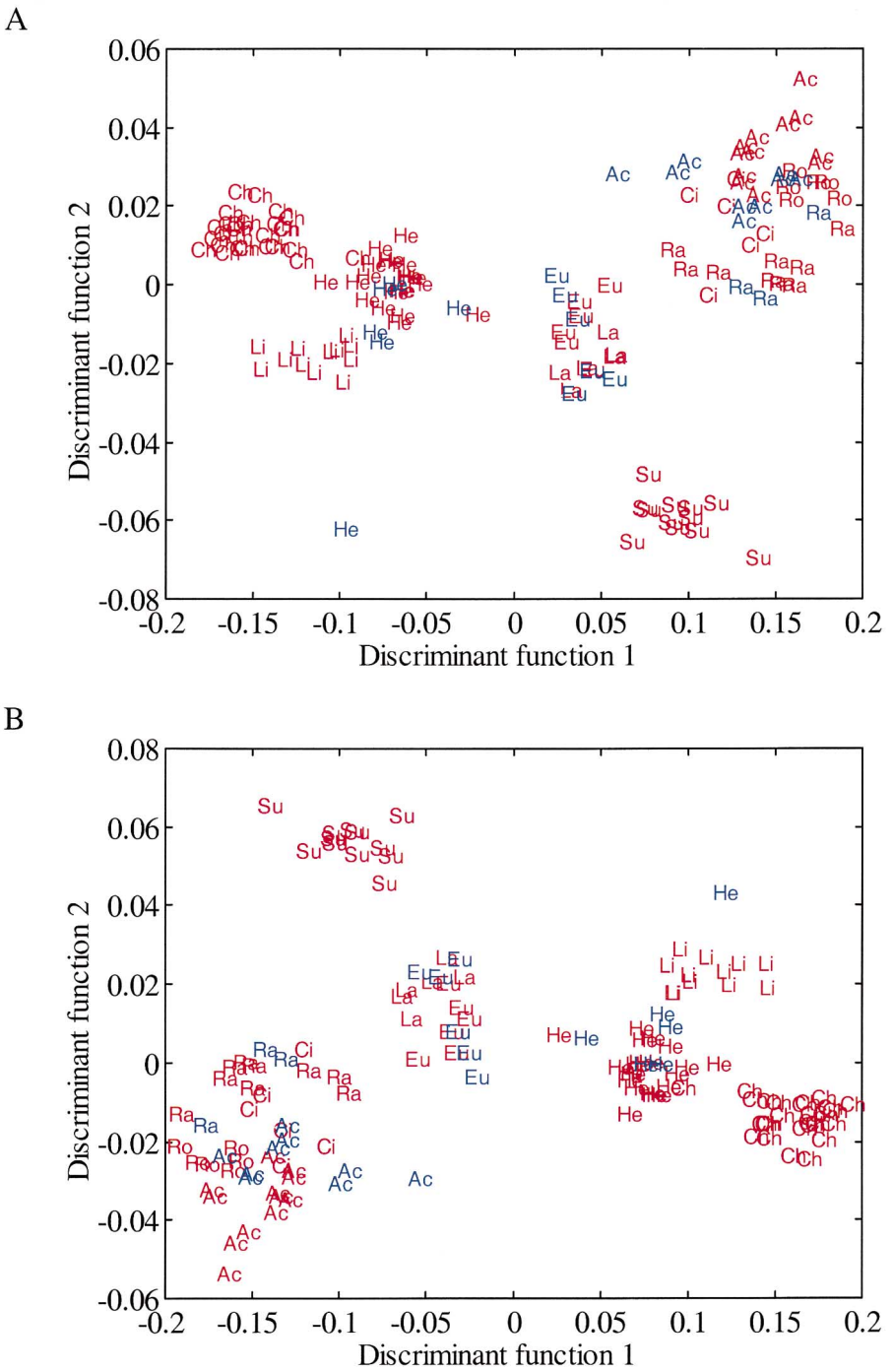

Fig. 2. Separation of the ten unifloral honey groups using full mass spectra and chemometric Method 1 (A) and Method 2 (B); see text for details. Codes: Ac: acacia; Ch: chestnut; Ci: citrus; Eu: eucalyptus; He: heather; La: lavender; Li: lime; Ra: rapeseed; Ro: rosemary; Su: sunflower. The training set is in red and the test set in blue.

sample was tested one would have to recompute the PCA before projection into DFA. The latter of course is a very simple matrix multiplication. Therefore a more elegant approach would be to calculate PCA on the training set only prior to DFA analysis. New test data would then be projected into the already constructed PCA space and the resultant PCs projected into the DFA space. This was carried out for the same training and test sets as used above and the results of the projection are displayed in Fig. 2B. Other than the simple 180° rotation these results are very similar to those shown in Fig. 2A.

One might also suppose that using the full 200 mass ions in the calculation of PCA is also computationally expense, especially when there is very little information shown in the latter 120 ions from 130 to 250 m/z (Fig. 1). Therefore the two methods of projection were calculated on the reduced mass spectrum and the results from Method 1 and Method 2 are shown in Fig. 3A and B respectively. Both PC-DFA plots and the projections therein are very similar to that seen in Fig. 2A, highlighting that no useful additional discriminatory information was found in mass ions greater than 130 m/z.

