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Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data

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Abstract

Forty-six artisanal honey samples, from different places of Madrid province (Central Spain), were characterized on the basis of their melissopalynological, physico-chemical and volatile composition data. Results were submitted to principal component analysis and stepwise discriminant analysis in order to evaluate the existence of data patterns and the possibility of differentiation of Madrid honey samples according to their botanical source (honeydew, nectar) and geographical collection place (mountain, plain). Colour, electrical conductivity, acidity, ash content and pH were the physicochemical parameters with higher discrimination power in the differentiation of nectar and honeydew honeys while, among the volatile components, concentrations of borneol, 1-(2-furanyl)-ethanone and 3-hydroxy-2-butanone were the most discriminant variables. In the differentiation of honey samples from mountain and plain zones, 2,3-butanediol and 1-(2-furanyl)-ethanone were the most significant volatiles, while physicochemical data were not useful for distinguishing between collection places. The honeydew percentage in a honey sample (*HD*%) was estimated from physicochemical measures and also from volatile concentrations; 2,3-butanediol, 3-hydroxy-2-butanone, 1-hydroxy-2-propanone and 1-(2-furanyl)-ethanone were found to be related to *HD*%.

Keywords: Honey; Geographical characterization; Melissopalynology; Physico-chemical parameters; Volatile composition; Multivariate statistical analysis

1. Introduction

Madrid (Central Spain) includes several rural areas, where artisanal honey production is an important economic activity. It is also a densely populated zone, with many consumers potentially interested in local high quality honeys, artisanally produced and with specific characteristics. Several honey types are produced, due to the wide variety of botanical sources associated with the markedly different climatological and orographic conditions which can be observed in Madrid rural areas, in spite of its relatively reduced surface (Guadalix & de Lorenzo, 2002). While mountain zones (>900 m altitude) are mainly covered by shrubs and trees, *Pinus* and *Quercus* sp. being the most representative, nectar-producing plants (e.g. *Rosmarinus officinalis*, *Thymus* sp., *Echium* sp., *Rosaceae* shrubs) are more abundant in the plain zones. The scenario offered by these different natural conditions in Madrid made it interesting to determine what different compounds from particular botanical sources and/or physiographic areas could be found.

Honey characterization is based on the determination of its chemical, physical or biological properties. Several studies have attempted to establish suitable ranges of some of these properties for honeys from the same botanical source by using different techniques.

Melissopalynology has been traditionally used for ascertaining the botanical origin of honey (Louveaux, Maurizio, & Vorwohl, 1978; Louveaux & Vergeron, 1964; Maurizio, 1975; Pourtallier & Taliercio, 1970) and remains nowadays as the reference method, in spite of

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several disadvantages: counting, identification and interpretation of pollen analysis data are tedious, difficult and require trained analysts. Since honey can only be derived from a single botanical source with difficulty, the term ''unifloral honey'' is used to describe honey mostly produced from one plant species, which generally represents more than 45% of the total pollen (P) content (Maurizio, 1975). This percentage is not valid for over- or under-represented pollen plants; e.g., rosemary, lavender and citrus honeys are considered to be unifloral when only 10–20% of pollen from these species is present (Pérez-Arquillué, Conchello, Ariño, Juan, & Herrera, 1994; Serra Bonvehí & Ventura Coll, 1993, 1995). On the other hand, honeydew honeys are often microscopically characterized by the presence of honeydew elements (HDE), such as microalgae, fungal mycelia and spores. A ratio of HDE/P higher than 3 is generally required to establish a honey sample as honeydew honey (Louveaux et al., 1978). However, this parameter fails in its application to some honeydew honeys, such as those from *Quercus sp*. (Serra Bonvehí, Gómez Pajuelo, & Gonell Galindo, 1987).

When compared to nectar honeys, honeydew honeys are generally distinguished on the basis of their higher values of pH, acidity, ash, electrical conductivity and darker colour, as well as by a lower monosaccharide and a higher di- and trisaccharide content (Mateo & Bosch-Reig, 1997, 1998; Mateo, Jiménez, & Bosch-Reig, 1992; Mateo & Bosch-Reig, 1997, 1998).

Several studies have been published on the use of physicochemical parameters and mineral content data for the characterization of honeys from several Spanish production areas, such as Galicia (North-West) (Latorre, Peña, García, & Herrero, 2000; Latorre, Peña, Pita, Botana, García, & Herrero, 1999; Peña & Herrero, 1993); La Rioja and Basque Country (North) (Sancho, Muniategui, Huidobro, & Simal, 1991a; Sanz, Pérez, Herrera, Sanz, & Juan, 1995a); Salamanca, Zamora and Cáceres (West) (Gómez Bárez, García-Villanova, Elvira García, Rivas Palá, González Paramás, & Sánchez Sánchez, 2000; González Paramás, Gómez Bárez, García-Villanova, Rivas Palá, Ardanuy Albajar, & Sánchez Sánchez, 2000).

Characterization of Spanish honeys of different floral type (e.g. rosemary, lavender, willow, thyme) has also been done by using physicochemical parameters (Mateo et al., 1992; Pérez-Arquillué et al., 1994; Pérez-Arquillué, Conchello, Ariño, Juan, & Herrera, 1995; Serra Bonvehí et al., 1987; Serra Bonvehí, & Ventura Coll, 1995) and sugar composition (Mateo & Bosch-Reig, 1998; Serra Bonvehí & Ventura Coll, 1995) data. However, dispersion and overlapping of these variables for samples from different floral nectar, reduce their usefulness for honey source classification.

