Evaluation of Some Fixed Components for Unifloral Honey Characterization

Lanfranco S. Conte,* Mauro Miorini, Andrea Giomo, Giovanna Bertacco, and Roberto Zironi

Department of Food Science, Università degli Studi di Udine, Via Marangoni 97, 33100 Udine, Italy

The classification of honeys according to their botanical origin is a problem usually solved by means of microscopic observation of pollens and by evaluation of organoleptic characteristics such as color, taste, and others. In this paper, a number of chemical parameters including water activity, free amino acid composition, reducing sugars, total sugars, and pH were evaluated for a number of samples of some different botanical origin honeys. The results were evaluated by chemometric methods, and this allowed discrimination between the different origins, also for some critical cases such as chestnut and lime tree.

Keywords: *Honey; botanical origin; free amino acids; chemometric methods*

1. INTRODUCTION

With the exception of their intrinsic characteristics which are determined by very specific physicochemical parameters, honey types are differentiated according to their botanical and geographic source. The latter can be determined with techniques which differ from the methods normally used, especially when employed for melissopalynology and honey judging.

Apart from being particularly complex, melissopalynology is not always practical for example, when there is a limited amount of pollen present. On the other hand, an evaluation of the organoleptic characteristics of honey may be influenced by subjectivity and involves methods not yet completely standardized.

The groundwork of the present study was an analysis of the free amino acid fraction, after a similar approach had been adopted both to characterize other food matrixes (Bertacco et al., 1992; Monastero et al., 1991; Vasconcelos and Chaves Das Neves, 1990) and more specifically, to characterize pollens and honey types (Bosi and Battaglini, 1978; Curti and Riganti, 1966; Davies, 1975, 1976, 1978; Davies and Harris, 1982; Fontanarrosa and Vigil, 1982; Gilliam et al., 1980; Pirini et al., 1992; Ricciarelli D'albore and Tonini D'Ambrosio, 1979; Speer and Montag, 1986). In this context, the objective was to further develop the separation method, namely, the separation of optical isomers through chiral columns, to evaluate whether honey contains D-isomers (Brucker and Hausch, 1989). In addition to providing more precise information on the product, the D-form evaluation is interesting as a means of monitoring bacterial proliferation in any single honey production stage.

In addition to the amino acid spectrum, other analyses were used to characterize the honey types: pH, water activity (Aw), reducing sugars, and total sugar content. A statistics-oriented approach was used to correlate the results of the various analyses with the botanical species and the botanical source of the honey types to highlight any interdependence.

* Address correspondence to this author [e-mail Lanfranco.Conte@DSA.Uniud.it; fax (+39) 432 501 637].

Methods used in the present work, of course, would represent furhter experiences on unifloral honey classification that could eventually be carried out as a confirmation of results of routine unifloral characterization, as, for example, the ones reported by Accorti et al. (1986) and Persano Oddo et al. (1988) for Italian honeys.

2. EXPERIMENTAL PROCEDURES

2.1. Sampling. This study was carried out on 92 samples of honey taken from 17 different botanical sources in 4 different areas.

Table 1 lists the samples used with their declared botanical source and geographic origin. Samples were at first classified according to indications given by expert institutes: in details, all samples from Emilia Romagna were certified for their botanical origin by palynological analysis, while those from Friuli-Venezia-Giulia were classified according to an evaluation of their organoleptic characteristics given by local tasters.

2.2. Determination of Amino Acids. The extraction and purification processes for amino acids were carried out as described in a previous work (Pirini et al., 1992) based on the Adams (1974) method; the derivatization was carried out using a method already described in the literature (Pirini et al., 1992; Mc Kenzie and Tenaschuck, 1974; De Ming, 1989) and which has already proved useful for the evaluation of D-forms as well (Bertacco et al., 1992). Standard solutions of L-isomers and D-isomers were prepared starting from the Sigma standards (Sigma Chemical Co., St. Louis, MO, code 87F-9000 for L-isomers and 103H9006 for D-isomers) with the addition of GABA to obtain a concentration of 10 mg/mL for each amino acid in 2 M ammonium hydroxide.

