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A contribution to the differentiation between nectar honey and honeydew honey

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Abstract

Thirty genuine honey samples were analyzed for pH, acidity, water, ash, net absorbance, total polyphenols (Folin–Ciocalteau method) and glucose, fructose, melezitose and erlose (as their trimethylsilyl oximes and trimethylsilyl ethers) by capillary gas chromatography. The resulting data were used, along with palynological analysis, to characterize the samples in relation to their possible source (nectar, honeydew and mixture honeys).

Some minor components (carboxylic acids and cyclitols), eluting before monosaccharides, were also determined. One of these compounds was quercitol (1,3,4/2,5-cyclohexane-pentol), a deoxyinositol which has been previously determined in *Quercus sp*. samples. Quercitol was present in a broad concentration range (0.01–1.50 g/100 g) in honeys whose major source was honeydew but it was never higher than 0.01 g/100 g in samples characterized as nectar honeys. Quercitol concentrations appear to be related to the presence and amount of *Quercus sp.* honeydew as honey source, although further research is required to confirm this.

Keywords: Nectar honey; Honeydew honey; Quercitol; Characterization

1. Introduction

Floral honey is made by honeybees from the nectar of blossoms, while honeydew honey is prepared from secretions of living parts of plants or excretions of plant-sucking insects on the living part of plants. Differentiation between floral and honeydew honey is a response to consumer demands; in many countries nectar honey is valued more highly than honeydew honey but, in other countries, honeydew honey is preferred (Prodolliet & Hischenhuber, 1998).

Melissopalynological analysis contributes to honey differentiation (Prodolliet & Hischenhuber, 1998) but is a laborious task requiring skilled personnel. Several different physicochemical parameters have been used to characterize honeys. According to Community Directive 74/409/EEC, for some of these parameters, value ranges have been proposed as characteristic of each type of honey source. Proline content was considered by Biino (1971) as a good indicator of the origin of the honey, but recent studies have demonstrated that the variability observed for proline makes it almost impossible to characterize honey origin using this parameter (Sánchez, Huidobro, Mateo, Muniategui, & Sancho, 2001).

pH, acidity, ash content, color and electrical conductivity have been considered as useful characteristics for the differentiation of the two types of honey (Campos, della Modesta, da Silva, & Raslan, 2001). Kirkwood, Mitchell, and Smith (1960) employed the relationship between pH, ash and reducing sugars as an index to

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differentiate floral honeys from honeydew honeys. However, some studies on classification of honeys of different origins, carried out by Krauze and Zalewski (1991), using physicochemical parameters, showed that the division of nectar and honeydew honey was not clear.

Optical rotation is a characteristic of both nectar and honeydew honeys (White, 1980), related to their carbohydrate composition. Honeydew honeys present lower values of glucose and fructose and higher levels of oligosaccharides, mainly melezitose or erlose (Földházi, 1994); thus, carbohydrate profiles have been used to characterize both types of honey (Bogdanov & Baumann, 1988; Weston & Brocklebank, 1999). Nevertheless, a correct classification is not always possible using mono- and oligosaccharide concentrations.

Recently, several cyclitols have been detected in honey (Sanz, Sanz, & Martínez-Castro, 2004). Some of these compounds could probably serve as markers of botanical origin of honey since they have been found in diverse plant families (Anderson, 1972; Dittrich, Gietl, & Kandler, 1971). The aim of the present work was to assess whether these compounds could be used to distinguish between honeydew honeys and nectar honeys.

2. Materials and methods

2.1. Samples

Thirty honeys from different locations, collected through 1999 to 2001 were analyzed (Table 1): eight of them were high quality commercial products and twenty-two were artisanal products obtained directly from beekeepers.

2.2. Physicochemical analysis

The following determinations were carried out according to Spanish Official Methods of Analysis for Honey (1986): pH; free, lactonic and total acidities in a solution of 10 g of honey in 75 ml of water; water content (%) by refractive index measurement and correlation with Chataway Charts; ash by calcination at 550 °C to constant weight; electrical conductivity in two different solutions: (i) the official 20% dry weight and (ii) the same solution used for acidity measurements; net absorbance (involving absorbance at 560 and 720 nm) according to Huidobro and Simal (1984), and polyphenols (Folin–Ciocalteau method).