Estimation of honey quality by consumers depends markedly on its aroma, which in turn is related to its volatile composition. Qualitative and quantitative determination of honey volatiles is usually carried out by GC–MS, but a previous fractionation step is always necessary. Several studies have been reported on different techniques for fractionation of honey volatiles; such as solvent extraction (Bicchi, Belliardo, & Frattini, 1983; Bonaga, Giumanini, & Gliozzi, 1986; D'Arcy, Rintoul, Rowland, & Blackman, 1997; Graddon, Morrison, & Smith, 1979; Rowland, Blackman, D'Arcy, & Rintoul, 1995), simultaneous steam distillation-extraction (SDE) (Bicchi et al., 1983; Bouseta & Collin, 1995) and purge and trap (Bouseta, Collin, & Dufour, 1992; Overton & Manura, 1994).

Solid phase microextraction (SPME), followed by GC–MS has recently proved to be useful for extraction of the volatile fraction from different honeys (Guidotti & Vitali, 1998; Pérez, Sánchez-Brunete, Calvo, & Tadeo, 2002; Soria, Martínez-Castro, & Sanz, 2003; Verzera, Campisi, Zappalà, & Bonaccorsi, 2001). However, the common presence, at different concentrations, of most of the volatiles fractionated, in honeys of different type, limits the use of marker volatiles for honey characterization. SPME has also been assayed for estimation of the honeydew ratio in a honey blend (Soria, González, de Lorenzo, Martínez-Castro, & Sanz, submitted for publication).

Taking into account that honey is a complex natural food obtained under conditions which are difficult to control, an unequivocal characterization of honey samples requires the use of most of the previously described parameters. In this case, multivariate statistical analysis can be applied for finding trends or correlations among the characterization data, or for establishing the combinations of parameters which are highly related to the objective pretended by the honey characterization.

The objective of this work is the characterization of artisanal honeys from Madrid province in Central Spain, by using data obtained from melissopalynological, physicochemical and volatile analysis. The presence of possible correlations among these values and their relationship to honey source (nectar or honeydew) and to collection zone in Madrid (mountain or plain) have also been evaluated by multivariate statistical analysis.

2. Materials and methods

2.1. Honey sampling

This study was carried out on 46 artisanal honey samples collected in Madrid province (Central Spain) during the 2001 season. The sampling area (see columns 1–2 in Table 1) covered the most important artisanal production zones in this province. Honeys were stored at room temperature for less than 6 months until analysis.

Table 1
Collection place (M: mountain and P: plain), main pollen contributions in melissopalynological analysis, source classification (N: nectar, H: honeydew and B: blend) and honeydew percentage (*HD%*) estimated according to expression 1 from honey samples collected in Madrid province

Honey sample	Collection place	Melissopalynological analysis	Source classification	*HD* %
H1	M	Honeydew, *Rosaceae*	H	70.8
H2	M	Honeydew, *Rosaceae*	H	85.8
H3	M	Honeydew, *Rosaceae*, heather	H	82.2
H4	P	*Rosaceae*	N	40.3
H5	P	Multiflower	N	48.8
H6	M	Multiflower, honeydew	B	51.3
H7	M	Multiflower, honeydew	B	60.0
H8	M	Heather, *Rosaceae*, honeydew	B	64.6
H9	P	*Rosaceae*	N	31.9
H10	P	Multiflower	N	25.4
H11	P	*Rosaceae*, honeydew	B	63.3
H12	M	*Rosaceae*	N	54.0
H13	P	Honeydew, *Rosaceae*	H	87.2
H14	P	*Rosaceae*, eucalyptus	N	31.0
H15	P	Echium	N	33.9
H16	P	*Rosaceae*, honeydew	B	60.3
H17	M	Multiflower, honeydew	B	48.6
H18	M	Multiflower	N	7.5
H19	M	Honeydew, *Rosaceae*	H	66.7
H20	M	Honeydew, *Rosaceae*	H	71.4
H21	M	Honeydew, heather, rhododendron, dandelion	H	100
H22	P	Multiflower	N	6.2
H23	M	Honeydew, heather	H	78.1
H24	M	Honeydew, *Rosaceae*	H	96.7
H25	M	Honeydew, *Rosaceae*	H	94.9
H26	M	Honeydew, multiflower	H	88.0
H27	M/P	*Labiatae*	N	6.4
H28	M	Honeydew, *Rosaceae*	H	96.5
H29	M	Multiflower	N	39.7
H30	P	Eucalyptus, honeydew	B	72.1
H31	M	Honeydew, *Rosaceae*	H	98.1
H32	M	Heather, *Labiatae*, honeydew	B	68.0
H33	M/P	*Echium*, *Labiatae*	N	42.6
H34	P	*Rosmarinus*	N	−3.9
H35	M	Multiflower, honeydew	B	85.8
H36	M	Multiflower, honeydew	B	45.5
H37	M	Honeydew	H	65.8
H38	M	Multiflower, honeydew	B	37.4
H39	M	Rosmarinus	N	21.4
H40	M	Honeydew, *Labiatae*	H	71.4
H41	M	Multiflower	N	36.7
H42	M	Multiflower	N	40.2
H43	P	Multiflower	N	−0.02
H44	P	*Rosaceae*, honeydew	B	66.2
H45	P	Honeydew	H	77.9
H46	P	Multiflower, honeydew	B	52.4

2.2. Standard compounds

Mixtures of standard compounds of analytical GC grade were used for retention index calculations and confirmation of tentative identifications.