A Fison's 5300 Mega series gas chromatograph was used, equipped with a 25 m fused silica capillary column with an internal diameter of 0.25 mm, coated with 0.12 μm chiral stationary phase film (Chirasil L-Val, Chrompack, Belgium). The conditions were the following: oven temperature programmed from 60 to 200 °C with a 2 min initial isotherm and subsequent program of 5 °C/min, with a final isotherm of 10 min. The injector (split) and detector (FID) temperatures were 280 °C; the gas flows were carrier gas (He) 2.8 mL/min, split ratio 1:50; the auxiliary gases were hydrogen 25 mL/min and air 300 mL/min.

Ten aliquots of standard amino acid solution were analyzed under the same conditions adopted for the samples. Average values, variation coefficients, and variance could thus be calculated.

S0021-8561(97)00837-6 CCC: $15.00
Published on Web 04/14/1998

Table 1. Sampling Plane

scientific name	area of origin	no. of samples
Robinia pseudoacacia	Emilia Romagna (I)	10
	Friuli-Venezia-Giulia (I)	6
Citrus	Italy (several areas)	10
Rosmarinus officinalis	Emilia Romagna (I)	4
Thymus spp.	Emilia Romagna (I)	4
Lavandula stoechas	Emilia Romagna (I)	3
Tilia spp.	Friuli-Venezia-Giulia (I)	6
Castanea sativa	Emilia Romagna (I)	8
	Friuli-Venezia-Giulia (I)	9
Eucalyptus sp.	Emilia Romagna (I)	10
Taraxacum officinalis	Friuli-Venezia-Giulia (I)	9
Satureja ortensis	Emilia Romagna (I)	2

2.3. pH Determination. The pH was determined potentiometrically on a 60% honey solution in double-distilled water. The pH measurements was carried out immediately after dilution, to avoid any pH change.

2.4. Water Activity Determination. Aw was determined on 15 g of honey using a PBI Hygroscope DT probe (PBI, Italy). The honey was left inside the probe until hygroscopic equilibrium was reached (approximately 2 h).

2.5. Sugar Determination. Reducing sugars were determined potentiometrically with a methodology previously used for wines (Zironi et al., 1989), on honey solutions in double-distilled water (2%) in order to obtain sugar concentrations lower than 25 g/L. Inverted sugars were evaluated on 10 mL of the solution used to determine reducing sugars that were treated with 0.3 mL of 37% hydrochloric acid and boiled for 2 min. After cooling, this solution was neutralized with 12 M sodium hydroxide (0.3 mL) and was subsequently titrated as described for reducing sugars.

2.6. Statistical Analysis of Data. Classical linear methodologies (Fischer's linear discriminating analysis) were employed (Mardia et al., 1979), together with experimental methodologies for the exploration data analysis (EDA) (Tukey, 1977; McGill et al., 1978; Wilkinson, 1990). Calculations were carried out with the aid of the Systat statistics package from SYSTAT Inc., Evanston, IL (Wilkinson, 1990).

3. RESULTS AND DISCUSSION

The composition results, i.e., the quantities of free amino acids in the honey samples used in this work shown in Table 2, display rather high variation coefficients for honey types from the same botanical source; this concurs with the results of previous published research (Davies and Harris, 1982; Pirini et al., 1992) which can be attributed to the remarkable number of variables which can influence the presence of amino acids in a honey type.

Table 3 shows data for honey types whose low sample numbers prevented calculations of averages, variance, etc.

It can be noted, however, that thyme honey was the richest in amino acids of all the honeys tested, with a content of approximately 125 mg/100 g, followed by chestnut honey, summer savory, and eucalyptus honeys. Apart from the expected predominance of proline, high concentrations of arginine, aspartic acid + asparagine (Asx), and glutamic acid + glutamine (Glx) were found in almost all the honeys tested even if it can be seen that the ratio of Asx to Glx is higher than Asx in chestnut, citrus, eucalyptus, and acacia honeys. Thyme honey should be considered separately, given its high content of serine, tyrosine, and lysine, making the ratios of Asx and Glx equal. In contrast, tyrosine proved to be the predominant amino acid in rosemary honey.

No sample showed traces of D-amino acids, which confirms the good microbiological condition of the honeys analyzed. Accordingly, racemization of amino acids in food matrixes from high physicochemical treatments can be excluded, as already reported (Brucker and Hausch, 1989).

Given the difficulty of interpretation for the size of the dataset at our disposal, it was necessary to employ a statistical analysis system.

