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Quality evaluation of Spanish rosemary (*Rosmarinus officinalis*) honey

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The qualities of 27 samples of rosemary (*Rosmarinus officinalis* L.) honey from Aragón (Spain) was evaluated. Most samples showed a proper maturity considering the low moisture content. The low electrical conductivity and ash content were typical of pale honeys. Optical rotation was mostly levorotatory except for four samples with high sucrose content. Since the hydroxymethylfurfural content was low and the diastase activity was high, a good freshness was estimated. The total acidity (below 40 meq/kg) indicated absence of undesirable fermentation; additionally the mean pH around 3·70 is usual in this kind of honey. The fructose/glucose ratio was 1·17 ± 0·01 and sucrose content averaged 1·97 ± 0·27%. The analysis of sediment showed a very low honeydew indication which explains the low trisaccharide content found in the samples by GC–FID quantitation.

INTRODUCTION

Honey is produced in almost every country of the world. Aragón is a region in north-east Spain on the mid-section of the river Ebro's valley. Next to the river, three different plant associations are found: sabine grove, pinewood and kermes oak. Anthropological, eolic and rain effects led to the current settlement of rosemary plants in shallow soils and rockrose species and different crops in other areas. Rosemary plants are widely distributed, showing two flowering seasons in fall and early spring. Most beekeepers in Aragón, on the basis of organoleptical character and location of hives, sell what they claim to be 'rosemary honey' which is broadly appreciated by consumers willing to pay more for such a description.

The composition of a particular honey sample will greatly depend on the composition of the nectar(s) whence it originates. Rosemary honey is elaborated from the nectar of rosemary flowers (*Rosmarinus officinalis* L.) by honey-bees. Its production is the second largest in Spain (MAPA, 1988) after sunflower honey (*Helianthus annuus* L.). A number of investigations have been done on the most important Spanish unifloral honeys (Mateo *et al.*, 1987; Serra *et al.*, 1987; Serra & Cañas, 1988; Sancho *et al.*, 1991) and other unifloral honey types (Abu-Tarboush *et al.*, 1993).

In the present study the physicochemical analysis of 27 rosemary honey samples which are compared with the Spanish standards of honey and other published data worldwide, is reported.

MATERIALS AND METHODS

Sample collection

In a previous work, 168 samples of honey were classified according to their botanical origin (unpublished data). Thirty-six of them had been claimed as 'rosemary honey' by beekeepers on the basis of organoleptical characteristics. Microscopic analyses of sediment, physicochemical parameters and organoleptical study were made according to several authors (Pourtallier & Taliercio,1970; Mohamed *et al.*, 1982; Serra *et al.*, 1987) to correctly typify those samples. After this laboratory study, only 27 of the original 36 samples were reclassified as 'rosemary honey'.

The samples were taken directly from the containers that beekeepers use for storage of honey. All samples were unpasteurised and were taken no more than three months after extraction from the hives by beekeepers. After removal of the superficial layer, about 100 g of honey were aseptically transferred to a plastic bottle. Special care was taken to avoid air bubbles. The bottles were transported to the laboratory in portable coolers and then stored at −20°C until analysis, which was not delayed more than one month. Geographical distribution of sampling locations related to the total volume of production and specific interest of some areas.

Analysis of sediment for the identification of honey samples

The method followed for pollen analysis was described by Louveaux *et al.* (1978). Briefly, a subsample of 10 g

of crude honey is dissolved in 20 ml of warm distilled water (around 40°C) and centrifuged twice (2000 × *g*) for 10 min. The dry sediment is mounted on a slide with glycerine/gelatine slightly stained with an alcoholic solution of fuchsine. Slides were microscopically observed and compared with the reference for identification. Each sample belonging to the 'rosemary honey' group should contain more than 20% of rosemary (*Rosmarinus officinalis* L.) pollen.

Physicochemical analysis

The samples of honey were analysed according to the official Spanish methods (BOE, 1986) and the AOAC methods (AOAC, 1990) in order to determine moisture, optical rotation, electrical conductivity, ash content, hydroxymethylfurfural, diastase activity, pH, acidity (free, lactone and total) and sugar composition.