2.3. Melissopalynological analysis

Melissopalynology was essentially performed according to Louveaux et al. (1978), using the non-acetolytic method. The modifications proposed by Terradillos, Muniategui, Sancho, and Simal-Lozano (1994) were tested and successfully adopted. For a precise identification of palynomorphs, reference pollens were collected in Madrid through spring-summer. Microscopical examination was carried out in a Leica DMR light microscope fitted to a digital camera and coupled to an Image Analyser system (Leica Qwin Standard software) for morphometry of pollen grains. Two independent slides were prepared for each sample, and 400 pollen grains were identified on each slide. Pollen dominance classes were established acoording to Zander (1951). The number and type of honeydew elements (mainly fungal spores and mycelia) were also recorded. However, honeydew honeys derived from *Quercus* sp., such as those from Madrid, are reported to be extremely low in honeydew elements (Ricciardelli D'Albore, 1998) and the classic index HDE/P for estimation of honeydew contribution to honey is not completely suitable. Quantitation of *Quercus* sp. pollen grains was also taken into account as an additional indicator of the presence of honeydew honey.

2.4. Physicochemical analysis

The physicochemical analysis of artisanal honey samples consisted of the following basic determinations, which were carried out in triplicate according to the Spanish Official Methods (B.O.E., 1986): pH, free, lactonic and total acidities, in a 10 g 75 ml^{-1} honey solution in deionized H_2O; water content, by refractive index measurement and correlation with Chataway Charts (B.O.E., 1986), ash content, by calcination at 550 °C until constant weight is reached, and electrical conductivity in two different solutions: (1) the official 20% solution of honey (dry weight) in deionized water and (2) the same solution used for acidity measurements (Sancho, Muniategui, Huidobro, & Simal, 1991b).

The assessment of honey freshness involved the determination of hydroxymethylfurfural (HMF), diastase number and activity of the enzymes α-glucosidase and β-glucosidase. HMF and diastase number were spectrophotometrically assessed according to the Spanish Official Methods (B.O.E., 1986); α-glucosidase and β-glucosidase were assayed according to Siegenthaler (1977) and Low, Va Vong, and Sporns (1986), respectively. For this last assay, a 0.1 M citrate/phosphate buffer was used to reach pH 4.2.

Determination of glucose and fructose in honey samples was carried out by HPLC, using a REZEX-Monosaccharide precolumn and column at 90 °C, with

deionized H_2O as eluent (1.0 ml min^{-1} flow rate) and using refractive index for detection.

Two different textural tests (force in compression and adherence) were designed and carried out with a Texture Analyser TA.XT2 texturometer (Stable Micro-Systems, U.K.) using a backward extrusion cell and probe. In the first case, the probe pushed the honey over 20 mm, causing extrusion of the sample, and then returned to its original position. The parameters measured were defined as firmness (maximum positive strength), cohesiveness (maximum negative strength), consistence (area of positive curve) and viscosity (area of negative curve). For the adherence test, the probe was pushed over the honey surface for 2 s with a 6 g strength, and then it ascended at 8 mm s^{-1}. Adherence was defined as the maximum force needed for complete separation between the probe and the honey sample. In both cases, the operation parameters of the texturometer (e.g. force over the honey sample, probe velocities) were experimentally optimised.

Colour parameters were assessed by two methodologies: UV–Vis spectrophotometry and CIE-$L^*a^*b^*$ colorimetry. Spectrophotometry involved transmitance measurements at 445, 495, 550, 625 nm and mathematical combinations of these to obtain x and y coordinates in the chromaticity diagram, turbidity, measured as absorbance at 720 nm, and net absorbance, which involved absorbance at 560 and 720 nm. All these determinations were obtained according to Huidobro and Simal (1984). Total polyphenols were measured with the Folin-Ciocalteau reagent (Rentschler & Tanner, 1976) by absorbance at 670 nm and using gallic acid as standard. CIE-$L^*a^*b^*$ and x and y coordinates were obtained by means of a Minolta CR-200 triestimulus colorimeter, using cylindrical cuvettes (diameter 5 cm; height 3 cm) made out from optic glass, a D65 iluminant and a standard observant of 2°.

2.5. Solid phase microextraction (SPME)

Fractionation of volatiles from honey headspace was carried out by using a manual SPME holder (Supelco, Bellefonte, PA) equipped with two different fibre coatings: 75 μm Carboxen™-Polydimethylsiloxane and 85 μm Polyacrylate (both from Supelco, Bellefonte, PA). Fibres desorbed at the GC–MS injection port, at the manufacturer recommended conditioning temperature, were tested for volatile artifacts before honey analysis. Experimental procedure was as previously described by Soria et al. (2003): 1.5-2.0 g of honey were dissolved until complete homogenization in 1 ml of milli-Q water and sonicated for 5 min. After an equilibrium time of 15 min, at 60 °C, fibre was exposed to honey headspace for 30 min. Agitation was used throughout the fractionation process in order to improve the extraction efficiency.