A close examination of the literature shows how possible correlations between the compositional characteristics and botanical and geographic origins of honey have gained validity together with the availability of appropriate methods of data interpretation (Bosi and Battaglini, 1978; Davies and Harris, 1982; Pirini et al., 1992; Gilbert et al., 1981; Guidetti et al., 1995).

A first approach was to study the development of the considered variables (amino acids, pH, Aw, reducing sugars, inverted sugars, and apparent sucrose) within the various classes of honey. Figures 1−3 show examples of the graphic representations of Aw, pH, and reducing sugar content. An extremely important factor in microorganism development inhibition is water activity (Aw) (data in Figure 1), whose distribution was associated with botanical source and whose values registered between 0.64 (citrus honey) and 0.55 (thyme honey). The minimum single value was 0.54 (*Taraxacum officinalis*) and the maximum 0.68 (*Eucalyptus spp*).

The pH values (Figure 2) were between 3.40 and 6.00 with a distribution associated with botanical sources, given that the highest pH levels were recorded in chestnut honeys and the lowest in summer savory, thyme, rosemary, and lime, confirming previously published research (Persano Oddo et al., 1986).

These graphs, called "Notched Box Plots", allow identification of the most discriminating variables between the various honey classes and those which have the lowest variability within a single class. The "Notched Box Plot" technique does not imply any hypothesis regarding the kind of density distribution; they show data probability by means of a graph which calculates the median confidence interval at 95%, while the box represents 50% of the data (area with a density or probability = 0.5), from the 25th percentile to the 75th percentile, and the whiskers represent one and one-half times the span of the box. The data outside the whiskers, within a span 3 times that of the box (outside values), are considered near outlier values; those outside of the external enclosure are considered far outlier values (Figure 4).

If the confidence intervals of the two groups of box diagrams do not overlap, this means that the two groups are significantly different.

The median confidence interval span of the notch box plots is a considerable calculus problem since every technique employed is valid only for a particular class of cases and cannot represent a generalization.

A rough estimate of the standard deviation of an unknown distribution is used to calculate the confidence interval according to Kendall and Stuart (1967).

The next step was to separate the samples into restricted clusters using a grouping analysis (nonlinear technique, data not shown). This allowed the elimination of samples showing anomalous characteristics. This double step was necessary to focus on the statistics system, so that it could sort the various classes of honey according to previously chosen variables.

Table 2. Statistical Summary of Free Amino Acids Composition of *Robinia, Citrus, Castanea, Eucalyptus, Taraxacum,* and *Tilia* Honeys (Data as mg/100 g)