In a final chemometric procedure the same data were split as detailed in Tables 1 and 2 to test for the seven geographical origin. However it was only after 40 PCs that any clusters relating to geographical origin could be seen (data not shown) and this only split half the samples from France away from one big heterogenous group. There was obviously no point in projecting the test set into this 'artificial' separation. The reason that it was not possible to detect the geographical origin was that the number of representative samples from each country was too small. For example, whilst nine honeys were supplied from France, six different botanical origins of honey were represented; two heather, two chestnut, two sunflower, one lavender, one acacia, and one rapeseed. As we have shown that it is clearly possible to separate the honeys according to their botanical origin, it is likely that having this very large (bio)chemical difference within regions will necessarily mean that it will be more difficult to separate between regions, a phenomenon observed when using Py-MS to investigate the geographical origin of olive oils [26].

In conclusion, this study shows that Py-MS is a very useful tool for the rapid discrimination of honey samples from the ten different floral (botanical) origins. Rather than a failure of Py-MS to separate successfully the honeys into seven different geographical origins it may be concluded that the sample homogeneity per geographic area was insufficient due to the diversity of botanical origins.

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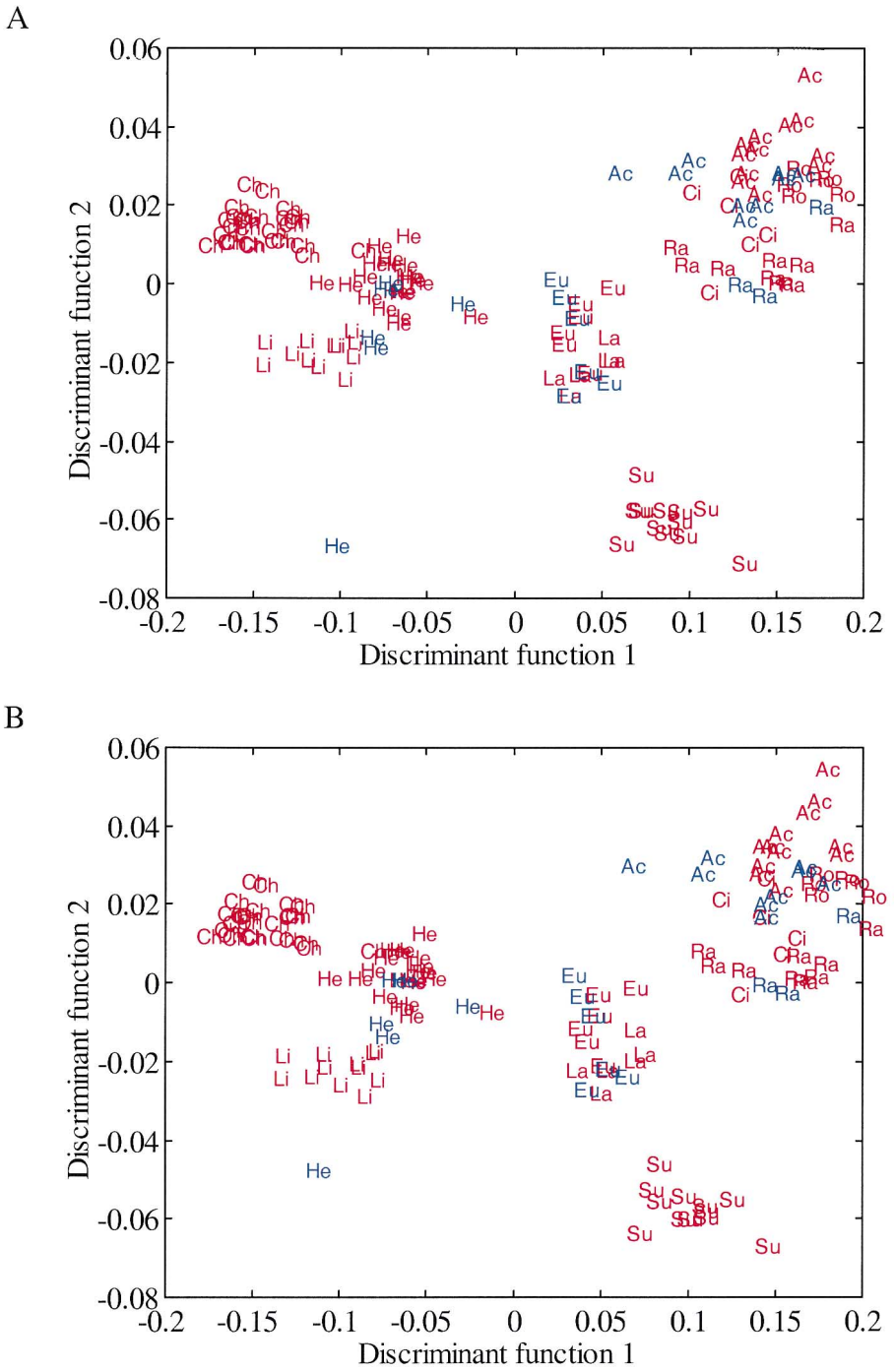

Fig. 3. Separation of the ten unifloral honey groups using partial m/z (51–130) and chemometric Method 1 (A) and Method 2 (B); see text for details. Codes: Ac: acacia; Ch: chestnut; Ci: citrus; Eu: eucalyptus; He: heather; La: lavender; Li: lime; Ra: rapeseed; Ro: rosemary; Su: sunflower. The training set is in red and the test set in blue.

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