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Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents

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Abstract

The qualities of 25 thyme honey samples from Spain were evaluated. Eight common physicochemical parameters were analysed, namely water content, pH, acidity (free, lactonic and total), sugar content, ash, electrical conductivity and mineral composition, including potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S). Most samples showed proper maturity, considering the low water content. The total acidity (below 50 meq/kg) indicated the absence of undesirable fermentation; also the mean pH, around 4.2, is usual in this kind of honey. The values for ash and electrical conductivity were medium (0.32% and 395 $\mu S\,cm^{-1}$, respectively), typical of dark amber honeys. Within the mineral content, potassium was quantitatively the most important mineral, having an average content of 679 ppm; sodium and calcium were present in moderate amounts in the honeys and accounted for 27% and 13% of the minerals, respectively.

Keywords: Thyme honey; Pollen analysis; Physicochemical properties; ICP-OES; Mineral content

1. Introduction

Honey is one of the most complex foodstuffs produced by nature, and certainly the only sweetening agent that can be used by humans without processing. Honey is produced by honey bees from carbohydrate-containing exudates produced by plants. Characterisation of unifloral commercial honeys is a hard task initiated in Europe in response to consumers' demands. Those have demanded, not only a basic quality level, but also a clear determination of geographical and botanical origin. This has occurred in different countries and has led to widespread regulation. In the E.U., the honey regulation (The Council of the European Union, 2002) states that the geographical and botanical origin of this product must be shown on package labels. Control of honey requires the determination of parameters that could unequivocally establish origin and calls for efforts to improve honey characterisation.

Melissopalynology, the analysis and identification of pollen grains contained in honey, was the first technique to be used and, currently complemented by sensory analysis, continues as a reference tool for this purposes. However, its known limitations have encouraged the search for physicochemical parameters.

Some unifloral honeys have specific chemical or physical properties, which may be used to confirm the results of microscopical analysis. Methyl anthranilate, and the flavonoid hesperetin, have been considered as indicators of citrus honey (Serra Bonvehi & Ventura Coll, 1995) and the norisoprenoid, *S*-dehydrovomifoliol for heather honey (Tan, Wilkins, Holland, & McGhie, 1989). Honeydew honeys have a high electrical conductivity and contain much melezitose (Kirkwood, Mitchell, & Smith, 1960).

In Spain, and thanks to the melliferous tradition, the study of honey has been well developed, and many studies, both palynological and physicochemical, have

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been carried out for characterisation of the main unifloral honeys produced, such as lavender, eucalyptus, rosemary, sunflower, citrus, heather and forest (Mateo & Bosch-Reig, 1997; Pérez-Arquillué, Conchillo, Ariño, Juan, & Herrero, 1994; Serra Bonvehi, 1988, 1989; Serra Bonvehi & Ventura Coll, 1995; Serra Bonvehi, Gómez Pajuelo, & Gonell Galindo, 1987). However, other honey types have been poorly studied; that is the case for thyme honey, despite the fact that it is one of the most important honey due to its appreciation by consumers (Sáinz Laín & Gómez Ferreras, 2000).

In the Mediterranean area, the thyme honeys are mainly produced in Greece, Italy, Morocco and Spain (Ricciardelli D'Albore, 1998). Although there are some melissopalynological studies (Damblon, 1987, 1988; Oddo, Piazza, Sabatini, & Accorti, 1995; Roselló Caselles, Burgaz Moreno, Mateu Andrés, & Gómez Ferreras, 1996; Thrasyvoulou & Manikis, 1995; Tsigouri & Passaloglou-Katrali, 2000), the only study known for the physicochemical characterisation of this honey type was carried out by Accorti, Persano, Piazza, and Sabatini (1986) in Italian honeys. In Spain, only few studies have been developed at local level: the first was a research work on the quality of 10 types of Spanish unifloral honeys from Aragon (northern Spain), but it only included one sample of thyme honey (Pérez-Arquillué, Conchello, Ariño, Juan, & Herrero (1995); in the second study, Sanz, Pérez, Herrera, Sanz, & Juan (1994) analysed 48 honey samples from La Rioja (northern Spain), and seven of them proved to be unifloral, of thyme.

Thus, the goal of the present work is the physicochemical characterisation of the thyme honeys produced in Spain, by the analysis of eight common physicochemical parameters (water content, pH, free, lactonic and total acidity, ash, electrical conductivity and sugar content), and the principal mineral elements (K, Na, Ca, Mg, P and S).