honey	no. of samples		L-Ala	L-Val	L-Thr	Gly	L-Ile	L-Pro	L-Leu	L-Ser	L-Met	L-Asx	L-Phe	L-Glx	L-Tyr	L-Orn	L-Lys	AA tot
Robinia pseudoacacia	16	average	0.53	0.50	0.61	0.66	0.48	22.32	0.38	0.60	0.34	2.96	19.01	1.53	1.56	1.58	1.49	54.56
		maximum	0.75	1.39	1.36	1.42	1.44	50.80	0.57	1.03	0.69	4.09	54.42	2.48	10.19	6.07	3.00	128.43
		minimum	0.29	0.29	0.28	0.26	0.16	15.60	0.17	0.19	0.19	2.15	3.50	0.17	0.52	0.00	0.87	34.57
		RSD	27.44	48.73	50.84	42.25	68.31	37.58	30.59	39.19	34.90	19.38	85.40	39.78	145.58	108.56	49.33	44.49
		median	0.50	0.47	0.55	0.55	0.41	19.68	0.33	0.55	0.32	2.80	12.05	1.54	0.95	1.28	1.20	47.30
		variance	0.02	0.06	0.10	0.08	0.11	70.34	0.01	0.05	0.01	0.33	263.48	0.37	5.14	2.95	0.54	586.12
Citrus	10	average	0.72	0.57	0.61	0.72	0.44	31.77	0.31	1.16	0.36	4.44	9.18	2.63	0.99	1.47	1.60	56.96
		maximum	1.39	1.15	0.84	1.11	0.80	49.11	0.55	1.93	0.54	6.47	17.52	4.53	1.71	4.58	2.50	82.81
		minimum	0.43	0.27	0.44	0.48	0.22	17.49	0.20	0.78	0.14	2.78	3.91	1.27	0.57	0.40	0.23	35.52
		RSD	40.90	42.94	24.65	32.09	39.07	31.18	33.24	33.26	31.96	26.52	41.21	40.72	34.63	87.16	39.95	26.86
		median	0.64	0.52	0.56	0.69	0.40	30.91	0.28	1.04	0.36	4.36	8.56	2.54	0.96	0.93	1.53	57.39
		variance	0.09	0.06	0.02	0.05	0.03	98.13	0.01	0.15	0.00	1.38	14.32	1.14	0.12	1.64	0.41	234.02
Castanea sativa	17	average	1.21	0.94	0.53	0.97	0.76	64.06	0.63	0.93	0.42	4.05	16.24	3.79	1.30	0.73	1.62	98.19
		maximum	2.12	1.53	0.95	1.64	1.17	98.51	0.96	2.15	0.79	7.30	32.15	6.37	2.78	1.71	2.76	148.93
		minimum	0.50	0.69	0.22	0.63	0.54	48.19	0.50	0.30	0.18	1.89	4.10	1.44	0.75	0.14	0.52	77.16
		RSD	28.33	23.04	35.23	33.23	20.70	19.15	17.61	51.05	39.68	38.16	44.32	41.24	37.19	56.42	39.63	17.70
		median	1.25	0.90	0.52	0.86	0.73	61.73	0.62	0.77	0.42	3.88	14.08	3.46	1.19	0.70	1.49	97.99
		variance	0.12	0.05	0.04	0.10	0.02	150.48	0.01	0.22	0.03	2.39	51.82	2.45	0.23	0.17	0.41	301.86
Eucalyptus spp.	10	average	1.04	0.89	0.79	1.11	0.96	52.09	0.73	1.71	0.63	3.08	16.04	2.82	1.43	1.07	2.34	86.71
		maximum	1.99	1.52	2.30	2.35	1.45	79.51	1.08	2.97	1.14	4.84	32.90	4.72	3.52	2.90	2.91	118.59
		minimum	0.15	0.20	0.19	0.40	0.44	16.30	0.31	0.59	0.20	1.27	4.14	1.46	0.74	0.00	1.56	32.47
		RSD	51.32	43.93	72.55	49.51	27.06	33.82	30.93	49.10	46.83	39.91	63.42	32.60	56.72	76.76	15.58	29.57
		median	0.87	0.88	0.69	0.90	0.99	49.76	0.78	1.62	0.66	3.16	13.73	2.74	1.38	0.89	2.42	94.63
		variance	0.29	0.15	0.33	0.30	0.07	310.33	0.05	0.70	0.09	1.51	103.42	0.85	0.66	0.67	0.13	657.66
Taraxacum officinalis	9	average	0.57	0.64	0.61	0.68	0.76	24.87	0.59	0.86	0.41	2.83	12.22	3.99	1.40	1.20	1.84	53.47
		maximum	0.94	0.89	0.74	0.94	1.03	36.17	0.82	1.06	0.69	3.85	26.65	4.84	2.46	2.95	5.76	64.83
		minimum	0.31	0.50	0.51	0.43	0.41	17.84	0.47	0.48	0.02	1.91	5.52	2.70	0.76	0.40	1.05	35.76
		RSD	35.05	18.78	10.87	25.20	28.71	27.87	21.13	22.64	38.51	19.51	55.93	20.29	36.18	66.84	80.26	19.10
		median	0.60	0.62	0.61	0.66	0.80	24.55	0.55	0.88	0.36	2.85	9.50	4.40	1.26	1.05	1.37	53.08
		variance	0.04	0.01	0.00	0.03	0.05	48.03	0.02	0.04	0.02	0.31	46.67	0.65	0.26	0.64	2.19	104.29
Tilia spp.	6	average	0.56	0.47	0.44	0.66	0.43	35.06	0.41	0.60	0.25	2.35	8.71	2.58	0.72	0.91	1.73	55.86
		maximum	0.77	0.63	0.57	0.82	0.54	56.06	0.69	0.98	0.37	3.93	17.39	4.36	0.97	1.53	2.43	72.21
		minimum	0.42	0.36	0.31	0.50	0.35	20.93	0.29	0.30	0.14	1.44	2.56	1.71	0.48	0.38	0.98	36.34
		RSD	23.30	21.70	24.60	18.49	17.00	38.31	35.61	49.74	34.68	41.14	69.31	38.80	24.45	41.38	27.79	22.58
		median	0.57	0.45	0.43	0.67	0.42	35.19	0.36	0.56	0.25	2.21	5.90	2.24	0.74	0.84	1.73	56.80
		variance	0.02	0.01	0.01	0.01	0.01	180.32	0.02	0.09	0.01	0.93	36.43	1.00	0.03	0.14	0.23	159.10

