

CHAPTER 2

Authentication of the Botanical Origin of Honey Using Profiles of Classical Measurands and Discriminant Analysis*

ABSTRACT

The potential of physical and chemical measurands for the determination of the botanical origin of honey by using both, the classical profiling approach as well as chemometrics was evaluated for the authentication of ten unifloral (acacia, rhododendron, chestnut, dandelion, heather, lime, rape, fir honeydew, metcalfa honeydew,) and polyfloral honey types (in total $n = 693$ samples). The classical approach using a profile for the determination of the botanical origin of honey revealed that the physical and chemical measurands alone do not allow a reliable determination. Pollen analysis is therefore essential for discrimination between unifloral and polyfloral honeys. Chemometric evaluation of the physical and chemical data by linear discriminant analysis allowed however a reliable authentication with neither specialized expertise nor pollen or sensory analysis. The error rates calculated by Bayes' theorem ranged from 1.1% (rape and lime honeys) up to 9.9% (acacia honey).

2.1 INTRODUCTION

2.1.1 DEFINITION OF UNIFLORAL AND POLYFLORAL HONEYS

The overwhelming majority of the honeys on the market contain significant nectar or honeydew contributions from several plant species and are therefore called polyfloral or multifloral honeys. Normally they are just labelled with the word "honey". The term unifloral honey is used to describe a honey in which the major part of nectar or honeydew is derived from a single plant species. Honey composition, flavour and colour varies considerably depending on the botanical source it originates from (1).

At present there is an increasing commercial interest to produce unifloral honeys. Indeed many consumers prefer unifloral to polyfloral honeys and appreciate the possibility to choose between different honey types. The production of unifloral honeys offers the beekeepers also an opportunity to compete with low priced polyfloral honeys imported from abroad. Moreover the increasing interest in the therapeutic or technological uses of certain honey varieties may also contribute to the demand of a reliable determination of their botanical origin.

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According to the Codex Alimentarius Standard for Honey (2) and the EU Council Directive (3) relating to honey, the use of a botanical designation of honey is allowed if it originates predominately from the indicated floral source. Physical, chemical and pollen analytical characteristics of the most important European unifloral honeys have been described in various papers (1, 4-7).

2.1.2 TRADITIONAL CLASSIFICATION OF HONEYS BASED ON A PROFILE OF MEASURANDS

Traditionally the botanical origin of honey is determined by experts evaluating several physical, chemical, pollen analytical as well as sensory characteristics (4, 8, 9). The analytical results of a honey sample have relatively unconsciously been compared with profiles describing the data ranges of different unifloral honeys. When all the values of the measurands considered fit into the respective ranges described for a unifloral honey type, it is assigned to this corresponding honey type. On the contrary if the characteristics of the sample do not fit into the profiles of the unifloral honey types considered, the sample is classified as polyfloral honey. Thus the group of polyfloral honeys represents a miscellaneous pool of samples of various botanical origins with significant nectar or honeydew contributions from several plant species. However, the amount of honeydew should not prevail, otherwise it is regarded as honeydew honey. Unfortunately up to now neither the measurands to be considered nor their corresponding ranges for the individual unifloral honeys have been defined and internationally accepted. Usually only few physical and chemical measurands, in particular electrical conductivity, sugar composition and pollen analytical results are used together for this purpose.

This profiling approach used for decades, has recently been described in more detail by Persano Oddo and Piro (1). However, only physical and chemical measurands were considered and the presentation of the data ranges was not optimal. The classification with a profile works because unifloral honeys generally express, at least in respect to some measurands, specific properties that are generally not found in other honey types. The purest samples of unifloral honeys are therefore easily recognized. However, unifloral honeys are hardly ever pure and generally contain minor nectar or honeydew contributions from other botanical origins. The proportion of different sources continuously increases towards the polyfloral honeys. Where the limit between unifloral and polyfloral honeys is set depends on definitions and is ultimately arbitrary. Consequently there will always be some overlapping between unifloral and polyfloral honeys. In other words the main problem in the authentication of unifloral honeys is to discriminate between unifloral and polyfloral honeys, rather than between different unifloral honeys.

2.1.3 DISCRIMINATION BETWEEN HONEY TYPES USING CHEMOMETRICS

Several attempts have already been made to predict the botanical origin of honey using their physical and chemical properties in combination with multivariate analysis. The first paper on classification of floral and honeydew honeys using discriminant functions considering pH-value, ash and monosaccharide content was published over forty years ago (10). Later electrical conductivity, monosaccharide content as well as glutamic acid concentration were found to be the most useful measurands for the discrimination between floral and honeydew honeys (11, 12). High fructose and glucose concentrations and low values in lactone and free acidity, electrical

conductivity, polyphenol content and net absorbance (visible spectroscopy) were described to be characteristic for floral honeys. Low glucose and fructose and high melezitose concentrations as well as high values for free acidity together with high polyphenol content and net absorbance characterised honeydew honeys (13).

Numerous studies have treated the subject of the chemometric classification of unifloral honeys. Linear discriminant analysis on sugar composition data of rosemary (*Rosmarinus* sp.), orange (*Citrus* sp.), lavender (*Lavandula* sp.), sunflower (*Helianthus annuus*), eucalyptus (*Eucalyptus* sp.), heather (*Calluna vulgaris*) and honeydew honeys allowed only a discrimination between floral and honeydew honeys (14). When the same honey types were studied using water content, electrical conductivity, pH-value, colour (x, y, L chromatic coordinates) and sugar composition, jackknife classification rates higher than 90 % were found for all unifloral honeys. The most important characteristics were electrical conductivity followed by colour and fructose content. Classification functions were presented using water content, electrical conductivity, fructose, sucrose, and colour (15).