2.3. GC analysis

Glucose, fructose, melezitose and erlose were determined by GC, the first two monosaccharides as their trimethylsilyl oximes and the trisaccharides as trimethylsilyl ethers. Standard solutions, containing different proportions of each carbohydrate were prepared in order to obtain their FID response factor (RF) relative to phenyl-β-D-glucoside (internal standard) over the expected range. For non-identified peaks, the relative RF was assumed to be 1, since it was not possible to use effective carbon number (ECN) values as proposed by Scanlon and Willis (1985). Samples were prepared by diluting 0.5 g of honey to 25 ml with 80% ethanol; 1 ml of solution was mixed with 1 ml of phenyl-β-D-glucoside (1 mg/ml ethanol) and evaporated under vacuum; oximes were formed as previously described (Brobst & Lott, 1966). After reaction, samples were centrifuged at 7000*g* for 5 min at 5 °C (Li & Schumann, 1981). Gas chromatographic separation was carried out using a SPB-1 fused silica capillary column, 25 m × 0.25 mm i.d., 0.25 μm film thickness from Supelco (Bellefonte, PA) installed in a Perkin–Elmer Autosystem GC equipped with a flame ionisation detector (Perkin–Elmer, Norwalk, CT). The injector and detector temperatures were 300 °C. The oven temperature was held at 200 °C for 20 min, then programmed to 270 °C at a heating rate of 15 °C min^{-1}, to 290 °C at 1 °C min^{-1} and finally to 300 °C at 15 °C min^{-1}, where it was held for 40 min. Nitrogen was used as carrier gas, and injections were made in split mode, with a split flow of 40 ml/min. Chromatographic peaks were measured using a Chrom-Card 1.20 acquisition system (CE Instruments, Milan, Italy).

GC–MS analysis was performed using the same capillary column, installed in a HP5890 series with a

Table 1
Site of origin, date and number of honey samples studied

Region	Number of samples	Collection date	Type
Castilla-León	2	2000	Multifloral
Extremadura	2	2000	Honeydew and oak honeydew
Canary Islands	1	1999	*Retama sphaerocarpa*
Asturias	1	2000	*Leather + eucaliptus*
Castilla-La Mancha	1	2000	Multifloral
Madrid	20	2000 and 2001	Multifloral, *Echium* and honeydew
Spain (indeterminate location)	1	2000	Honeydew
Italy	2	2000 and 2001	Acacia

MD5971 quadrupole mass detector (both from Hewlett–Packard, Palo Alto, CA, USA) working in EI mode at 70 eV and using helium as carrier gas. Chromatographic conditions were the same as above.

2.4. Statistical analysis

Statistical analysis was carried out with the statistical package BMDP, using the BMDP 4M factorial analysis programme (BMDP Statistical Software, 1992).

3. Results and discussion

3.1. Physicochemical characterization

To distinguish between honey sources requires prior classification, but honey reference samples are not available. Palynological analyses were performed on artisanal samples, but these present problems in the case of honeys from mixed sources; also, some of the industrial samples may have been filtered, causing subsequent pollen loss. For these reasons, physicochemical analysis was preferred for honey characterization. Several parameters, which other authors have considered to be related to the honey source (Campos et al., 2001; Krauze & Zalewski, 1991; Mateo & Bosch-Reig, 1997), were determined for the studied samples; these are summarized in Table 2. The ranges found by us agreed with those proposed by the cited authors as typical of honeydew honeys and floral honeys.

Table 3 summarizes the glucose, fructose, erlose and melezitose contents of the honeys. Glucose+fructose content ranged from 54.1% to 83.6%. Samples presenting low monosaccharide contents generally had high concentrations of melezitose.

Principal component analysis (PCA) was run on a matrix including physicochemical data and sugar content. The first component, which explained 57.3% of variance, correlated positively with pH, free acidity, total acidity, electrical conductivity, ash, polyphenols and net absorbance and negatively with glucose, fructose and erlose. Glucose and fructose presented the highest positive contributions to the second component, which explained 14% of variance. Fig. 1 shows a plot of the 30 honeys on axes representing the first two principal components. Samples fell into two differing groups based on the first component scores. Group A comprised samples H1 to H13, whose first component scores were negative because of high glucose and fructose content and low values of acidity, electrical conductivity, polyphenols and net absorbance. These characteristics are usually associated with floral honeys. Samples H14 to H30 (group B), in which first component scores were positive, should correspond to honeydew honeys since they presented relatively low values for glucose and fructose and high values for melezitose, acidity, polyphenols and net absorbance. Although erlose has been considered a typical compound of certain honeydew honeys (Doner, 1977), the erlose content in our samples was rather variable and did not correlate well with the other parameters. The lack of clear groups in the PC plot in Fig. 1 could have been caused by the presence of honeys from mixed sources, which would present intermediate

Table 2
Mean values and range of chemical and physical parameters in 30 honey samples.