Table 3. Free Amino Acid Composition of Rosemary, *Thymus, Lavandula, Satureja,* and Buckwheat (All Data as mg/100 g)

honey	L-Ala	L-Val	L-Thr	Gly	L-Ile	L-Pro	L-Leu	L-Ser	L-Met	L-Asx	L-Phe	L-Glx	L-Tyr	L-Orn	L-Lys	AA tot
rosemary 2	0.23	0.39	0.24	0.19	0.66	25.86	0.28	0.73	0.22	1.04	7.65	1.69	4.51	0.86	1.59	46.14
rosemary 10	0.54	0.42	0.48	0.55	0.55	26.32	0.43	0.86	0.47	0.92	17.85	2.41	2.27	0.34	1.88	56.29
rosemary 11	4.17	0.64	0.65	0.64	0.49	45.14	3.83	1.84	0.38	2.78	13.44	2.53	14.17	1.51	3.38	95.59
rosemary 14	0.43	0.46	0.43	0.36	0.46	26.87	0.31	0.94	0.18	0.94	9.29	1.54	4.16	0.95	1.49	48.81
Thymus 3	1.13	1.09	1.11	0.85	0.91	98.14	0.87	2.41	0.49	4.52	14.52	3.56	3.03	0.68	3.15	136.46
Thymus 9	1.85	1.03	1.05	0.96	0.54	101.75	0.93	2.76	0.40	2.34	35.77	2.31	4.67	0.52	3.14	160.02
Thymus 12	1.83	1.33	1.24	1.02	0.76	93.13	0.90	3.37	0.51	3.69	10.08	3.56	3.28	0.40	2.89	127.99
Thymus 13	1.81	1.32	1.09	0.90	0.64	90.17	0.87	2.60	0.46	3.53	10.30	3.47	3.33	0.52	3.15	124.16
Lavandula	0.64	0.53	0.46	0.47	0.51	37.38	0.57	1.16	0.41	3.17	10.74	4.55	3.52	0.63	2.47	67.21
Lavandula	1.04	0.80	0.78	0.78	0.61	45.96	0.47	2.20	0.50	5.17	15.54	4.63	2.23	0.78	2.85	84.34
Lavandula	0.37	0.50	0.55	0.40	0.46	50.21	0.42	1.59	0.59	3.91	12.42	4.51	4.16	0.99	2.72	83.80
Satureja ortensis 7	1.14	0.80	0.86	0.68	0.53	79.19	0.57	1.47	0.72	1.55	13.43	2.38	2.42	0.32	2.74	108.80
Satureja ortensis 8	1.12	0.73	0.77	0.70	0.53	64.66	0.53	1.57	0.55	1.54	16.63	2.61	1.02	0.40	2.43	95.79
buckwheat	11.00	1.80	6.10	3.23	1.54	0.00	35.53	32.60	5.60	0.11	3.33	22.09	10.91	28.65	0.35	1.54
buckwheat	11.00	1.46	5.03	2.58	1.09	0.00	29.58	17.59	3.67	1.24	2.51	27.52	9.08	16.90	2.22	1.89

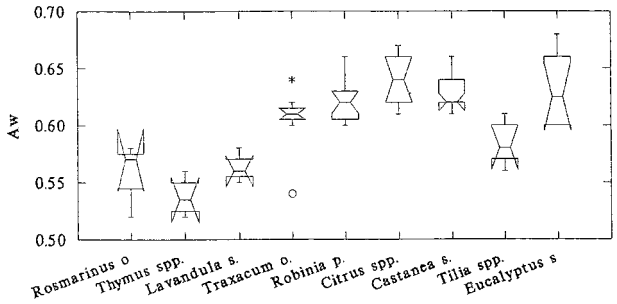

Figure 1. Notched box plot representing water activity (Aw) in honey types which underwent statistical analysis.