2. Materials and methods

2.1. Honey samples

The present study made use of 25 thyme honey samples from *Thymus mastichina* (L.) L. and *Thymus capitatus* (L.) Hoffmanns & Link (= *Thymbra capitata* (L.) Cav.) collected in 17 provinces of Spain, between 2002 and 2003. The samples were taken directly from the beekeepers, all professionals, and the extraction of honeys from combs was done by centrifugation. All samples were unpasteurised and were taken no more than three months after extraction, stored in holders and immediately transferred to the laboratory and kept at 4–5 °C. Analyses were done within a 6-month time period after harvesting.

2.2. Pollen analysis

The botanical origin of the honeys was studied using the techniques described by Maurizio (1979) and Erdtman (1960). Slides were prepared with acetolysis by centrifuging 10g of honey dissolved in 20 ml of diluted sulphuric acid (5 g H_2SO_4/l) for 10 min at 2500 rpm. The supernatant was decanted, and the sediment washed twice with 10 ml distilled water and then centrifuged. The sediment was extended on a slide and dried at 70 °C, then mounted with stained glycerine gelatine. Following the results obtained by Behm, VonderOhe, & Henrich (1996), at least 500 pollen grains were counted among four different slides for each honey sample. For the pollen identification, the general key to pollen types from Díez (1987) was used. A minimum of 15% pollen from *Thymus* sp. (eliminating the pollen grains from anemophilous and nectarless plants) was required to consider these honeys as unifloral from a melissopalynological point of view, according to Roselló Caselles et al. (1996) and Sáinz Laín & Gómez Ferreras (2000).

2.3. Physicochemical parameters

Water content (moisture) was determined with an Erma refractometer, reading at 20 °C, using the Wedmore table (AOAC, 1990), and the results were expressed as percentages.

pH was measured in a pH-meter (Orion 420 A) from a solution containing 10 g of honey in 75 ml of CO_2-free distilled water (AOAC, 1990).

The free, lactonic and total acidity were determined as follows, by the titrimetric method: the addition of 0.05 M NaOH was stopped at pH 8.50 (free acidity), immediately a volume of 10 ml 0.05 M NaOH was added and, without delay, back-titrated with 0.05 M HCl to pH 8.30 (lactonic acidity). Total acidity results were obtained by adding free and lactone acidities (AOAC, 1990).

Sugar content was determined utilising a special refractometer with direct reading display, and the results were expressed as brix degrees.

Ash percentage was determined by calcination, at 550 °C in a furnace (Heron), until constant mass was attained (AOAC, 1990), and the results were expressed as percentages.

Electrical conductivity of a honey solution at 20% (dry matter basis) in CO_2-free deionised distilled water was measured at 20 °C (AOAC, 1990) in a Crison Basic 30 conductimeter, and the results were expressed as $\mu S\,cm^{-1}$.

2.4. Determination of mineral elements

A Jobin Yvon Ultima 2 inductively coupled plasma-optical spectrophotometer was used for metal determinations. The instrument was operated in the following conditions: RF frequency, 27 MHz; operating power, 1200 W; pump rate, 20 rpm; plasma argon flow rate, 2 l/min; carrier argon flow rate, 2 l/min; burner type Minitorch; ultrasonic nebuliser type Cetac; sample flow rate, 0.02 ml/min; and nebulisation pressure, 1 bar.

Five ml of nitric acid (0.1 N) were added to the resultant ash, and the mixture was stirred on a heating plate to almost complete dryness. Then, 10 ml of the same acid were added, and brought up to 25 ml with distilled water. The minerals (Na, K, Ca, Mg, P and S) were determined by inductively coupled plasma optical emission spectrophotometer (*ICP-OES*). The emission wavelength (nm) for the determination of each metal, together with its linear working range and correlation coefficient from the calibration graph, were as follows: K (766.490; 0–400.87 mg/l; 0.9999), Mg (382.935, 0.04–28.34 mg/l, 0.9999), Ca (422.673, 0.33–99.93 mg/l, 0.9999), Na (589.592, 0.85–249.71 mg/l, 0.9998), P (255.473, 0–2000 mg/l, 1) and S (181.978, 0.82–40.32 mg/l, 0.9991).

2.5. Sensorial analysis

In addition to the identification of honey type by the pollen analysis, the samples were subjected to a sensory panel. Thyme honey is golden/dark amber-coloured; it smells of thyme flowers with soft odour, persistent flavour, fine and coarse crystallisation.

3. Results and discussion

3.1. Pollen analysis

The results of microscopical analysis of the sediment from the honeys used in this work are briefly summarised. Percentages are always referred to pollen from nectar plants. Thyme honeys contained between 15% and 73% of the pollen of *Thymus* (*Thymus mastichina* and *Thymus capitatus*), accordingly under-representing presence of this pollen type in the honeys. The most characteristic accompanying species are Leguminosae (*Calicotome villosa*, *Cytisus baeticus*, *Genista hirsute* and *Ulex borgiae*), present in 100% of the samples, followed by *Echium* sp. and *Reseda luteola* (90%) and *Eucalyptus* sp. (80%). Other taxa were Compositae (mainly *Helianthus annuus*), *Olea europaea* and *Cistus monspeliensis*. Our results agree with data reported by other authors (Roselló Caselles et al., 1996), who analysed the pollen contained in seven thyme honeys from Valencia (eastern Spain).