In a recent study classification functions for as many as 16 different unifloral honeys using diastase activity, electrical conductivity, specific rotation, total acidity, fructose, glucose and colour (Pfund scale and CIE L.a.b) were reported. The average correct classification rate was as high as 89.6 % and all honey types except thistle honey were correctly classified at a rate higher than 80 %. Electrical conductivity, glucose and fructose concentration as well as colour were found to be the most important variables for the classification of unifloral honeys (16).

In a recent study on a large sample set fir (*Abies* spp.), cinder heather (*Erica carnea*), chestnut (*Castanea sativa*), lavender, acacia (*Robinia pseudoacacia*) rape (*Brassica* spp.) and sunflower honeys were analyzed. Principal component analysis (PCA) showed that samples of fir, chestnut, lavender and acacia honeys formed well separated groups in the plot of the first two PC's while samples of rape, cinder heather and sunflower honeys clustered together. Stepwise discriminant analysis was used to select the most important measurands among water, hydroxymethylfurfural (HMF), fructose, glucose, sucrose, erlose, raffinose and melezitose content as well as electrical conductivity, pH-value, free acidity, diastase activity and colour (Pfund scale). The botanical origin of the samples could be perfectly predicted using electrical conductivity, pH-value, free acidity, fructose, glucose and raffinose content (17).

The above mentioned approaches using physical and chemical measurands and chemometrics allow clear discrimination between the main honey types or even between several types of unifloral honeys, but none of them accounts for the polyfloral honeys that represent the most important majority (about 80 %) of the honeys produced. As already noted the main problem in the authentication of unifloral honeys is to discriminate between polyfloral and unifloral honeys, rather than between different unifloral honeys. This means that the above-mentioned methods are inadequate for analytical practice. This also explains why until now none of these methods is commonly applied to determine the botanical origin of honey and pollen analysis is referred to be the fundamental tool for authentication of the botanical origin of honey (15).

The only paper on chemometric evaluation of physical and chemical measurands considering unifloral and polyfloral honeys was published by Krause and Zalewski using PCA. Electrical conductivity, proline, free acidity and pH-value were found to be most important measurands for classifying honeys according to their botanical origin. The authors were able to distinguish between rape, acacia and honeydew honeys but failed to differentiate between polyfloral, lime (*Tilia* spp.) and heather honeys (18). Enzyme activities and HMF content are depending on honey processing and storage conditions and are therefore not useful for the determination of the botanical origin (17, 18).

As several analytical methods have to be simultaneously used for a reliable authentication of the botanical origin, it is consequently very time consuming and costly. In addition currently very specialised expertise is needed for the interpretation of the pollen analytical results and the physical and chemical measurands determined. Thus, there is a need for new analytical tools that allow a rapid and reproducible authentication of the botanical origin of honey (19, 20).

In this context the aims of the current work were to evaluate the potential of two different approaches for authentication of unifloral and polyfloral honeys. These were on the one hand measurand profiles considering classical physical, chemical and pollen analytical characteristics and on the other hand chemometric evaluation of the physical and chemical measurands in order to verify the most important characteristics and to develop a mathematical procedure for the determination of the botanical origin of honey.

2.2. MATERIALS AND METHODS

2.2.1 SAMPLING

A total of 646 honey samples produced between 1998 and 2004 were collected and stored at 4°C until analysis. They originated predominantly from Switzerland (CH) but samples from Germany (D), Italy (I), France (F) and Denmark (DK) were also considered.

2.2.2 DETERMINATION OF PHYSICAL AND CHEMICAL MEASURANDS, POLLEN ANALYSIS

To classify these honey samples, the following measurands were determined according to the harmonized methods of the European Honey Commission (21): electrical conductivity, sugar composition, fructose/glucose ratio, pH-value, free acidity, and proline content. Pollen analysis was carried out according to DIN 10760 (22, 23).

2.2.3 BOTANICAL CLASSIFICATION BY REFERENCE METHODS

The honey samples were assigned to one of the following honey types according to their fructose/glucose ratio, melezitose content, electrical conductivity as well as pollen analytical results: acacia (*Robinia pseudoacacia*) (CH, n = 26; D, n = 7; F, n = 3), rhododendron (*Rhododendron* spp.) (CH, n = 24; I, n = 5), sweet chestnut (*Castanea sativa*) (CH, n = 52; I, n = 5; F, n = 3), rape (*Brassica napus* var. *oleifera*) (CH, n = 36), fir honeydew from (*Picea* spp. and *Abies* spp.) (CH, n = 110; D, n = 22), Metcalfa honeydew from *Metcalfa pruinosa* (I, n = 14), heather (*Calluna vulgaris*) (D, n = 19; DK, n = 3), lime (*Tilia* spp.) (CH, n = 22; D, n = 12; I, n = 5), dandelion (*Taraxacum* s.l.) (CH, n = 22; D, n = 7; I, n = 2) and polyfloral honeys (CH, n = 294). The ranges of the physical

and chemical measurands mostly corresponded to the ranges presented by Persano and Piro (Persano Oddo and Piro, 2004). In case of uncertain classification based on physical, chemical and pollen analytical criteria the decision was made by sensory evaluation by experts.

2.2.4 CLASSIFICATION USING DIFFERENT PROFILES

Three types of profiles with three different sets of measurands were tested and compared for classification of unifloral and polyfloral honeys. The measurands considered in the Profiles I and II with the corresponding ranges defined for the different unifloral honeys are presented in **Table 1**. With profile I a classification of the honey types was attempted by using only physical and chemical characteristics. As this was known to be very difficult especially regarding the discrimination between unifloral and polyfloral honey types as many as possible measurands were included in the profile. Commonly used physical, chemical and pollen analytical measurands were incorporated in profile II. In profile III the ranges of the measurands presented by Persano Oddo and Piro (1) were used as far as they were available: i.e. fructose, glucose and sucrose content, fructose/glucose and glucose/water ratio, pH-value, free acidity and electrical conductivity. With respect to pollen analytical results only the minimum percentage of the specific pollen form of each unifloral honey type was considered.