Moisture in honey was determined with a Shibuya refractometer reading at 20°C and obtaining corresponding % moisture from the Chataway table (Chataway, 1935), revised and updated (BOE, 1986; AOAC, 1990; Crane, 1990).

Optical rotation was measured in a polarimeter (Carl Zeiss 811753) as follows: 10 g of honey sample was clarified with Carrez reagents (I and II) and distilled water was added to get a final volume of 100 ml. Then, this solution was inserted into the polarimeter and results were stated in angular degrees on a 200 mm basis.

Electrical conductivity of a honey solution at 20% (dry matter basis) in CO_2-free deionised distilled water was measured at 20°C in a Crison 522 instrument. Results were expressed as 10^{-4} S × cm^{-1} (BOE, 1986)

Ash percentage was measured by calcination, overnight at 550°C in a furnace, to constant mass (BOE, 1986; AOAC, 1990).

Hydroxymethylfurfural was determined after clarifying samples with Carrez reagents (I and II) and addition of sodium bisulphite (based on methodology described in AOAC (1990). Absorbance was determined at 284 and 336 nm in a 1 cm quartz cuvette in a Kontron spectrophotometer. Results were expressed as mg/kg.

Diastase activity was measured using a buffered solution of soluble starch and honey which was incubated in a specially designed glass tube, shaped to end in an inverted 'V', in a thermostatic bath until the endpoint was determined photometrically (Spectronic 20). Results were expressed (as Gothe degrees) as ml of 1% starch hydrolysed by an enzyme in 1 g honey in 1 h (AOAC, 1990).

pH was measured in a pH meter (Crison 2001) from a solution containing 10 g honey in 75 ml of CO_2-free, distilled water.

Free, lactone and total acidity were determined by the titrimetric method first add 0·05 N NaOH and stop at pH 8·50 (free acidity), immediately pipet in 10 ml 0·05 N NaOH, and without delay back-titrate with 0·05 N HCl to pH 8·30 (lactone acidity). Total acidity results from adding free plus lactone acidities (BOE, 1986; AOAC, 1990). Results were expressed as meq/kg.

The sugar composition was determined by gas–liquid chromatography with flame ionisation detector (GC–FID) based originally on the method by Pourtallier and Rognone (1977) modified by Serra and Bosch (1989). Trimethylsilyl derivatives of sugar oximes were baseline separated and quantitated in a gas chromatograph HP 5890 Series II and an HP 3396A integrator under the following conditions: 3 m stainless-steel column (⅛-in. o.d.) packed with 4% SE-52 on Chromosorb WAWDNCS 100/120 mesh, carrier gas flow 25 ml N_2/min, FID with H_2 at 30 ml/min and O_2 at 400 ml/min, temperatures (°C): injector 280, detector 290 and column 205, rate 2°C/min to 280°C, held for 20 min, internal standard calibration with xylose. All standard sugars were obtained from Sigma Chemical Company. Results were expressed as grams of each sugar in 100 g of honey (percentage).

Statistical analysis was designed using StatView™ SE + Graphics (Abacus Concepts, Inc., 1988, Berkeley, CA, USA).

Organoleptical quality

In addition to the identification of honey type by analysis of sediment, the honey samples were subjected to a sensory panel. Rosemary honey is light-coloured, white after crystallisation and smells of rosemary flowers with some balsamic odour. The natural crystallisation is fine to medium coarse. This honey is light-flavoured at first taste, and tastes of wet flour after a while in the mouth.