2.6. Gas chromatography–mass spectrometry

GC–MS analyses were performed on a Hewlett-Packard 5890 (Palo Alto, CA, USA) gas chromatograph coupled to a Hewlett-Packard 5971 quadrupole mass detector.

The SPME fibre was desorbed at 250 °C for 2 min, in splitless mode, and chromatographic separation was carried out on a 50 m×0.20 mm×0.20 μm film thickness polyethyleneglycol capillary column (HP-Innowax, Agilent Technologies, USA). The oven was temperature programmed from 45 °C (2 min) to 190 °C (50 min) at 4 °C min^{-1}. He at ~1 ml $min.^{-1}$ was used as carrier gas.

Mass spectra were recorded in EI mode at 70 eV, scanning the 35–450 m/z range. Interface and source temperature were 280 and 230 °C, respectively.

Qualitative analysis was based on comparison of the obtained spectra with those of the Wiley mass spectral library (Wiley, 1989) and with published data, and was confirmed, when possible, by using retention indices (RI). As chromatographic separation included isothermal and temperature programmed steps, RI values were interpolated using a polynomic fit of isothermal RI data for standard compounds vs. their experimental retention times. Available standard compounds were also used for further confirmation.

Relative quantitative values (percentage of total volatile composition) were directly obtained from total ion current (TIC) peak area, using the average value of two replicates.

2.7. Statistical analysis

The BMDP statistical package (BMDP, 1992) was used for the multivariate statistical analysis of quantitative data. The programmes Principal Component Analysis (4M), Stepwise Discriminant Analysis (7M) and Multiple Linear Regression in the stepwise mode (2R) were used.

3. Results and discussion

3.1. Melissopalynological analysis

Main pollen contributions for the studied honeys appear listed according to their importance in the third column of Table 1. Many honey samples were predominantly *Quercus sp*. honeydew honeys with different contributions from *Rosaceae* or multiflower pollen. Nectar honeys were mainly classified as *Rosaceae* or as multiflower, the most important pollen contributors being in the last case *Echium*, *Rosaceae* and *Labiatae* species.

Melissopalynological analysis of honey samples showed a wide variability, with samples from different

honey sources (nectar or honeydew) being collected at neighbouring places. Column 4 in Table 1 lists the assignation of the samples under study to honeydew (H), nectar (N) or blend (B) types, based on data from their melissopalynological analysis. Results from physicochemical analysis were also considered as a complementary criterion.

These variable palynological characteristics are caused by the different nectar and honeydew plants which grow in Madrid province. In the high altitude honey-producing areas, honeydew sources, such as *Quercus* sp., are the most important. Nectar sources, such as *Rosaceae*, which grow up to 1400 m, are more frequent in the 900–1200 m zone. *Labiatae* (*Rosmarinus*, *Thymus* and *Lavandula* sp.) and *Echium* sp. are more abundant between 600 and 900 m, but even in some of these areas there is an important presence of *Quercus* sp.

3.2. Physicochemical analysis

Table 2 lists the minimum and maximum values obtained for each of the physicochemical variables (average of three determinations) previously described under Section 2 for the 46 honey samples studied. The wide range observed for most of them agree with the variability of honey sources observed in Table 1 for honey samples produced in Madrid province.

Table 2
Range for average ($n = 3$ replicates per sample) physicochemical data

Physicochemical parameter	Minimum	Maximum
pH	3.63	5.01
Free acidity (meq kg^{-1})	13.1	51.2
Lactonic acidity (meq kg^{-1})	0.00	13.9
Total acidity (meq kg^{-1})	14.5	59.6
Water content (%)	13.0	18.7
Ash content (%)	0.003	0.990
Electrical conductivity 20% (S cm^{-1} 10^{-4})	0.119	1.515
Electrical conductivity 10g 75mL^{-1} (S cm^{-1} 10^{-4})	0.117	1.116
HMF (mg l^{-1})	0.00	15.65
Diastase number (° Gothe)	10.17	63.7
α-glucosidase (U kg^{-1} min $^{-1}$)	45.3	273
β-glucosidase (U kg^{-1} min $^{-1}$)	24.2	97.1
Glucose (%)	19.3	31.2
Fructose (%)	23.2	39.9
Fructose + Glucose (%)	42.5	71.1
Fructose/Glucose	1.13	1.36
Glucose/water content	1.14	2.10
Firmness (g)	24	213
Cohesiveness (g)	8	98
Consistence (arbitrary units)	407	2938
Viscosity (arbitrary units)	68	1786
Adherence (g)	23	264
x spectrophotometry	0.347	0.558
y spectrophotometry	0.362	0.462
Turbidity	0.061	2.691
Net Absorbance	0.053	0.706
Polyphenols (mg g^{-1})	0.23	1.49
L^*	23.24	33.66
a^*	−2.19	2.32
b^*	1.24	9.96
x colorimetry	0.311	0.358
y colorimetry	0.336	0.376

Even honeys considered as predominantly "nectar" or "honeydew", on the basis of their melissopalynological analysis, could be, to different extents, honey blends. According to Soria et al. (submitted for publication), a simple expression [Eq. (1)] based on the electrical conductivity, 10 g 75 ml^{-1} (EC_{1075}), and on the percentage of fructose plus glucose (%FG) was applied for the estimation of the percentage of honeydew (HD%) in the 46 honey samples now studied. Results obtained are listed in column 5 of Table 1. Most of the honey samples grouped as honeydew or nectar (see column 4 in Table 1) from their melissopalynological analysis were characterized by HD% values higher than 70% and lower than 45%, respectively, proving the usefulness of Eq. (1) for the estimation of honey source. Intermediate HD% results were obtained for honey blends.