Table 1
Distribution data for common physicochemical parameters in Spanish thyme honeys

Sample	pH	Moisture (%)	°Brix (%)	Ash (%)	Electrical conductivity ($\mu S\,cm^{-1}$)	Free acidity (meq/kg)	Lactonic acidity (meq/kg)	Total acidity (meq/kg)
1	4.28	17.2	81.1	0.33	400	23.0	7.1	30.1
2	3.71	17.1	81.2	0.19	323	31.9	9.8	41.7
3	4.13	16.0	82.1	0.33	410	25.9	7.3	33.2
4	4.05	16.0	82.4	0.19	300	25.0	7.3	32.3
5	4.38	16.5	81.8	0.29	460	28.7	6.3	35.0
6	4.28	16.6	80.9	0.30	427	27.8	6.3	34.1
7	4.30	15.0	83.5	0.28	384	29.6	4.3	33.9
8	4.39	15.6	82.7	0.37	443	30.0	6.3	36.4
9	3.56	17.2	81.1	0.23	326	27.3	10.8	38.1
10	4.24	16.6	81.7	0.51	400	27.8	6.8	34.6
11	3.85	14.4	83.8	0.24	372	17.6	8.3	25.9
12	4.79	16.2	82.1	0.32	418	39.8	8.8	48.6
13	3.74	16.2	82.0	0.35	453	25.0	11.3	36.3
14	4.74	15.6	82.7	0.44	498	30.6	6.3	36.9
15	4.26	16.5	82.0	0.60	413	26.9	4.8	31.6
16	3.71	16.8	81.7	0.16	288	25.5	8.8	34.3
17	4.45	14.2	84.0	0.50	382	27.8	6.3	34.1
18	3.81	19.8	78.8	0.27	340	23.2	8.8	32.0
19	4.63	15.8	82.4	0.45	559	34.7	5.3	40.0
20	4.11	16.8	81.6	0.25	342	24.1	10.3	34.4
21	3.99	16.3	82.0	0.18	337	21.8	6.3	28.1
22	4.53	15.3	82.5	0.44	460	34.7	5.8	40.5
23	4.64	16.0	82.3	0.30	453	27.8	4.3	32.1
24	4.25	16.9	82.0	0.20	345	20.4	5.2	25.6
25	4.24	18.0	80.3	0.42	355	24.1	7.8	31.9
Mean	4.2	16.3	81.9	0.32	395	27.2	7.22	34.5
SD[a]	0.33	1.13	1.1	0.11	65	4.8	1.9	4.9
Range	3.56–4.79	14.2–19.8	78.8–84	0.16–0.60	288–559	17.59–39.81	4.3–11.3	25.6–48.6

[a] Standard deviation.

3.2. Physicochemical parameters

Table 1 shows the means, standard deviations and ranges of the data obtained from the analysis of the different physicochemical parameters. Water content, a parameter that is related to the climatic conditions, the season of the year and the degree of maturity (White, 1978), showed similar values (between 16% and 18%) in 70% of the samples; nevertheless, none of the honeys exceeded the permitted limit established by the European Community Directive (The Council of the European Union, 2002). The values found correspond to mature honeys, partially due to the current use of modern hives by beekeepers in Spain and finding the proper time of extraction; the mean value corresponds to honeys extracted in the summer.

The sugar content, a parameter for which anomalous values may be a reliable index of adulteration, presents normal values between 78.8% and 84%, the mean being 81.9%. On the other hand, the moisture and sugar contents of honey are strictly correlated (Conti, 2000), which is confirmed in this study when the correlation between both parameters is studied, this correlation being 0.97 at $p < 0.05$.

pH values, which are of great importance during the extraction and storage of honey, as they influence the texture, stability and shelf life, range between 3.55 and 4.79; these are typical pH values in floral honeys, the mean being 4.2, which is slightly higher than that found by Accorti et al. (1986) in Italian thyme honeys (mean 3.8), but very similar to the pH value described by Sanz et al. (1994) in the honeys from La Rioja

Owing to the presence of organic acids in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions such as phosphate, or sulphate, the values for the free acidity ranged between 17.6 and 39.8 meq/kg; the lactonic acidity (considered as the acidity reserve when the honeys become alkaline) ranges between 4.3 and 11.3 meq/kg, while the mean of the total acidity was 34.5 meq/kg. Nevertheless, none of the samples exceeded the acidity limit established by the European Community regulations (The Council of the European Union, 2002). The results obtained for the acidity are slightly low when compared to those observed in Italian honeys (mean of total acidity = 43.3 meq/kg) and honeys from La Rioja (mean of total acidity = 39.5 meq/kg).