	Mean	Maximum	Minimum
pH	4.09	4.88	3.29
Free acidity (meq/kg)	34.0	53.5	11.2
Lactonic acidity (meq/kg)	3.91	11.83	0.00
Total acidity (meq/kg)	37.9	57.3	11.2
EC (10^{-4} S cm^{-1})	5.13	11.7	0.09
Ash (%)	0.29	0.73	0.00
Moisture (%)	16.22	18.70	13.00
Polyphenol (mg/kg honey)	0.78	1.98	0.21
Net absorbance ($A_{560} - A_{720}$)	0.36	0.99	0.03

Table 3
Glucose, fructose, melezitose and erlose composition of 30 honey samples (expressed as g/100 g of honey)

	Mean	Maximum	Minimum
Fructose (%)	35.8	45.2	29.2
Glucose (%)	29.7	38.0	22.3
Glucose + fructose (%)	65.5	80.0	51.5
Erlose (%)	0.55	2.48	0.15
Melezitose (%)	0.55	2.60	0.00

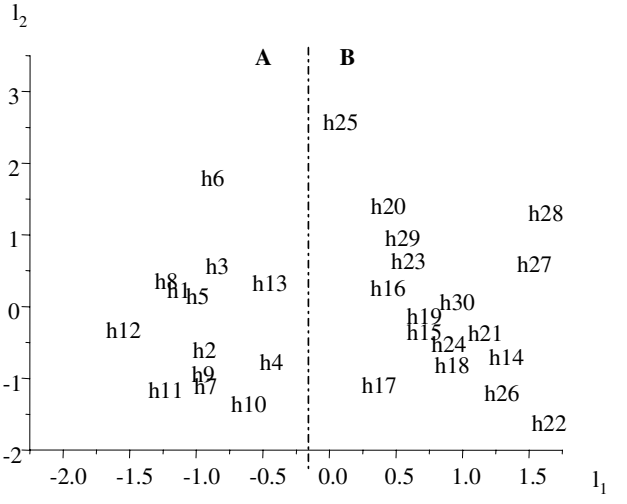

Fig. 1. Plot of principal components (l_2 vs l_1) of the 30 honey samples using 14 descriptors: pH, free acidity, lactonic acidity, total acidity, electrical conductivity, moisture, ash, polyphenols, net absorbance, glucose, fructose, glucose + fructose, erlose and melezitose.

characteristics (Soria, Gonzalez, de Lorenzo, Martinez-Castro, & Sanz, in press), and by natural dispersion in the physicochemical parameters of different honeys from the same source.

3.2. Classification based on minor components

Besides sugar derivatives, chromatograms used for carbohydrate determination showed several small peaks eluting before glucose and fructose. Fig. 2 shows the chromatographic profile of this zone in a floral honey (a) and in a honeydew honey (b). The sum of peak areas 1–11 was higher for samples H14 to H30 (group B in Fig. 1), which were presumed to be honeydew honeys from their physicochemical data. Some of those peaks were identified as *per*-TMS derivatives of some carboxylic acids (citric, quinic and gluconic) described in honeys by Horváth and Molnár-Perl (1998). Some peaks were TMS-cyclitols (quercitol, methyl-*muco*-inositol, *muco*-inositol and pinitol) which were recently identified in honey (Sanz et al., 2004); others could not be identified. Citric acid appeared as a small shoulder of the quercitol peak in some samples; however, the mass spectra of both compounds are clearly different, so that it was possible to distinguish them on the basis of ions at 273, 347 and 363. Table 4 shows the ranges and the average concentrations of quercitol and other minor compounds. Quercitol appeared in 20 samples, ranging from 0.003 to 1.5 g/100 g. This compound is a deoxyinositol (1,3,4/2,5-cyclohexane-pentol) called "acorn sugar"; it is a major component of carbohydrates in *Quercus* acorns, leaves and bark (all possible sources of honeydew) (Sanz et al., 2004). The genus *Quercus* is abundant in Spain; it is seen as representative of Mediterranean flora and its honeydew is present in many Spanish honeys classified as such. Concentrations of honeydew elements are sometimes low in *Quercus* honeydew (Ricciardelli D'Albore, 1998), which makes clear identification difficult. Quercitol seemed to be a good marker for *Quercus sp.* honeydew, since all samples in B group (Fig. 1) displayed this peak, ranging from 0.013 to 1.5 g/100 g. On the other hand, it was absent in most honeys