$$HD\% = 104.6\ EC_{1075} - 1.353\%FG + 65.47 \quad (1)$$

A data matrix containing the physicochemical parameter data was submitted to principal component analysis (PCA) in order to show possible trends in their values. The first component explained 39.3% of data variance and was positively related to colour (x spectrophotometry, net absorbance and polyphenols), both electrical conductivities, free acidity and ash content. Most of the parameters which presented positive contributions to F1 have been previously described as related to honey source (nectar or honeydew) (Mateo & Bosch-Reig, 1997, 1998; Mateo et al., 1992; Serra Bonvehí et al., 1987). b^* and y colorimetry were the main negative contributions to F1.

Fig. 1 plots, using as coordinate axes the first (F1) and second (F2) principal components, the honey samples

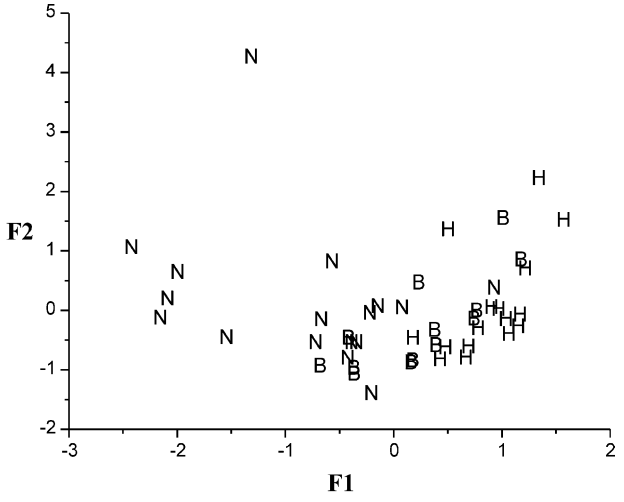

Fig. 1. PCA plot for physicochemical analysis data of Madrid province honeys.

palynologically characterized as nectar (N), honeydew (H) and honey blends (B). Although no clear division into groups was found, F1 was clearly related to honey source: honeydew honeys presented high F1 scores (reflecting dark colour and high conductivity, free acidity and ash content values) and nectar honeys were plotted at low F1 values, while honey blends were dispersed through the centre of the plot. No pattern related to nectar source was observed when considering higher principal components.

Stepwise discriminant analysis (SDA) was also applied to physicochemical data in order to show which parameters were more clearly associated with the honeydew and nectar groups obtained by melissopalynological analysis (column 4 in Table 1). The value of the F statistic obtained for each variable in the first step of SDA is a measure of the variable discriminant power and of its relative importance in the differentiation into groups, which is being sought.

When the group of sixteen honeydew honeys ("H" in Table 1) was discriminated from the other honeys, ash content, electrical conductivities and net absorbance presented F values higher than 30, indicating a high discrimination power; these four parameters showed higher mean values in honeydew samples. The joint use of EC_{1075}, lactonic acidity and y spectrophotometry correctly classified 94% of the samples. The seventeen samples classified by their pollen content as nectar honeys ("N" in Table 1) were characterized by low values of the four parameters previously mentioned, in addition to pH, their F values being around 70. Only one of the 46 samples was incorrectly classified when using pH, electrical conductivity (20%) and HMF content.

Physicochemical parameter measures could also be related to the different botanical and climatological characteristics of the honey collection places, but no relationship was found in the PCA plots for samples from neighbouring places, and no clear trend appeared when considering the different mountain and plain areas (see column 2 in Table 1). Discriminant analysis was also applied to groups formed by the samples belonging to mountain and plain zones. The parameters with higher discrimination power were ash content, electrical conductivities and net absorbance, but their F values were only between 7 and 12. The joint use of 2–5 parameters allowed us to correctly classify only 80–85% of the samples.

3.3. Volatile composition

Two different fibre coatings, Carboxen™-Polydimethylsiloxane (C/PDMS) and Polyacrylate, were tested for SPME fractionation of honey volatiles. As total volatile amount fractionated by using C/PDMS fibre was higher, according to results previously reported by Soria et al. (2003), and C/PDMS fibre showed fewer interference peaks in blank runs after conditioning, it was selected for subsequent analyses.

Differences in TIC profiles were observed when comparing honey samples from different sources and collection places [Fig. 2: nectar and honeydew honeys from North of Madrid (mountain); Fig. 3: nectar and honeydew honeys from South of Madrid (plain)]. Table 3 lists the volatile compounds identified by their GC retention and their mass spectral data in the honey samples collected through Madrid province during the 2001 season. Column 1 in this Table lists retention indices (RI) data obtained as described in Section 2.

Most of the compounds summarized in this Table were present in all honey samples, independently of the source or collection place considered, the differences in TIC profiles being mainly due to quantitative variations in their volatile composition.

Twenty-eight volatile compounds chosen among those shown in Table 3 were selected for characterization purposes because of their common presence in most of honey samples under study or their major concentration in at least one of them. Ranges for average ($n=2$ replicates per sample) percent concentrations of these volatile compounds are listed in Table 4.