The classification was achieved by comparing the values of the honey samples with each of the nine profiles of the unifloral honey types considered. They were assigned to the corresponding honey type if all values were within the ranges defined in the profile. Samples that did not fit into any of the profiles were regarded as polyfloral (principle of exclusion).

2.2.5 DATA PROCESSING AND CHEMOMETRICS

The following 17 measurands were originally included in data evaluation: fructose, glucose, total monosaccharides, sucrose, maltose, trehalose, isomaltose, erlose, melezitose, maltotriose, raffinose and water content, electrical conductivity, free acidity, pH-value, fructose/glucose and glucose/water ratio. Because of missing values the number of samples had to be reduced to a total of 646 considered in the chemometric data evaluation. The values of each measurand were standardised (by subtracting the mean and subsequent division by the standard deviation). The equations for the standardisation of the variables are given in the Appendix A. This information is important for using the classification functions. The variables designated with a subscript uppercase "S" indicate standardised variables.

In order to select the most important variables for the classification of the unifloral honeys linear discriminant analysis (LDA) was applied. Backward elimination on the 17 initial variables was based on the partial F-values in the discriminant models. Forward selection on the same variables was used to confirm the results. The models were then optimised for maximum correct classification in jackknife classification. The validation was carried out with about one third of the samples, selected randomly, and not present in the group of samples used to build the model (SYSTAT® Version 11, Systat Software Inc., Richmond, USA).

Table 1. Data ranges of different unifloral honeys used in the profiles I and II.

	Unit	Considered in		Acacia (n = 36)		Rhododen- drdon (n = 29)		Chestnut (n = 60)		Dandelion (n = 31)		Heather (n = 22)		Lime (n = 39)		Rape (n = 36)		Fir honeydew (n = 132)		Metcalfa honeydew (n = 14)	
		Profile I	Profile II	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Electrical conductivity	mScm ⁻¹	x	x	0.10	0.27	0.15	0.45	0.62	1.70	0.37	0.64	0.65	1.07	0.38	0.95	0.14	0.28	0.70	1.33	1.48	2.57
pH-Value		x		3.7	4.1	3.7	4.6	4.4	6.4	4.2	5.0	3.9	5.1	4.1	6.2	3.9	4.4	4.1	5.4	4.6	5.8
Free acidity	meq/kg	x		6	23	5	25	4	30	7	13	14	42	4	21	8	17	17	46	21	41
Fructose	g/100g	x	x	22.4	46.9	34.2	41.0	36.6	44.6	32.3	39.5	34.9	41.0	32.9	41.6	34.6	39.5	20.9	39.4	26.8	33.1
Glucose	g/100g	x	x	13.8	29.4	27.7	33.6	21.4	30.0	32.0	43.2	26.2	31.5	26.2	42.9	31.5	40.0	14.8	31.5	20.3	26.8
Sucrose	g/100g	x		0.0	8.8	0.0	2.6	0.0	3.6	0.0	0.3	0.0	0.6	0.0	4.5	0.0	2.0	0.0	2.7	0.0	0.1
Maltose	g/100g	x		0.0	3.6	0.0	8.6	0.0	5.6	0.0	5.7	0.0	1.9	0.0	5.7	0.0	2.2	0.0	4.9	4.7	7.7
Isomaltose	g/100g	x		0.0	1.2	0.2	2.5	0.0	2.4	0.0	1.7	0.0	1.3	0.1	2.2	0.0	1.5	0.0	3.4	0.9	4.4
Erlöse	g/100g	x		0.4	2.8	0.0	3.7	0.0	4.3	0.0	0.8	0.0	0.2	0.0	0.9	0.0	1.4	0.0	4.5	0.1	1.2
Melezitose	g/100g	x		0.0	0.5	0.0	0.8	0.0	3.8	0.0	0.7	0.0	1.4	0.0	1.1	0.0	0.4	0.0	11.7	0.0	0.7
Maltotriose	g/100g	x		0.0	0.6	0.0	0.4	0.0	0.3	0.0	0.3	0.0	0.0	0.0	1.0	0.0	1.1	0.0	2.8	0.0	1.5
Raffinose	g/100g	x		0.0	0.6	0.0	0.5	0.0	0.6	0.0	0.3	0.0	0.0	0.0	0.3	0.0	0.2	0.0	2.8	0.0	0.4
Fructose/Glucose ratio		x	x	1.28	1.88	1.10	1.39	1.36	1.86	0.85	1.15	1.22	1.57	0.97	1.41	0.95	1.24	1.07	1.53	1.10	1.45
Robinia pollen	%	x		10.9	64.3	0.0	6.4	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0
Rhododendron pollen	%	x		0.0	0.0	6.0	58.2	0.0	2.8	0.0	0.0	0.0	0.0	0.0	19.0	0.0	0.0	0.0	5.1	0.0	0.0
Calluna pollen	%	x		0.0	0.0	0.0	1.9	0.0	0.0	0.0	0.0	8.0	81.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Castanea pollen	%	x		0.0	56.5	0.0	83.5	92.0	100.0	0.0	1.1	0.0	0.0	0.0	93.2	0.0	5.2	0.0	84.0	0.0	0.0
Tilia pollen	%	x		0.0	0.6	0.0	34.0	0.0	3.6	0.0	0.0	0.0	0.0	2.0	80.0	0.0	0.0	0.0	11.5	0.0	0.0
Taraxacum pollen	%	x		0.0	1.9	0.0	5.2	0.0	0.2	1.6	58.1	0.0	2.7	0.0	1.7	0.0	2.0	0.0	11.2	0.0	0.0
Brassica pollen	%	x		0.0	9.2	0.0	8.3	0.0	0.0	0.0	41.5	0.0	0.0	0.0	35.1	68.1	97.8	0.0	73.9	0.0	0.0