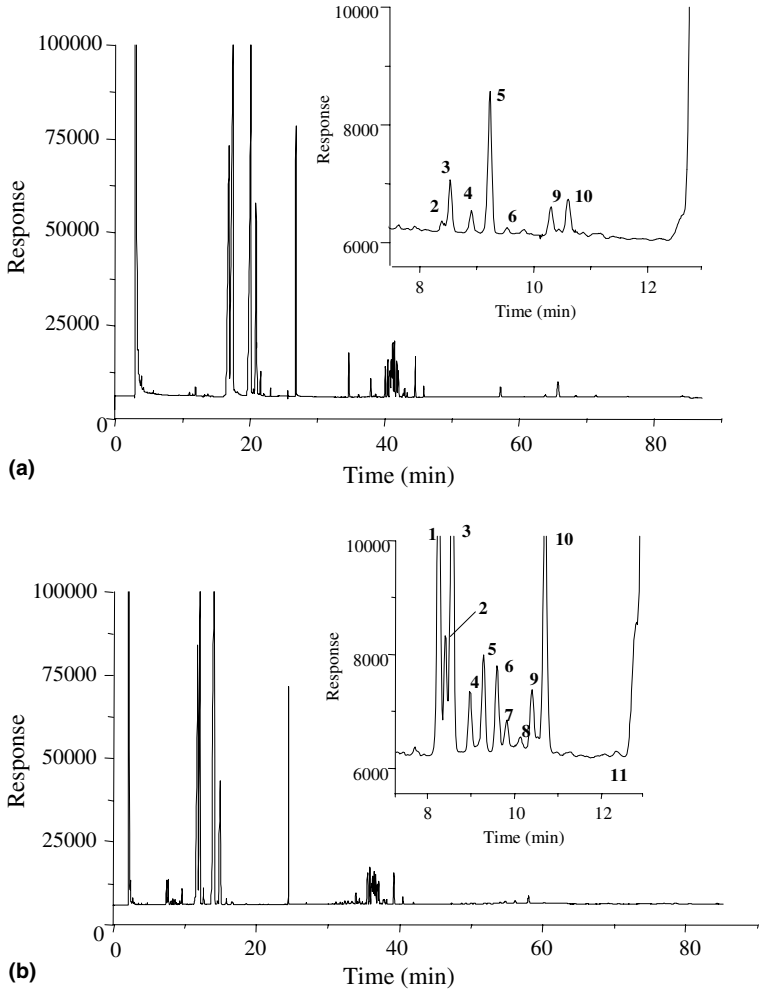

Fig. 2. Chromatogram profiles obtained by GC analysis of TMS oximes of honeydew honey (a) and nectar honey (b). 1: quercitol; 2,3,8, and 10: not identified; 4: methyl-*muco*-inositol; 5: pinitol; 6: artifact; 7: quinic acid; 9: gluconic acid; 11: *muco*-inositol.

Table 4
Carboxylic acids and cyclitols in honeys (expressed as g/100 g of honey)

Source		Quercitol	Methyl-*muco*-inositol	Pinitol	*muco*-Inositol	Quinic acid	Gluconic acid
Group A ($n = 13$)	Mean	0.001	0.015	0.096	0.013	0.014	0.086
	Minimum	0.000	0.000	0.000	0.000	0.000	0.019
	Maximum	0.010	0.066	0.425	0.149	0.080	0.212
Group B ($n = 17$)	Mean	0.325	0.124	0.327	0.006	0.055	0.118
	Minimum	0.013	0.000	0.000	0.000	0.000	0.056
	Maximum	1.519	0.327	0.833	0.018	0.201	0.224

classified as "floral" (A group in Fig. 1); it was detected at very low levels (0.003, 0.004 and 0.010 g/100 g) in only three of the thirteen samples in this group. Small amounts of honeydew honey were probably present in these floral honeys, but at too low a level to be detectable using either palynological or physicochemical analysis. These results are consistent with a previous study, in which many Spanish honeys were found to contain mixtures of nectar and honeydew (Soria et al., in press).

A classification based on the selected minor components is therefore consistent with the results of analysis of physicochemical parameters. A routine GC analysis of honey carbohydrates may allow the determination of acids (as stated by Horváth & Molnár-Perl (1998)) and cyclitols (Sanz et al., 2004). Quercitol therefore seems to be a good marker for *Quercus* honey. Determination as described above appears to be a fast, simple way to roughly estimate the relative amount of *Quercus* honeydew honey in a blend; however, a larger number of honeys would need to be analyzed to establish more reliable ranges and limits for this compound.