Relative composition data for the 28 compounds in the 46 studied honey samples were submitted to PCA in

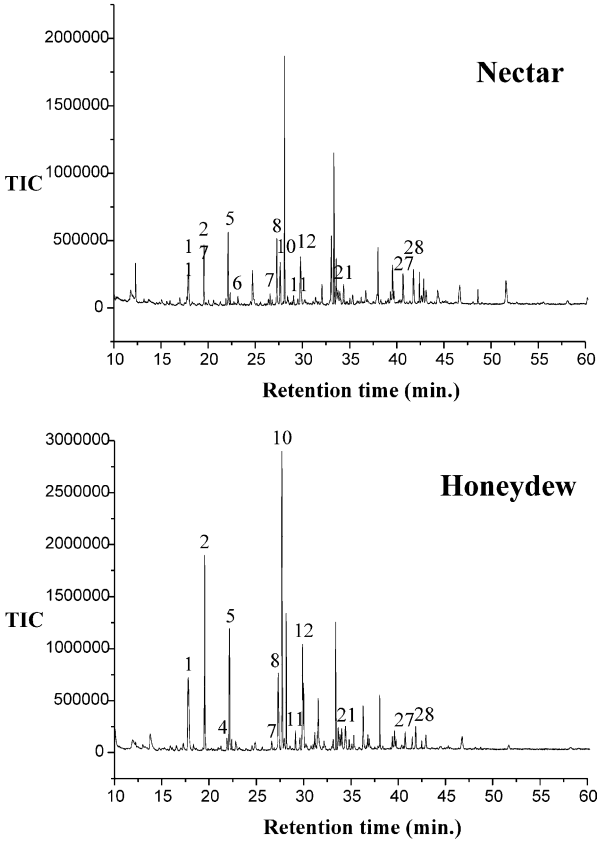

Fig. 2. TIC profiles for a nectar and a honeydew honey collected from mountain zones of Madrid province.

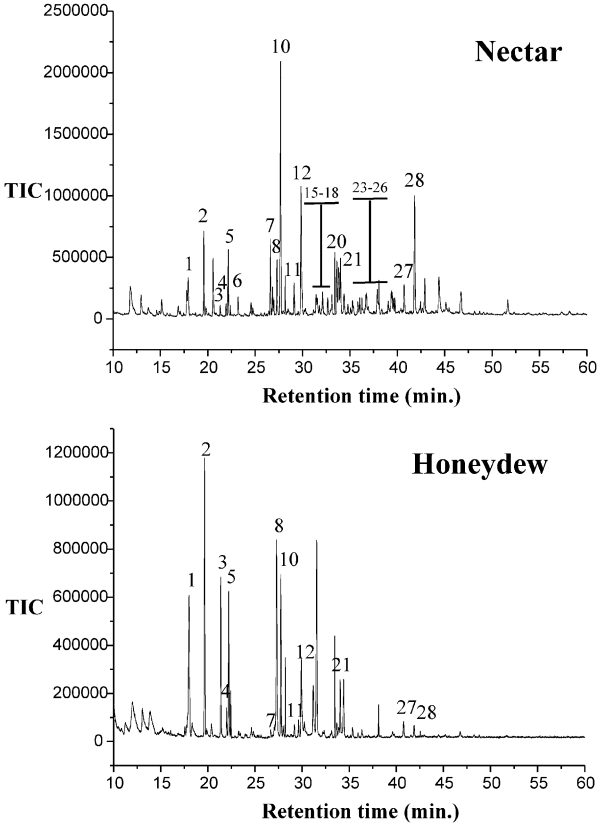

Fig. 3. TIC profiles for a nectar and a honeydew honey collected from plain zones of Madrid province.

Table 3
Volatile compounds identified in Madrid province honey samples collected during 2001 season

RI	Compound
963	2,3-Butanedione
1009	Decane
1020	Toluene
1054	Dimethyl disulfide
1084	2-methyl-2-butenal
1104	Ethylbenzene
1113	*p*-Xylene
1118	3-Hexen-2-one
1121	*m*-Xylene
1173	*o*-Xylene
1173	Limonene
1209	Pyridine
1201	2,3-Dihydro-4-methylfuran
1212	2-Methyl-1-butanol
1214	3-Methyl-1-butanol
1253	3-Methyl-3-buten-1-ol
1257	Styrene
1275	Dihydro-2-methyl-3(2H)-furanone
1276	3-Methylbutenenitrile
1276	Trimethylbenzene
1310	3-Hydroxy-2-butanone
1318	1-Hydroxy-2-propanone
1328	2- and 3-Methyl-2-buten-1-ol
1356	1-Hexanol
1372	Ethynylbenzene
1379	Not identified (45,57,82)
1390	Nonanal
1391	3-Hexen-1-ol
1397	1-Hydroxy-2-butanone
1398	Dimethyl trisulfide
1431	Tetramethylbenzene
1443	Tetramethylbenzene
1446	$C_{10}H_{12}$
1451	*cis*-Linalool oxide (furan)
1454	1-Heptanol
1471	Acetic acid
1483	*trans*-Linalool oxide (furan)
1483	Furfural
1487	Not identified (150,135,91,43,107,109,105)
1503	Tetramethylbenzene
1506	3,9-Epoxy-*p*-menthene
1524	1-(2-Furanyl)-ethanone
1536	Camphor
1545	Benzaldehyde
1548	Linalool
1548	2,3-Butanediol (*threo*-)
1549	Lilac aldehyde (isomer I)
1558	Propanoic acid
1564	Lilac aldehyde (isomer II)
1573	Lilac aldehyde (isomer III)
1584	2-Methylpropanoic acid
1587	2,3-Butanediol (*erythro*-)
1595	5-Methylfurfural
1596	Dimethyl sulfoxide
1598	Lilac aldehyde (isomer IV)
1614	Hotrienol
1615	Isophorone
1646	Butanoic acid
1662	1-Nonanol
1665	Dihydro-2(3H)-furanone
1668	Phenylacetaldehyde
1675	Furfuryl alcohol
1710	α-Terpineol
1717	4-Butyl-cyclohexen-3-one[a]
1717	Borneol
1731	Verbenone
1736	Lilac alcohol (isomer I)
1746	Phenylmethyl acetate
1753	Linalool oxide I (pyran)
1758	Lilac alcohol (isomer II)
1772	Linalool oxide II (pyran)
1801	Lilac alcohol (isomer III)
1841	β-Damascenone
1845	Lilac alcohol (isomer IV)
1869	Hexanoic acid
1908	Benzyl alcohol
1944	2-Phenylethanol
1957	$C_{10}H_{18}O$
1977	1-Dodecanol
2022	Furan-2,5-dicarbaldehyde[a]
2045	Phenol
2089	Octanoic acid
2211	Nonanoic acid
2236	Carvacrol
2498	Benzoic acid
2536	5-Hydroxymethylfurfural