2.3. RESULTS

2.3.1 CLASSIFICATION BY PROFILE

In the profile considering only physical and chemical measurands it is obvious that most of unifloral honey samples are correctly assigned since almost the whole range of the values found was used (**Table 1**; **Table 2**, Profile I). Interestingly misclassifications between different unifloral honeys occurred rarely, clearly indicating that the physical and chemical properties of the unifloral honeys are distinctly different. Since nearly the whole data range found was considered, in principle all unifloral honey samples should therefore be correctly classified. Nevertheless, some unifloral honeys were assigned to polyfloral honeys due to reasons arising from truncation in the ranges used (limits set at 2.5 % and 97.5 % percentiles of the values observed). The correct classification rate for the polyfloral honeys was only 49 %. This means that approximately half of the polyfloral honey samples were misclassified to various unifloral honey types. Samples were especially assigned to rhododendron, lime and fir honeydew honeys. These unifloral honey types do express highly variable chemical compositions, thus showing a relatively broad data range for all measurands considered and therefore allow many samples to meet the requirements of the profile. Because of the high rate of misclassification of the polyfloral honey samples the profile using only physical and chemical measurands is inadequate for a reliable determination of the botanical origin of honey.

In Profile II that was based on the relative frequencies of the specific pollen and a reduced number of physical and chemical measurands, high correct classification rates were found again for the unifloral honeys (**Table 2**, Profile II). The classification rate of the polyfloral honeys was significantly improved up to 70 %, showing that pollen analysis plays a key role in the discrimination between unifloral and polyfloral honeys. Therefore pollen analytical, physical and chemical measurands should be included in the same profile used for the determination of the botanical origin.

The profile established with data ranges recently published (1) showed notably lower classification rates for the unifloral honey types studied than obtained with the profiles I and II (**Table 2**, Profile III). For example none of the heather honey samples were considered to be unifloral. Interestingly the highest classification rate was found for the polyfloral honeys.

2.3.2 CHEMOMETRIC EVALUATION

Multivariate explorative data analysis revealed that electrical conductivity, fructose, raffinose and glucose concentration, together with free acidity, contributed most to the classification of the different unifloral honeys using a single linear discriminant model. Total monosaccharide content was found to be redundant for classification when the individual glucose and fructose concentrations were considered. Most of the unifloral honeys revealed rates of correct classification of higher than 80%, by using the above-mentioned variables. The rates were similar in jackknife classification and validation thus demonstrating that the models used were robust (**Table 3**). Heather honey samples were partly classified as chestnut or polyfloral honeys and exhibited the second lowest classification rate (80 %). Fir honeydew honeys were mostly assigned to the correct group except a few samples that were misclassified as heather honeys. Among the unifloral honeys lime honeys showed with 71 % the lowest jackknifed classification rate.

Table 2. Classification rates of the different profiles studied

	Classification rates (%)												
	Profile I ¹											Profile III ³	
	Acacia	Rhodo-dendron	Chestnut	Dandelion	Heather	Lime	Rape	Fir honedew	Metcalfa honedew	Polyfloral	Correct class.	Correct class.	
Acacia (n = 36)	100	0	0	0	0	0	0	0	0	0	100	92	83
Rhododendron (n = 29)	0	93	0	0	0	0	0	0	0	7	93	79	48
Chestnut (n = 60)	0	0	98	0	0	0	2	0	0	0	98	95	67
Dandelion (n = 31)	0	0	0	97	0	0	0	0	3	0	97	87	61
Heather (n = 22)	0	0	5	0	91	0	0	0	5	0	91	95	0
Lime (n = 39)	0	0	0	0	0	90	0	0	8	0	90	90	74
Rape (n = 36)	0	3	0	0	0	0	92	0	6	0	92	89	50
Fir honedew (n = 132)	0	0	0	0	0	0	0	99	2	0	99	95	69
Metcalfa honedew (n = 14)	0	0	0	0	0	0	0	0	93	7	93	93	64
Polyfloral (n = 294)	1	15	7	1	0	16	1	11	0	49	49	70	86

¹Considering fructose, glucose, sucrose, maltose, isomaltose, erlose, melezitose, maltotriose, raffinose, fructose/glucose ratio, pH-value, free acidity and electrical conductivity.

²Considering fructose, glucose, fructose/glucose ratio, electrical conductivity, as well as Brassica-, Castanea-, Calluna-, Rhododendron-, Robinia-, Tilia- and Taraxacum pollen.

³Considering fructose, glucose, sucrose, fructose/glucose and glucose/water ratio, pH-value, free acidity and electrical conductivity and minimum percentage of the specific pollen.

Eighteen percent of the lime honey samples were classified as polyfloral and 11 % as dandelion honeys. Jackknife classification and validation revealed that polyfloral honeys were very often classified into the groups of the unifloral honeys while the latter were rarely misclassified into the polyfloral honeys (**Table 3**).