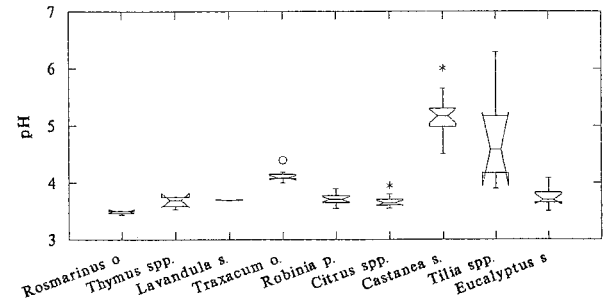

Figure 2. Notched box plot representing the pH values in honey types which underwent statistical analysis.

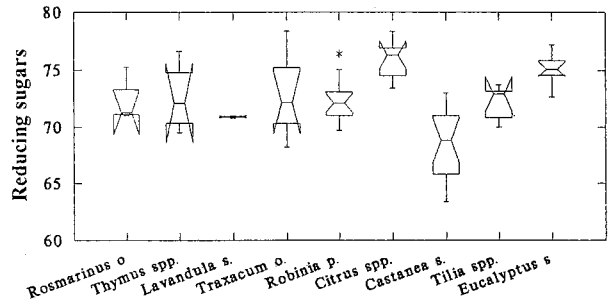

Figure 3. Notched box plot representing the reducing sugar content of honey types which underwent statistical analysis.

The separation of the samples into groups was optimized using linear discrimination analysis. These groups were established according to honey type and are shown by the graph in Figure 5. The graph highlights the confidence ellipses at 80% calculated from the group centroid (average of the averages). The ellipses provide an opportunity to verify graphically whether a group is significantly different from another.

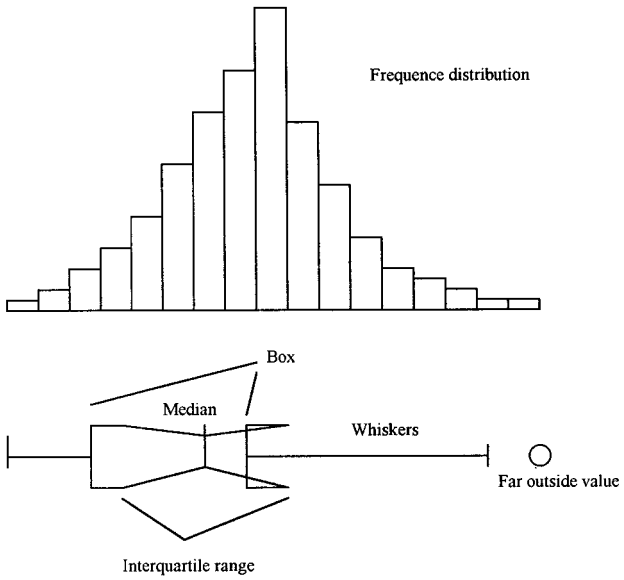

Figure 4. Notched box plot as frequency distribution representation.

Table 4, in contrast, shows the cases which were correctly classified by the linear algorithm of the discriminating analysis.

Chestnut and thyme honeys appeared distinctly separate, and dandelion samples were all grouped within a limited area. Citrus and acacia honeys were grouped in a sufficiently restricted area, although the area itself was difficult to identify since there were many categories of honey in this area.

Eucalyptus honeys were rather widespread and vague in distribution. If viewed superficially, the method used to separate lime honey from other honey types may appear disappointing; however, such evaluations should only be made from a strictly botanical point of view. Pollen from lime is undoubtedly underrepresented, but this is partly due to the inverted position of lime flowers where the pollen does not mix with the nectar. Furthermore, it must be considered that lime honey often contains chestnut pollen, which is an overrepresented species (Ricciarelli D'Albore and Persano Oddo, 1981). Melissopalynology of this honey is particularly difficult and at times impossible when lime pollen cannot be obtained, while the graph of Figure 5 shows that lime honeys can be separated roughly into two areas: a rather clean area containing samples which could

Table 4. Performance of Classification Obtained by Linear Algorithm of Discrimining Analysis