[a] Compounds tentatively identified based on mass spectral data reported by Graddon et al. (1979).

Table 4
Percent data range for volatile composition of 46 Madrid honey samples collected during 2001 season

Compound	Minimum	Maximum
2-Methyl- and 3-methylbutanol	0.00	16.90
3-Methyl-3-buten-1-ol	1.32	27.30
3-hydroxy-2-butanone	0.00	19.90
1-Hydroxy-2-propanone	0.00	3.76
2-Methyl- and 3-methyl-2-buten-1-ol	0.00	14.00
1-Hexanol	0.00	4.47
cis-Linalool oxide (furanyl ring)	0.00	13.50
Acetic acid	2.62	53.90
trans-Linalool oxide (furanyl ring)	0.00	7.65
Furfural	0.98	44.00
1-(2-Furanyl)-ethanone	0.00	3.09
Benzaldehyde	0.00	26.90
2,3-Butanediol (*threo*-)	0.00	17.30
2,3-Butanediol (*erythro*-)	0.00	25.80
Lilac aldehyde (isomer I)	0.00	1.74
Lilac aldehyde (isomer II)	0.00	2.29
Lilac aldehyde (isomer III)	0.00	2.05
Lilac aldehyde (isomer IV)	0.00	2.33
Isophorone	0.00	11.50
Phenylacetaldehyde	0.00	18.20
Furfuryl alcohol	0.00	12.00
Borneol	0.00	7.63
Lilac alcohol (isomer I)	0.00	4.05
Lilac alcohol (isomer II)	0.00	4.97
Lilac alcohol (isomer III)	0.00	4.92
Lilac alcohol (isomer IV)	0.00	2.71
Benzyl alcohol	0.19	8.57
2-Phenylethanol	0.14	10.30

order to show possible trends related to honey source or to collection zone, but no clear pattern was found in the plots when using the most important principal components. Although the continuous distribution of the samples in the PCA plots indicated the difficulty of using the volatile composition in order to distinguish among groups of honeys from different sources or origins, stepwise discriminant analysis (SDA) was also applied to percent volatile data in order to find whether the relative concentrations of some volatile compounds (used as variables in SDA calculations) could be associated with these characteristics.

When stepwise discriminant analysis was applied to the volatile concentration data of honeys collected from mountain zones (see column 2 in Table 1), *threo*- ($F = 17.35$) and *erythro*-2,3-butanediol ($F = 10.98$) and 1-(2-furanyl)-ethanone ($F = 15.93$) were the most significant single variables in the discrimination of the 28 mountain honeys from the remaining honey samples under study, mountain honeys being characterized by higher contents of both butanediol isomers and by a lower concentration of 1-(2-furanyl)-ethanone. *Threo*-2,3-butanediol, *cis*-linalool oxide, furfural, 1-(2-furanyl)-ethanone and isophorone were the variables with the highest joint discriminant power in the separation of mountain honeys, but they allowed only 83% of the samples to be correctly classified.

It is worth noting that some furan derivatives, considered for discrimination purposes, are known to arise from honey heat-processing or from honey storage. Since the SPME operation conditions were the same for all the samples analysed, relative values obtained for these volatiles were also useful for comparison.

Threo-2,3-butanediol also presented a relatively high F value (13.53) in the differentiation of the sixteen honey samples from plain zones, this volatile compound usually being present at lower concentration in these samples, which are identified as "P" in column 2 of Table 1. However, only 83% of samples were correctly classified when using 2,3-butanediol (*threo*-), 3-hydroxy-2-butanone, lilac alcohol (isomer I), *trans*-linalool oxide and isophorone as variables in the SDA of honey samples from plain zones.