The high rate of misclassified polyfloral honeys made it impossible to use a single discriminant model for the authentication of the botanical origin of honey and lead to the idea to develop a two-step procedure. In the first step the sample was attributed to one of the ten honey types considered, using the classification functions of the overall discriminant model with as many groups as honey varieties. The sample was assigned to the honey type showing the highest value in the classification functions taken into account. In the second step this classification was verified by using one or several two-group models consisting of a group formed by samples of a given unifloral honey and a group called "non-unifloral" consisting of all the other samples. Each two-group model was separately built using LDA backward elimination and forward selection. They were optimised for maximum correct classification rate together with a minimum number of necessary variables. For the verification of the classification by the first model at least the two-group model of the corresponding honey type was used. In addition one to four two-group models (indicated by bold-face numbers in **Table 3**) were used when a misclassification rate of higher than 3% was calculated in jackknifed classification or validation tables of the overall model. The probabilities for misclassification were calculated by applying Bayes' theorem on the conditional probabilities of disjoint events. The error probabilities cannot be directly taken from **Table 3**; they only quantify the conditional probabilities of correct classification given the corresponding honey type. By Bayes' theorem the posterior probabilities of finding the correct honey type given a distinct classification by the discriminant model was calculated, and the error rate being the complement to 1.

The classification rates for the unifloral honeys in the two-group models were generally > 90 %, except for lime honeys (**Table 4**). They showed with 79 % in jackknife classification respectively 56 % in validation the lowest rates. In general the high rates of correct classification for both, the unifloral and non-unifloral groups considered by the two-group models indicate that the botanical origin can be reliably determined by this procedure. Some overlapping is to be expected regarding dandelion, rape and lime honeys as about 15 % of the samples not belonging to these honey types are erroneously classified to these groups.

If a sample is assigned to the same honey type by the overall- and by the two-group model it is very likely that it belongs to this type of honey. If the classifications of the two models do not agree the sample has to be considered to be of polyfloral origin. When the sample is assigned to the same honey type by both, the overall model and the corresponding two-group model and is moreover considered to belong to the non-unifloral groups in all the other two-group models tested, the honey sample belongs almost certainly to the honey type indicated by the overall model. The respective error rates of this two-step procedure (for misclassification of a sample of unknown botanical origin), were found for the ten honey types studied except for acacia and fir honeydew honeys to be $\leq 5\%$ (**Table 5**).

Table 3. Jackknife classification and validation tables of the honey samples classified by the overall discriminant model. Bold numbers indicate misclassification rates of > 3% (two-group models to be used).

	Jackknife classification (%)										Total
	Acacia	Rhodo- dendron	Chestnut	Dandelion	Heather	Lime	Rape	Fir honeydew	Metcalfa honeydew	Polyfloral	
Acacia (n= 28)	100	0	0	0	0	0	0	0	0	0	100
Rhododendron (n= 29)	0	93	0	0	0	0	3	0	0	3	93
Chestnut (n= 56)	2	0	91	0	0	5	0	0	0	2	91
Dandelion (n= 31)	0	0	0	84	0	10	7	0	0	0	84
Heather (n= 15)	0	0	7	0	80	0	0	0	0	13	80
Lime (n= 28)	0	0	0	11	0	71	0	0	0	18	71
Rape (n= 36)	0	6	0	0	0	0	94	0	0	0	94
Fir honeydew (n= 126)	0	0	0	0	14	1	0	85	0	0	85
Metcalfa honeydew (n= 13)	0	0	0	0	0	0	0	0	100	0	100
Polyfloral (n= 284)	3	8	5	8	19	10	11	3	0	34	34

	Classification rate in validation (%)										Total
	Acacia	Rhodo- dendron	Chestnut	Dandelion	Heather	Lime	Rape	Fir ho- neydew	Metcalfa honeydew	Polyfloral	
Acacia (n= 9)	100	0	0	0	0	0	0	0	0	0	100
Rhododendron (n= 10)	0	90	0	0	0	0	10	0	0	0	90
Chestnut (n= 18)	0	0	94	0	0	6	0	0	0	0	94
Dandelion (n= 11)	0	0	0	91	0	9	0	0	0	0	91
Heather (n= 4)	0	0	0	0	75	0	0	0	0	25	75
Lime (n= 9)	0	0	0	0	0	78	0	0	0	22	78
Rape (n= 13)	0	0	0	8	0	0	92	0	0	0	92
Fir honeydew (n= 42)	0	0	0	0	14	0	0	83	0	2	83
Metcalfa honeydew (n= 4)	0	0	0	0	0	0	0	0	100	0	100
Polyfloral (n= 96)	2	17	1	1	19	9	10	4	0	37	37

2.4. DISCUSSION

2.4.1 CLASSIFICATION USING MEASURAND PROFILES

Profiles based on only physical and chemical measurands were shown to be inadequate for a reliable classification of the botanical origin of honey because of the high rate of misclassification of polyfloral honeys (**Table 2**, Profile I). However in combination with pollen analytical results the number of physical and chemical measurands can be considerably reduced as the results using profile II showed (**Table 2**, Profile II). Pollen analytical data can be included in profiles by just defining a range for the relative frequency of the specific pollen of a given unifloral honey type (1). However a more reliable profile would probably consider ranges for all the specific pollen of the unifloral honey types. Such a procedure would considerably simplify pollen analysis since only the relative frequencies of the characteristic pollen forms of the unifloral honey types would have to be considered. For a classification of the most important honey types in Europe it would be sufficient to identify and calculate the total number of pollen, the sum of the nectarless species and about 15 pollen forms characteristic for the honey types considered. By the use of a profile that includes physical, chemical and pollen analytical measurands with well-defined ranges the ambiguity resulting from the correction of over- and underrepresented pollen could be avoided and fully comprehensible classifications could be obtained. For the correct classification of most honeydew honeys the physical and chemical measurands are generally sufficient. Although honeydew honeys do not contain specific pollen, the relative frequencies of the specific pollen of the floral honeys should be considered for a reproducible procedure.