	Rosmarinus o.	*Thymus* spp.	*Lavandula* spp.	*Taraxacum officinalis*	*Robinia* spp.	*Citrus* spp.	*Castanea sativa*	*Tilia* spp.	*Eucalyptus* spp.	total
Rosmarinus o.	3									3
Thymus spp.		4								4
Lavandula s.			3							3
Taraxacum o.				8						8
Robinia p.					15	1				16
Citrus spp.			2		3	5				10
Castanea s.							18			18
Tilia spp.					1		1	4		6
Eucalyptus s.									10	10
total	3	4	5	8	19	6	19	4	10	78

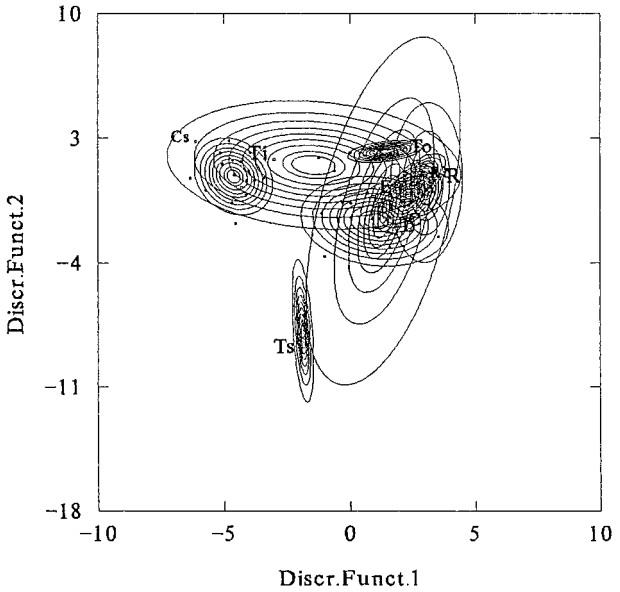

Figure 5. Graphic representation of the linear discriminating anaysis of honey samples which underwent statistical analysis; chestnut honey separation from all other honey types and lime honey distribution are also shown.

probably be considered pure lime, and another area, close to chestnut honeys, which could include lime samples with higher traces of chestnut. On one hand, this result promotes a qualitative evaluation of lime honey, while on the other, it supports the fact that the amino acid component is strictly connected to the types of pollen present.

4. CONCLUSIONS

In light of these results, it can be said that the characterization of honey types not only can be successfully carried out by means of parameters considered "unconventional" but also can be achieved even when conventional methods fail. The methodology presented in this work, however, provides a successful alternative for the separation of peculiar honey types and highlights their degree of purity. Given the good results obtained so far, this approach could be further developed by increasing the number of samples, including other honey types, so that the statistics program can be "trained" to elaborate a system capable of distinguishing the different honey types on the basis of data resulting from analyses of free amino acids, pH, Aw, and sugar content. Thus, it would become a useful tool available to staff not extensively trained in microscopic observation or in organoleptic skills, areas where results are considerably influenced by the operator's behavior.

LITERATURE CITED

Accorti, M.; Persano Oddo, L.; Piazza, M. G.; Sabatini, A. G.; Schede di classificazione delle principali qualità di miele italiano. *Apicaltura* **1986**, *2*, appendice.

Adams, R. F. Determination of amino acid profiles in biological samples by capillary gas chromatography. *J. Chromatogr.* **1974**, *95*, 189−212.

Bertacco, G, Boschelle, O.; Lercker, G. Gas chromatographic determination of free amino acids in cheese. *Milchwissenschaft* **1992**, *47*, 348−350.

Bosi, G.; Battaglini, M. Gas chromatographic analysis of free and protein amino acids in some unifloral honeys. *J. Apic. Res.* **1978**, *17*, 156−166.

Brucker, H.; Hausch, M. Detection of free D-amino acids in food by chirasil phase capillary gas chromatography. *J. High Resolut. Chromatogr.* **1989**, *12*, 680−684.

Curti, R.; Riganti, V. Ricerche sugli ammino acidi liberi del miele. *Rassegna Chimica* **1966**, *6*, 278−282.

Davies, A. M. C. Amino acid analysis of honeys from eleven countries. *J. Apic. Res.* **1975**, *14* (1), 23−89.

Davies, A. M. C. The application of amino acid analysis to the determination of geographycal origin of honey. *J. Food Technol.* **1976**, *11*, 515−523.