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References

Anderson, L. (1972). The cyclitols. In W. Pigman, & D. Horton (Eds.). *The carbohydrates, chemistry and biochemistry* (Vol. IA). London: Academic Press.

Biino, L. (1971). Amino acid analysis of two varieties of honey. *Rivista Italiana Essenze, Profumi, Piante Officianali, Aromi, Saponi, Cosmetici, 53*, 80–84.

BMDP Statistical Software Release 7. (1992). Los Angeles: University Press of California.

Bogdanov, S., & Baumann, E. (1988). Determination of sugar composition of honeys by HPLC. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene, 79*, 198–206.

Brobst, K. M., & Lott, C. E. (1966). Determination of some components in corn syrup by gas–liquid chromatography of trimethylsilyl derivatives. *Cereal Chemistry, 43*, 35–43.

Campos, G., della Modesta, R. C., da Silva, T. J. P., & Raslan, D. S. (2001). Comparison of some components between floral honey and honeydew honey. *Rivista do Instituto Adolfo Lutz, 60*, 59–64.

Dittrich, P., Gietl, M., & Kandler, O. (1971). *D*-1-*O*-Methyl-*muco* inositol in higher plants. *Phytochemistry, 11*, 245–250.

Doner, L. W. (1977). The sugars of honey – a review. *Journal of the Science of Food and Agriculture, 28*(5), 443–456.

Földházi, G. (1994). Analysis and quantitation of sugars in honey of different botanical origin using high performance liquid chromatography. *Acta Alimentaria, 23*, 299–311.

Horváth, K., & Molnár-Perl, I. (1998). Simultaneous GC–MS quantitation of o-phosphoric, aliphatic and aromatic carboxylic acids, proline, hydroxymethylfurfurol and sugars as their TMS derivatives: In honeys. *Chromatographia, 48*(1/2), 120–126.

Huidobro, J. F., & Simal, J. (1984). Determinación del color y la turbidez de las mieles. *Anales de Bromatología, XXXVI*, 225–245.

Kirkwood, K. C., Mitchell, T. J., & Smith, D. (1960). An examination of the occurrence of honeydew in honey. *Analyst, 85*, 412–416.

Krauze, A., & Zalewski, R. (1991). Classification of honeys by principal component analysis on the basis of chemical and physical parameters. *Zeitschrift fur Lebensmittel Untersuchung und Forschung A, 192*, 19–23.

Li, B. W., & Schumann, P. J. (1981). Gas chromatographic analysis of sugars in granola cereals. *Journal of Food Science, 46*, 425–427.

Mateo, R., & Bosch-Reig, F. (1997). Sugar profiles of Spanish unifloral honeys. *Food Chemistry, 60*(1), 33–41.

Prodolliet, J., & Hischenhuber, C. (1998). Food authentication by carbohydrate chromatography. *Zeitschrift fur Lebensmittel Untersuchung und Forschung A, 207*, 1–12.

Ricciardelli D'Albore, G. (1998). *Mediterranean melissopalynology*. Perugia: Universitá degli Studi.

Sánchez, M. P., Huidobro, J. F., Mateo, I., Muniategui, S., & Sancho, M. T. (2001). Correlation between proline content of honeys and botanical origin. *Deutsche Lebensmittel Rundschau, 97*(5), 171–175.

Sanz, M. L., Sanz, J., & Martínez-Castro, I. (2004). Presence of some cyclitols in honey. *Food Chemistry, 84*, 133–135.

Scanlon, J. T., & Willis, D. E. (1985). Calculation of flame ionization detector relative response factor using the effective carbon number concept. *Journal of Chromatographic Science, 23*, 333–340.

Spanish Official Methods for Honey Analysis. (1986). *Boletin Oficial del Estado*, 145, 22195–22202.

Soria, A. C., Gonzalez, M., de Lorenzo, C., Martinez-Castro, I., & Sanz, J. Differentiation of nectar and honeydew honey from their volatile composition obtained by SPME and GC–MS. *Journal of the Science of Food and Agriculture* (in press).

Weston, R. J., & Brocklebank, L. K. (1999). The oligosaccharide composition of some New Zealand honeys. *Food Chemistry, 64*, 33–37.

White, J. W. (1980). Detection of honey adulteration by carbohydrate analysis. *Journal of Association of the Official Analytical Chemistry, 63*, 11–18.