The variability in the relative frequency of the pollen forms found in this study is considerable and may have to be adjusted when more samples of unifloral honeys have been studied. Among the samples considered in the present study some pollen forms were not detected at all in some unifloral honey types. Therefore the maximum percentage is for some pollen types equal to zero. In these cases it can supposedly be raised to the minimum value of the specific pollen of the corresponding unifloral honey type.

The high misclassification rates observed using profile III may be explained with the unfavorable definition of the range (1). The ranges presented were calculated using the standard deviation. This procedure implies a normal distribution of the data in order to make sense. However the values of several measurands show a highly asymmetric distribution. The authors must have also been aware of this problem, since minimum or maximum values were presented if the 95 % confidence interval exceeded the former. If the range used in profiles is calculated from the mean value using the standard deviation for asymmetrically distributed data, the range may be delicately clipped on one end of the distribution. The ranges published are based on a huge number of samples certainly having a considerable variability. Therefore the ranges of the individual measurands seem at first sight to be very liberal. But when the ranges are used in a profile considering as many as 9 different measurands it is very likely that a value of a sample lies outside the 95 % confidence interval. This is especially true in the case of asymmetrically distributed data. When the number of measurands included in the profile were reduced to 5 (i.e. electrical conductivity, fructose and glucose content, fructose/glucose ratio and specific pollen) the rate of correct classification rose considerably for most honey types except for lime and

polyfloral honeys (data not shown). The total monosaccharide content, glucose/water ratio and diastase activity are probably not very useful to determine the botanical origin of honey. It is clear that a data range used for classification should not include extreme values such as outliers. In an asymmetrically distributed dataset it would be better to define the range for example by the 2.5 and 97.5 percentiles. To classify honey samples according to their botanical origin using a profile it is not necessary to standardise the values as proposed by Persano Oddo and Piro (1).

2.4.2 CLASSIFICATION USING DISCRIMINANT FUNCTIONS

Our results indicating that the most important measurands for a classification of unifloral and polyfloral honeys are electrical conductivity, fructose, raffinose and glucose concentration, together with free acidity are in agreement with those found in the literature (14, 17).

Table 4. Jackknife classification and validation table of the honey samples classified by the two-group discriminant models.

	Jackknife classification				Validation	
	Unifloral		Non-Unifloral		Unifloral	
	n	Correct (%)	n	Correct (%)	n	Correct (%)
Acacia	28	100	618	98	9	100
Rhododendron	29	90	616	92	10	90
Chestnut	56	95	590	97	18	100
Dandelion	31	90	615	85	11	100
Heather	15	93	631	94	4	88
Lime	28	79	582	84	9	56
Rape	36	92	610	85	13	100
Fir honeydew	126	92	517	97	42	88
Metcalfa honeydew	13	92	630	98	4	100
Polyfloral	282	79	329	65	96	82

The classification functions of the overall and two-group classification models are given in the Appendix B. The abbreviation "DG" designates the classification function values of the classification functions belonging to the general model. The honey samples were classified to the honey type whose corresponding classification function gave the highest classification function value. The quantity of the absolute values of classification function coefficients indicate which variables are particularly important for the discrimination between the honey types. Thus high fructose content, low electrical conductivity and glucose content are characteristic for acacia honeys and therefore the most important variables for the discrimination of acacia honeys as already shown by Piro et al. (16).

Interestingly electrical conductivity seems to be an important measurand for classification of metcalfa honeydew and chestnut honey but is not very relevant for the characterisation of fir honeys, while high raffinose content and high free acidity were found to be very characteristic for the latter. High glucose content was found to be important for the classification of rape and dandelion honeys

The classification functions of the two-group models are identified by the abbreviation "DT" in the classification function value. The difficulties in discrimination of lime honeys were indicated by the fact that eight variables were necessary in the model and none of the absolute values was particularly high. Maltose, isomaltose and erlose were found to be the most relevant measurands. The high rate of misclassification of lime honey samples to polyfloral and dandelion honeys may be explained by the variable chemical composition of this honey type as it often contains different amounts of honeydew and thus exhibits variable physical and chemical characteristics (**Table 3**). This makes it similar to polyfloral honey that may contain proportions of nectar and honeydew. In the two-group model, high glucose, low fructose concentration and a low fructose-glucose ratio were once again characteristic for rape honey. The most important variables for the classification of fir honeys were raffinose, melezitose and trehalose, which is in agreement with other studies (14, 17). For the discrimination between acacia honey and all the other honey types the fructose/glucose ratio was found to be the most relevant variable. Chestnut honey was characterised by a high fructose content, high electrical conductivity and pH-value. Glucose concentration was found to be the most important factor for classification between dandelion and other honey types. Metcalfa honeydew honey was characterised by high maltose and maltotriose contents as well as high electrical conductivity. The former findings confirm the results of previous studies (16). For heather honey the water content and free acidity were found to be the most discriminating variables. For identifying rhododendron honey pH-value, free acidity and erlose content were found to be the most important measurands. The classification of polyfloral and lime honeys needs a high number of measurands and none of them seems to play a very decisive role. This is also reflected by the low classification rates. The sub-optimal classification of polyfloral honeys is of less importance, as we are interested in the authentication of unifloral honeys.