RESULTS AND DISCUSSION

A descriptive analysis of the results is given in Tables 1 and 2. Moisture content of honey depends on harvest season along with the degree of maturity reached in the hive. This parameter is highly important for the shelf-life of the honey during storage. Twenty-three out of 27 samples, yielded moisture between 16·0–18·0% which means a proper degree of maturity, partially due to the current use of modern hives by beekeepers in Spain

Table 1. Analysis of some physiochemical parameters in 27 samples of rosemary honey

Parameter	Mean ± SE	Range	SD
Moisture (%)	16·8 ± 0·14	15·4–18·3	0·73
Optical rotation $\alpha^{20°C}$	−2·78 ± 0·58	−8·45–(+4·67)	2·99
Electrical conductance (10^{-4} S × cm^{-1})	1·55 ± 0·06	1·03–2·39	0·29
Ash content (%)	0·05 ± 0·005	0·02–0·11	0·02
HMF (mg/kg)	3·0 ± 0·7	0–18·26	3·56
Diastase activity (*G*°)	18·0 ± 0·9	10–29	4·88
pH[a]	3·71	3·42–3·94	—
Free acidity (meq/kg)	16·2 ± 0·43	10·6–20	2·22
Lactone acidity (meq/kg)	1·06 ± 0·26	0–6·11	1·35
Total acidity (meq/kg)	17·2 ± 0·40	10·6–21·5	2·45

[a] The value of pH is a geometric mean.

and finding the proper time of extraction. This mean value is lower than those previously reported in Spain (Serra *et al.*, 1987) and similar to that of < 17·5% reported by the Institut Technique Apicole (1975).

From the optical viewpoint, honey has the property of rotating the polarisation plane of polarised light. Floral honeys are levorotatory in contrast to honeydew and some adulterated honeys which are usually dextrorotatory. This is a consequence of the normal preponderance of fructose in floral honey, which shows a negative specific rotation over glucose (Table 1). Most samples (85·2%) were levorotatory and only four samples with high sucrose content (3·21% on average) were dextrorotatory. Due to the wide variation observed in the optical rotation, this parameter did not discriminate honey samples from different floral origins.

Electrical conductivity depends on the mineral content, organic acids, proteins and some complex sugars and polyols (Crane, 1975). Electrical conductivity varies with botanical origin, honeydew having the highest values. The conductivity mean value obtained (1·55 $S \times cm^{-1}$) is similar to the values published by Pourtallier and Taliercio (1970). However, Serra *et al.* (1987) reported higher conductivity in rosemary honey samples containing *Quercus* honeydew.

Rosemary honey, as is observed in all pale honeys, has a low mineral content. The ash content is influenced by the botanical origin as well as the technique used for the determination. Twenty-one out of 27 samples had ash contents below 0·08% in disagreement with data by Serra *et al.* (1987) in which ash averaged 0·15% in rosemary honey samples. Mohamed *et al.* (1982) reported a mean value of 0·11% in the same kind of honey.

The diastase activity and the hydroxymethylfurfural (HMF) content are widely recognised parameters in evaluating the freshness of honey (Schade *et al.*, 1958; Hadorn & Kovacs, 1960; White, 1979; Sancho *et al.*, 1992). Legal regulations in Spain set a minimum value for diastase activity of eight on Gothe's scale, and a maximum HMF content of 40 mg/kg. In honeys with low enzymatic content a diastase number of three on Gothe's scale is permissible as long as HMF content does not exceed 15 mg/kg.

Honey samples showed an appropriate diastase number ranging from 10 to 29°G, and their HMF content averaged 3·0 mg/kg with a maximum of 18·3 mg/kg (Table 1). Thus, all samples fell within the Spanish legal regulations for diastase number and HMF content. Most samples (96·3%) showed an HMF content below 10 mg/kg, and 63% of samples had HMF contents of less than 3 mg/kg. Only one sample exceeded 15 mg/kg of HMF (its diastase number was 29·1°G). These low values of HMF are in agreement with those reported by Sancho *et al.* (1992) in honeys from the Basque Country (Spain) whose HMF contents fell below 15 mg/kg in most samples. These authors suggested reducing the current maximum level of HMF (40 mg/kg) in a new regulation governing quality, since good manufacturing practices allow less HMF production during processing and storage of honey.