The wide variability of honey composition is reflected also in the ash content; this parameter, used for the determination of the botanical origin (floral, mixed or honeydew) (White, 1978), showed values between 0.16% and 0.60%. None of the sample surpassed 0.6%, the

Table 2
Distribution data for mineral content (ppm) in Spanish thyme honeys

Sample	Ca	K	Mg	Na	P	S
1	221	989	78	334	59	36
2	194	311	58	379	35	28
3	201	772	70	318	65	40
4	190	830	78	307	73	24
5	202	1380	139	376	48	40
6	187	933	74	405	48	31
7	191	844	67	403	35	34
8	192	622	57	322	32	27
9	124	261	59	376	32	18
10	129	483	49	353	26	19
11	157	603	56	393	34	21
12	180	326	58	458	30	32
13	165	410	65	440	32	24
14	190	666	77	462	63	43
15	178	1131	91	473	96	38
16	175	717	128	375	60	32
17	239	837	110	501	78	28
18	110	680	54	256	54	21
19	248	399	78	454	65	22
20	190	727	106	390	55	28
21	224	710	85	405	50	30
22	113	175	37	258	61	21
23	201	430	101	321	59	36
24	185	960	96	465	35	40
25	150	780	65	501	71	33
Mean	181	679	77	389	51	29
SD[a]	35	287	24.8	69.5	17.9	7.3
Range	110–248	261–1380	37–139	256–501	26–96	19–43

[a] Standard deviation.

permitted limit for flower honeys (The Council of the European Union, 2002). The mean value was 0.32%, similar to that described by Pérez-Arquillué et al. (1994) (0.29%), but quite higher than the value found by Sanz et al. (1994) (mean 0.18%). Although this parameter could be used to differentiate our thyme honeys from some light honey types, such as rosemary, willow, sainfoin or chickweed (Pérez-Arquillué et al., 1995), the range of the ash content of the most important honey types (e.g. eucalyptus, lavender) overlaps with these results.

The electrical conductivity of the honey is closely related to the concentration of mineral salts, organic acids and proteins; it is a parameter that shows great variability according to the floral origin and is considered one of the best parameters for differentiating between honeys with different floral origins (Krauze & Zalewski, 1991; Mateo & Bosch-Reig, 1998; Terrab, Díez, & Heredia, 2002). This parameter shows values between 288 and 559 $\mu S\,cm^{-1}$, the mean value being 395 $\mu S\,cm^{-1}$, quite similar to that of the Italian thyme honeys (mean 380 $\mu S\,cm^{-1}$). So, the electrical conductivity could be considered a reliable parameter for differentiating from other type honeys, such as rosemary (89–250 $\mu S\,cm^{-1}$), citrus (124–262 $\mu S\,cm^{-1}$), heather (815–1092 $\mu S\,cm^{-1}$) or honeydew honey (822–1213 $\mu S\,cm^{-1}$) (Mateo & Bosch-Reig, 1998).

3.3. Mineral elements

The results of the metals determined in thyme honey samples are summarized in Table 2; six minerals were identified and quantified: potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S). The potassium is, quantitatively, the most important mineral, and accounts for 48% of the total mineral quantified, having an average content of 679 ppm; sodium and calcium are present in moderate amounts in the honeys and account for 27% and 13% of the minerals, respectively. Magnesium ranges between 37 and 137 ppm (average: 77 ppm). Phosphorus and sulphur show a low average value, and represent less than 3% of the total mineral content.

4. Conclusions

The results obtained for the electrical conductivity, ash and mineral contents are in agreement with the dark amber colour characteristic of this honey type, since the amber honeys show a medium content of minerals and electrical conductivity values (White, 1978).

The thyme honeys produced in Spain are characterised by the presence of Leguminosae (*Calicotome villosa*, *Cytisus baeticus*, *Genista hirsuta* and *Ulex borgiae*), *Echium* sp. and *Reseda luteola*, *Eucalyptus* sp. as the main accompanying species. The physicochemical parameters such as water content, acidity and sugar content, closely related to the quality of the honey, showed values in agreement with the legal limits. On the other hand, parameters strongly related to the floral origin, such as ash and electrical conductivity, showed moderate values (0.32% and 395 $\mu S\,cm^{-1}$, respectively). Electrical conductivity is the parameter most useful for distinguishing thyme honeys from other honeys. Within the mineral contents, the high values for potassium and sodium (average: 679 and 389 ppm, respectively) must be highlighted.

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