Table 5. Error probabilities for the classification of unifloral and polyfloral honeys calculated by Bayes' theorem (two-step approach)

Honey type	Error probability	
	Jackknife	Validation
Acacia	0.099	0.060
Rhododendron	0.038	0.047
Chestnut	0.031	0.013
Dandelion	0.023	0.017
Heather	0.015	0.019
Lime	0.011	0.001
Rape	0.012	0.011
Fir honeydew	0.044	0.055
Metcalfa honeydew	0.017	0.017
Polyfloral	0.017	0.020

Data evaluation showed that electrical conductivity is not a very reliable criterion to discriminate between floral and honeydew honeys although this measurand is defined as important in the Codex Alimentarius Standard for Honey and the European Honey Directive. However, several exceptions are listed in the above mentioned standards thus indicating the limited value of this measurand for the discrimination

of honey types. Thus multivariate data evaluation of traditional physical and chemical measurands may also be helpful to establish new criteria for a more reliable description of the honey types and for the determination of their botanical origin.

The chemometric analysis of physical and chemical data demonstrated that the botanical origin of honey can be determined without considering pollen analytical results. Unfortunately this approach does not save very much time and costs as only pollen analysis can be abandoned. Indeed 14 physical and chemical measurands have still to be determined. Nevertheless pollen analysis is the technique which requires the most professional expertise and skill, the most time and cannot be automated. In case of doubt the traditional approach using physical, chemical and pollen analytical results and the expertise required for their interpretation will so far remain the reference method.

2.4.3 CONCLUSIONS

The classical approach using a defined profile would allow a reliable and reproducible determination of the botanical origin provided that an international agreement can be made on the measurands as well as the corresponding data ranges to be taken into account. Using such a procedure pollen analysis cannot be discarded and will in principle express the same inconsistencies. However, the difficulties in the interpretation of pollen analytical results may be overcome by including pollen analytical characteristics together with physical and chemical measurands into a distinct profile and appropriate definition of the data ranges. For straightforward classification the profiles can be programmed in a spreadsheet software. However the ranges presented in this study should be reconsidered by an even larger set of unifloral honeys as especially the pollen ranges may need to be adjusted.

Chemometric evaluation of the physical and chemical measurands revealed that a determination of the botanical origin of honey can be achieved with a mathematical procedure without considering pollen analytical results. The classification functions published in the Appendix can be used for this purpose without special expertise and statistical software.

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GENERAL SUMMARY

Currently the botanical origin of honey is determined by experts by a global interpretation of several physical, chemical, pollen analytical and sensorial properties. Unifloral honeys express distinctly different physical and chemical characteristics and can thus be easily distinguished from each other. However, the challenge in the authentication of unifloral honeys is to distinguish the minority of unifloral honeys from the overwhelming majority of polyfloral honeys.

The aim of the current work was to evaluate the potential of different profiles and to develop a chemometric approach for a comprehensible determination of the botanical origin of honey using various physical and chemical measurands.

Traditionally the botanical origin of honey is determined by comparing the values of physical and chemical measurands of a honey sample with profiles of unifloral honeys consisting of defined ranges of the measurands considered (principle of exclusion). A profile consisting of as many as 13 different physical and chemical criteria showed high correct classification rates for the unifloral honeys but half of the polyfloral honey samples were misclassified to various unifloral honey types (**Table 2, Profile I**). This clearly indicates that a profile using only physical and chemical measurands is inadequate for a reliable determination of the botanical origin of honey. When the number of physical and chemical criteria was reduced to four and ranges for the relative frequencies of specific pollen were included, the correct classification rate of the polyfloral honeys rose considerably (**Table 2, Profile II**). This demonstrates that pollen analysis plays a key role in the discrimination between unifloral and polyfloral honeys.

Multivariate explorative data analysis revealed that electrical conductivity, fructose, raffinose and glucose concentration, together with free acidity, contributed most to the classification of the different unifloral honeys. Correct classification rates of higher than 80 % were found for most of the unifloral honeys when the above-mentioned variables were used. Again polyfloral honeys were very often misclassified to different unifloral honey types (**Table 3**). These difficulties were resolved by a two-step procedure. The sample was first classified to a honey type by the general model considering all the honey types. This attribution was thereafter verified by at least one two-group model consisting of a group formed by samples of a given unifloral honey and a group called "non-unifloral" consisting of all the other samples (**Table 4**). If the sample is assigned to the same honey type by the overall- and by the two-group model it is very likely that it belongs to this honey type. Chemometric evaluation of the physical and chemical measurands revealed that a determination of the botanical origin of honey may be achieved without pollen analysis by using the standardisation and classification functions shown in the **Appendix**.

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2.6 APPENDIX

A = Free acidity (meq/kg)

E = Erlöse (g/100 g)

FG = Fructose / Glucose-Ratio

GW = Glucose / Water-Ratio

MA = Maltose (g/100 g)

MT = Maltotriose (g/100 g)

R = Raffinose (g/100 g)

T = Trehalose (g/100 g)

C = Electrical conductivity (mScm⁻¹)

F = Fructose (g/100 g)

G = Glucose (g/100 g)

I = Isomaltose (g/100 g)

ME = Melezitose (g/100 g)

P = pH-Value

S = Sucrose (g/100 g)

W = Water (g/100 g)