Table 2. Analysis of sugar composition (%) in 27 samples of rosemary honey

Sugar	Mean ± SE	Range	SD
Fructose	36·6 ± 0·47	31·9–42·5	2·43
Glucose	31·2 ± 0·44	26·3–35·98	2·26
Sucrose	1·97 ± 0·27	0·07–5·85	1·39
Maltose	7·20 ± 0·16	5·72–9·03	0·84
Erlose	0·61 ± 0·07	0–1·76	0·38
Melezitose	0·05 ± 0·04	0–0·33	0·11

Most honeys are acidic, having pH in the range 3·5–4·5. pH values obtained in rosemary honey agreed with data reported by Pourtallier and Taliercio (1970) who established pH ≤ 4·0 as the normal value.

The acidity of honey results from the presence of organic acids (mainly D-gluconic acid) in equilibrium with their lactones, or internal esters, and some inorganic ions such as phosphate, chloride and sulphate whose corresponding acids are honey constituents. A high titratable acidity figure may mean that the honey fermented at some time, and that the resulting alcohol was converted to organic acids. Special attention should be paid to the fact that these samples were obtained in the spring flowering season (February to June) when honey is often less acidic than in the autumn season which usually contains more honeydew.

Of the total sugars (see Table 2), the fructose and glucose mean values agreed with the results reported by Serra *et al.* (1987) of 35·26% fructose and 29·52% glucose. However, both Zurcher *et al.* (1975) and Gonnet (1979) reported higher values of these monosaccharides, in contrast to lower values obtained by Mohamed *et al.* (1982) of 32·90% for fructose and 24·95% for glucose.

The ratio fructose/glucose was 1·17 ± 0·01, a proportion which is slightly out of the range proposed by Pourtallier and Taliercio (1970) for rosemary honey (1·06–1·13). This parameter displayed a remarkably low variation among samples (SD 0·05). In consequence it is suggested that the ratio fructose/glucose be used to typify honey samples from different origins, especially those of rosemary as the main component.

The proportion of sucrose is similar to that found by Mohamed *et al.* (1982) and Serra *et al.* (1987), but higher than the 0·3% sucrose reported by Zurcher *et al.* (1975). Samples displayed a wide range of sucrose content, but only one sample was above the Spanish maximum legal limit of 5% sucrose for multifloral honeys (up to 10% for lavender, acacia, honeydew and its mixtures). The majority of previously reported data on sucrose content in Spanish honeys are below 1%. Thus, Serra and Cañas (1988) reported 0·26% sucrose in eucalyptus honeys. Battaglini and Bosi (1973) also reported sucrose content below 1% in several honey samples of different botanical origins: *Hedysarum coronarium*, *Onobrychis viciifolia*, *Medicago sativa*, *Citrus* sp., *Eucalyptus* sp., *Erica* sp., *Castanea sativa*, *Brassica napus* and *Ilex aquifolium*.

With respect to the disaccharide content, maltose was found in all samples, at higher levels than the 5·72% reported by Serra *et al.* (1987), but lower than the 8·7% maltose detected by Zurcher *et al.* (1975).

Two trisaccharides have been identified: erlose and melezitose. White and Maher (1953) gave special significance to erlose findings in honey samples with high sucrose content. Pourtallier and Taliercio (1970) also emphasised the importance of erlose level in rosemary honey with more than 1% sucrose. However, the authors were not able to correlate these sugars. In general, trisaccharide contents were lower than those reported by Serra *et al.* (1987), presumably due to the presence of fall honeydew in their samples.

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REFERENCES

Abu-Tarboush, H. M., Al-Kahtani, H. A. & El-Sarrage, M. S. (1993). Floral-type identification and quality evaluation of some honey types. *Food Chem.*, **46**, 13–17.

AOAC (1990). *Official Methods of Analysis* (15th edn), ed. K. Helrich. Association of Official Analytical Chemists, Inc., Arlington, VA, USA.

Battaglini, M. & Bosi, G. (1973). Caratterizzazione chimico-fisica dei mieli monoflora sulla base dello spettro glucidico e del potere rotatorio specifico. *Scienza e Tecnologia degli alimenti*, **III**, 217–21.

BOE (1986). Métodos oficiales de análisis para la miel. *Boletín Oficial del Estado*, 145, 22195-22202. Imprenta Nacional del Boletín Oficial del Estado, Madrid, Spain.