Davies, A. M. C. Proline in honey: an osmoregulatory hypothesis. *J. Apic. Res.* **1978**, *17*, 227−233.

Davies, A. M. C.; Harris, R. G. Free amino acid analysis of honeys from England and Wales: application to the determination of the geographical origin of honeys. *J. Apic. Res.* **1982**, *21*, 168−173.

De Ming, Liu. Capillary gas chromatographic analysis of amino acids in geological environments with electron capture detection. *J. High Resolut. Chromatogr.* **1989**, *12*, 239−243.

Fontanarrosa, M. E.; Vigil, J. B. Determinacion cualitativa de amionacidos en meles por cromatografia. *Rev. Fac. Ing. Quim.* **1982**, *45*, 73−80.

Gilliam, M.; Mc Caughey; Wintermute, B. Amino acids in pollens and nectars of citrus cultivars and in stored pollen and honey from honeybee colonies in citrus groves. *J. Apic. Res.* **1980**, *19*, 64−72.

Guidetti, G.; Piro, R.; Capolongo, F.; Baggio; Mutinelli, F. Analisi statistica discriminante dei parametri chimico-fisici per la classificazione dei mieli monoflorali. Proceedings of "2° Congresso Nazionale di Chimica degli Alimenti", *Giardini Naxos*, 28−30 Maggio, 1995.

Kendal, M. G.; Stuart, A. *The advanced theory of statistics*; N. Y. Publishing & Co.: New York, 1967; 2nd ed., Vol. I.

Mardia, K. V.; Kent, T.; Bibby, M. *Multivariate analysis*; Academic Press: New York, 1979.

McGill, R.; Tukey, J. W.; Larsen, W. A. Variation of Box plots, *Am. Stat.* **1978**, *32*, 12−16.

McKenzie, S. L.; Tenaschuck, D. Gas−liquid chromatography of N-heptafluoributyril isobutyl esters of amino acids. *J. Chromatogr.* **1974**, *97*, 19−24.

Monastero, E.; Deutsch, W.; Ellero, M. Caratterizzazione di albicocche con il contributo dell'analisi degli ammino acidi. *Ind. Aliment.* **1991**, *27*, 1077−1084.

Persano Oddo, L.; Stefanini, R.; Piazza, M. G.; Accorti, M. Diagnosis of Italian unifloral honeys. *Apicoltura* **1988**, *4*, 27−38.

Persano Oddo, L.; Baldi, E.; Piazza, M. G. Acidità e pH nei principali mieli uniflorali italiani. *Apicoltura* **1986**, *2*, 145−154.

Pirini, A.; Conte, L. S.; Francioso, O.; Lercker, G. Capillary gas chromatographic determination of free amino acids in honeys as a means of discriminating between different botanical sources. *J. High Resolut. Chromatogr.* **1992**, *15*, 165 −170.

Ricciardelli, D'Albore G.; Persano Oddo, L. Flora Apistica, Istituto Sperimentale per la Zoologia Agraria, Firenze, 1981.

Ricciarelli D'Albore G.; Tonini D' Ambrosio M. In "Annali Istituto Sperimentale per la Zoologia Agraria, Rome, 1979.

Speer, K.; Montag, A. Verteilung freier Aminosären in Honigen unter besonderer Berücksichtigung der deutschen un der französischen Heidehonige. *Dtsch. Lebensm.-Rundsch.* **1986**, *82* (8), 248−253.

Tukey, J. W. *Exploratory data analysis*; Addison-Wesley: Reading, MA, 1977.

Vasconcelos, A. M. P.; Chaves das Neves, H. J. HRGC of wine free amino acids as a tool for elementary wine characterization. *J. High Resolut. Chromatogr.* **1990**, *13*, 494−498.

Wilkinson, L. SYSTAT. The system for Statistics, Evanston, IL, SYSTAT Inc., 1990.

Zironi, R.; Buiatti, S.; Dosualdo, D.; Baroncini, P.; Guidotti, C.; Stefani, R. Determinazione automatica degli zuccheri riduttori nei mosti e nei vini con elettrodo Pt/redox. *Ind. Bevande* **1989**, *18*, 513−517.

Received for review September 25, 1997. Revised manuscript received January 28, 1998. Accepted February 19, 1998. This work was carried out by a grant from the National Researches Council (CNR) of Italy (Grant 94.01018.CT06).

JF970837M