2.6.1 STANDARDISATION FUNCTIONS USED:

$$A_s = \frac{A-18.68}{8.849}$$

$$C_s = \frac{C-0.6911}{0.391}$$

$$E_s = \frac{E-0.640}{0.848}$$

$$F_s = \frac{F-37.23}{3.458}$$

$$FG_s = \frac{FG-1.268}{0.184}$$

$$G_s = \frac{G-29.78}{3.884}$$

$$GW_s = \frac{GW-1.873}{0.277}$$

$$I_s = \frac{I-0.831}{0.680}$$

$$MA_s = \frac{MA-1.76}{1.29}$$

$$ME_s = \frac{ME-0.932}{1.40}$$

$$MT_s = \frac{MT-0.0798}{0.286}$$

$$P_s = \frac{P-4.48}{0.472}$$

$$R_s = \frac{R-0.234}{0.455}$$

$$S_s = \frac{S-0.373}{0.592}$$

$$T_s = \frac{T-0.850}{0.909}$$

$$W_s = \frac{W-16.05}{1.242}$$

2.6.2 CLASSIFICATION FUNCTIONS USED

DG_x = Classification function belonging to the general model

DT_x = Classification function belonging to the two group-model

$$DG_{Acacia} = -15.80 - 6.553 C_S - 1.131 A_S + 6.279 F_S - 5.267 G_S - 0.707 R_S$$

$$DG_{Rhododendron} = -5.691 - 4.367 C_S - 1.524 A_S + 0.4233 F_S - 1.908 G_S - 1.04 R_S$$

$$DG_{Chestnut} = -12.29 + 6.990 C_S - 3.461 A_S + 3.781 F_S - 1.803 G_S - 2.45 R_S$$

$$DG_{Dandelion} = -6.676 + 0.5799 C_S - 1.709 A_S - 1.607 F_S + 3.984 G_S - 0.854 R_S$$

$$DG_{Heather} = -4.095 + 0.6708 C_S + 2.243 A_S + 0.7035 F_S - 0.2699 G_S - 1.19 R_S$$

$$DG_{Lime} = -3.140 + 0.2620 C_S - 1.602 A_S - 0.2838 F_S - 0.2120 G_S - 1.57 R_S$$

$$DG_{Rape} = -6.758 - 3.399 C_S - 0.7763 A_S - 0.8761 F_S + 2.513 G_S - 0.465 R_S$$

$$DG_{Fir} = -8.960 + 1.191 C_S + 1.929 A_S - 3.053 F_S + 0.02895 G_S + 4.02 R_S$$

$$DG_{Metcalfa} = -24.65 + 10.97 C_S - 0.3305 A_S - 6.366 F_S + 0.1213 G_S - 2.86 R_S$$

$$DG_{Polyfloral} = -2.724 - 1.169 C_S + 0.4742 A_S + 0.4872 F_S + 0.2819 G_S - 0.612 R_S$$

$$DT_{Acacia} = -7.565 - 3.351 C_S + 4.337 FG_S$$

$$DT_{Non-Acacia} = -0.7060 + 0.1392 C_S - 0.1910 FG_S$$

$$\begin{aligned} DT_{\text{Rhododendron}} &= -4.336 - 3.792 P_S - 3.612 A_S - 2.879 G_S + 1.99 E_S - 2.032 FG_S - 1.17 S_S + 1.898 C_S \\ DT_{\text{Non-Rhododendron}} &= -0.7009 + 0.2182 P_S + 0.1911 A_S + 0.1065 G_S - 0.0885 E_S + 0.08556 FG_S + 0.0316 S_S - 0.1581 C_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Chestnut}} &= -7.521 + 2.260 C_S + 3.273 P_S + 3.999 F_S \\ DT_{\text{Non-Chestnut}} &= -0.7617 - 0.2497 C_S - 0.3074 P_S - 0.4043 F_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Dandelion}} &= -2.912 - 1.20 P_S + 2.587 G_S \\ DT_{\text{Non-Dandelion}} &= -0.6987 - 0.0675 P_S - 0.1292 G_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Heather}} &= -5.837 + 3.091 W_S + 2.694 A_S - 1.40 ME_S \\ DT_{\text{Non-Heather}} &= -0.7028 - 0.1547 W_S - 0.07763 A_S + 0.0266 ME_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Lime}} &= -2.074 - 0.6357 A_S + 0.766 S_S + 0.989 MA_S + 0.964 I_S - 1.35 E_S - 0.931 R_S - 0.4547 C_S - 0.2105 FG_S \\ DT_{\text{Non-Lime}} &= -0.7095 + 0.09442 A_S - 0.0134 S_S - 0.0105 MA_S + 0.0443 I_S - 0.0949 E_S - 0.196 R_S - 0.06527 C_S + 0.04987 FG_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Rape}} &= -2.862 - 1.280 C_S - 5.501 F_S + 7.363 G_S - 0.515 E_S + 7.2005 FG_S \\ DT_{\text{Non-Rape}} &= -0.7005 + 0.04757 C_S + 0.3211 F_S - 0.4674 G_S + 0.0211 E_S - 0.4397 FG_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Fir}} &= -6.811 + 0.8622 A_S - 1.286 F_S + 1.17 T_S + 1.75 ME_S - 0.595 MT_S + 3.63 R_S \\ DT_{\text{Non-Fir}} &= -1.052 - 0.1992 A_S + 0.3252 F_S - 0.296 T_S - 0.415 ME_S + 0.150 MT_S - 0.872 R_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Metcalfa}} &= -16.17 + 2.611 C_S + 4.14 MA_S + 3.38 MT_S \\ DT_{\text{Non-Metcalfa}} &= -0.7000 - 0.05311 C_S - 0.0916 MA_S - 0.0678 MT_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Polyfloral}} &= -0.9999 - 0.2374 C_S + 0.5129 A_S + 1.220 F_S - 1.647 G_S - 0.332 MA_S - 0.276 T_S + 0.222 I_S - 0.514 R_S - 1.527 FG_S \\ &\quad + 0.5209 GW_S \end{aligned}$$

$$\begin{aligned} DT_{\text{Non-Polyfloral}} &= -0.8230 + 0.08931 C_S - 0.2962 A_S - 1.310 F_S + 1.549 G_S + 0.271 MA_S + 0.243 T_S - 0.0994 I_S - 0.0681 R_S + 1.626 FG_S \\ &\quad - 0.3674 GW_S \end{aligned}$$