Chataway, H. D. (1935). Honey tables showing the relationship between various hidrometer scales and refractive index to moisture content and weight per gallon of honey. *Can. Bee J.*, **43**, 215.

Crane, E. (1975). *Honey: A Comprehensive Survey*. International Bee Research Association, Heinemann, London, UK.

Crane, E. (1990). *Bees and Beekeeping: Science, Practice and World Resources*. International Bee Research Association, Comstock Publishing Associates, Ithaca, New York, USA.

Gonnet, M. (1979). Aplication au miel d'une méthode de dosage par voie enzymatique des monosaccharides reducteurs. *Apidologie*, **10**, 395–401.

Hadorn, H. & Kovacs, A. S. (1960). Zur Untersuchung und Beurteilung von auslandischem Bienenhonig auf Grund des Hydroxymethyl furfurol seines Diastase gehaltes. *Lebensm. Hyg.*, **53**, 6–28.

Institut Technique Apicole (Groupe de Travail) (1975). *Caractéristiques de quelques Miels Monofloraux et Miellats de la Production Française*. Apimondia, Bucarest.

Louveaux, J., Maurizio, A. & Vorwhol, G. (1978). Methods of melissopalynology. *Bee World*, **59**,139–57.

MAPA (Ministerio de Agricultura Pesca y Alimentación) (1988). *Análisis del Sector de la Miel*. Madrid, Spain, Doc. num. 2, pp. 1–53.

Mateo, R., Bosch, F. & Pastor, A. (1987). Capillary column gas chromatographic identification of sugars in home as trimethylsilyl derivatives. *J. Chromatogr.*, **410**, 319–28.

Mohamed, M. A., Ahmed, A. A. & Mazid, M. M. (1982). Studies on Libyan honeys. *J. Food Qual.*, **4**, 185–201.

Pourtallier, J. & Taliercio, Y. (1970). Les caracteristiques physicochimiques des miels en fonction de leur origine florale. 1. Aplication à un projet de normes pour les grandes variétés de miels. *Bull. Apic. Doc. Sci. Technol.*, **13**, 58–67.

Pourtallier, J. & Rognone, C. (1977). Methode ameliorée de dosage des sucres des miels par chromatographie en pase gazeuse. Symp. Int. Apic. Bologna, Italy.

Sancho, M. T., Muniategui, S., Huidobro, J. F. & Simal, J. (1991). Mieles del País Vasco. V: origen floral. *Ann. Bromatol.*, **43**, 151–63.

Sancho, M. T., Muniategui, S., Huidobro, J. F. & Simal, J. (1992). Aging of honey. *J. Agric. Food Chem.*, **40**, 134–8.

Schade, J. W, Marsh, G. L. & Eckert, J. E. (1958). Diastase activity and hydroxymethyl furfural in honey and their usefulness in detecting heat alteration. *Food Res.*, **23**, 446–63.

Serra, J. & Bosch, J. (1989). Determinación de azúcares de la miel mediante cromatografía de gases. *Anales de Química*, **1**, 37–46.

Serra, J. & Cañas, S. (1988). Caratteristiche fisico-chimiche, composizione e spettro pollinico del miele di eucalipto (*Eucalyptus* sp.) prodotto in Spagna. *Apicoltura*, **4**, 59–81.

Serra, J., Gómez, A. & Gonell, J. (1987). Composición, propiedades físico-químicas y espectro polínico de algunas mieles monoflorales de España. *Alimentaria*, **185**, 61–84.

White Jr, J. W. (1979). Spectrophotometric method for hydroxymethylfurfural in honey. *J. AOAC*, **62**, 509–14.

White Jr, J. W. & Maher, J. (1953). Transglucosilation by honey invertase. *Arch. Biochem. Biophys.*, **48**, 360–7.

Zurcher, J., Maurizio, A. & Hadorn, H. (1975). Untersuchungen an Handelshonigen mit Spezieller Berucksichtigung des Zuckerspektrums. *Apidologie*, **6**, 59–90.