Sixteen samples were classified from their palynological analysis as honeydew honeys ("H", column 4 in Table 1). Borneol ($F = 14.10$), which was present at higher concentration in honeydew honeys, presented the highest discrimination power in SDA analysis. 1-(2-furanyl)-ethanone ($F = 13.37$) was found to be the most characteristic compound for the seventeen samples considered to be pure nectar honeys ("N", column 4 in Table 1). 3-hydroxy-2-butanone ($F = 11.69$) was present at lower concentrations in nectar samples. SDA was also applied to these groups, trying to find combinations of up to five variables able to classify most honey samples. However, only 70% of honeys from both sources could be correctly classified.

Since both physicochemical parameter measures and volatile composition data are supposed to depend on the honey sample characteristics, multiple linear regression in the stepwise mode (MLR) was used in order to find possible relationships among the two sets of parameter values; physicochemical parameter data were taken as dependent variables and volatile composition data were used as independent variables. The calculated F ratios in the first regression step, in a similar way to that described for SDA, are related to the extent to which independent variables explain the variation of dependent variables.

Calculated honeydew percentage (HD%) was also considered as a dependent variable since its values, listed in Table 1, were derived from physicochemical parameters. Relative concentrations of *threo*- ($F = 26.19$) and *erythro*-2,3-butanediol ($F = 17.25$) and 3-hydroxy-2-butanone ($F = 22.20$) were positively correlated with HD%, while 1-(2-furanyl)-ethanone ($F = 23.74$) was negatively associated with this parameter. When the concentration of 5 volatile compounds (*threo* - 2,3 - butanediol, 1 - hydroxy - 2 - propanone, 3 - hydroxy-2-butanone, borneol and furfuryl alcohol) was considered for the prediction of HD%, a linear regression coefficient, $r = 0.73$ and a standard error of estimation of 19.69, was achieved.

Polyphenol values showed a relatively high correlation with volatile data. Concentrations of *threo*- ($F = 79.78$) and *erythro*-2,3-butanediol ($F = 77.10$) were the most important variables, and were positively correlated. The concentrations of these compounds, usually higher in honeydew honeys, could be related to the darker colour (higher polyphenol content) of honey samples from this source. Both 2,3-butanediol isomers were also the most significant single variables in the prediction of other physicochemical parameters related to colour, such as net absorbance, b^*, x spectrophotometry, x and y colorimetry. Variables negatively correlated with polyphenols were 1-(2-furanyl)-ethanone ($F = 25.43$), 2-methyl- and 3-methyl-2-buten-1-ol ($F = 19.59$) and 3-methyl-3-buten-1-ol ($F = 18.25$). Stepwise multiple linear regression of polyphenols afforded a correlation coefficient of $r = 0.84$ when using up to five parameters (*threo*-2,3-butanediol, furfuryl alcohol, 1-(2-furanyl)-ethanone and 2-methyl-and 3-methylbutanol concentrations) as independent variables.

Erythro- and *threo*-2,3-butanediol (both positive), 3-hydroxy-2-butanone (positive), 1-hydroxy-2-propanone (positive) and 1-(2-furanyl)-ethanone (negative) were the most clearly correlated ($F > 15$) single volatile components in the calculation of physicochemical parameters such as free and total acidity, both electrical conductivities, pH and ash content, which have been described as related to honey source (nectar or honeydew).

Phenylacetaldehyde ($F = 110.73$) presented a strong positive association ($r = 0.74$) to honey turbidity. When considering up to five variables (phenylacetaldehyde, benzaldehyde, 1-(2-furanyl)-ethanone, 1-hydroxy-2-propanone and *trans*-linalool oxide) in the stepwise linear regression of turbidity, a correlation coefficient $r = 0.87$ was obtained.

In the prediction of rheological properties, both isomers of 2,3-butanediol were the most significant positive contributors, while 2-methyl- and 3-methylbutanol were negatively related to them.

Other physicochemical parameters presented lower correlations with volatile concentration data, both when these last parameters were considered as single variables or as multiple variables in stepwise linear regression.

A wide variability is a common characteristic of the results obtained for the 46 honey samples from Madrid in their melissopalynological analysis, physicochemical determinations and study of their quantitative volatile composition presented in Tables 1, 2 and 4, respectively. The comparative analysis of these results indicated that the main cause of their variability was the different honey source (honeydew or nectar). The existence of beekeeping zones with very different climatic characteristics seemed to present a smaller influence; however, they were reflected in the relative abundance of nectar- and honeydew-producing plants (e.g. most of the honeydew honeys were produced in mountain zones).

The common occurrence of honeydew-nectar blends was also an important characteristic shown in data from Table 1. Results from honey source classification, mainly based on melissopalynological analysis (see columns 3 and 4), agree with those of the *HD%* calculated from physicochemical measures (see column 5).

The broad range of variation of the volatile data summarized in Table 4 and graphically shown in the chromatographic profiles of Figs. 2 and 3, indicated their usefulness for honey sample characterization. Some of these compounds have been found to be related to honey source or geographical origin. Volatile compound concentrations can also be used in the estimation of *HD%*.

While melissopalynogical analysis remains nowadays as the only technique which allows a direct botanical source characterization, physicochemical parameters afford quantitative results and allow an approximate estimation of the presence of honey blends. Volatile data, besides their previously mentioned advantages in honey characterization, are related to honey aroma and hence to honey acceptance by the consumer.

No previous report of the characterization of Madrid honey samples has been publisged. Our approach, using the three types of parameters previously mentioned seems to be advisable e.g. for comparison with artisanal honey samples produced in other Spanish honeys, or with Madrid honeys produced in years with different climatic characteristics.

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