Plant Phenolic Metabolites and Floral Origin of Rosemary Honey

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Phenolic metabolites present in rosemary honey and floral nectar were studied to find biochemical markers for honey floral origin. Rosemary nectar, from bee honey stomach, contained kaempferol 3-sophoroside (93%) and quercetin 3-sophoroside (7%) as the only significant constituents. All samples had a common flavonoid profile comprised of 15 flavonoids. Nectar glycosides were not detected in honey suggesting that they are hydrolyzed by the bee enzymes to render the corresponding aglycons. Honey flavonoid profiles were similar to those found for propolis, a plant resin collected by bees, and confirmed that the majority of the flavonoids present in honey originate from this source. The amount of kaempferol in the honey samples ranged between 0.4 and 1.2 μg/g. The coefficient of variation of kaempferol in the honey samples was much smaller than those observed for the rest of flavonoids, supporting its floral origin, and the propolis origin for the rest of flavonoids. The presence of kaempferol in rosemary honey cannot be considered as proof of its floral origin because this flavonol can originate from different flower nectars. However, its absence or presence in small levels (<0.3 μg/g of honey) could be considered additional evidence of a different floral origin.

Keywords: *Honey; nectar; flavonoids; kaempferol; rosemary; Rosmarinus officinalis; botanical origin; classification; HPLC; biochemical characterization*

INTRODUCTION

In the past few years there has been an increasing interest in finding objective analytical methods that could complement pollen analysis in the determination of the floral origin of honey. In this context it has been suggested that the next step in this type of research will be an attempt to correlate floral source with the presence of certain compounds originating either in the nectar or in some biochemical modifications of nectar compounds carried out by the bee (Bonaga and Giumanini, 1986). Volatile compounds (Bonaga et al., 1986), aromatic and degraded carotenoid-like substances (Tan et al., 1988, 1989a,b, 1990; Wilkins et al., 1993), amino acids (Davies, 1975; Bosi and Battaglini, 1978), degradation products of phenylalanine (Speer and Montag, 1987), aromatic aldehydes and heterocycles (Häusler and Montag, 1990), aromatic acids and their esters (Speer and Montag, 1984; Steeg and Montag, 1988), and phenolic compounds (Amiot et al., 1989; Ferreres et al., 1992, 1994a,b; Sabatier et al., 1992) have been found in honey and related to the floral origin. In fact, the flavonoid hesperetin proved to be a useful marker for the floral origin of citrus honey (Ferreres et al., 1993) and, as a marker, showed some advantages over methyl anthranilate, another biochemical marker of the floral origin of citrus honey (Ferreres et al., 1994b).

Rosemary honey is produced from *Rosmarinus officinalis* L. (Lamiaceae). This honey has a very good consumer acceptance and commercial value in European countries because of its mild flavor and light color (Tomás-Barberán et al., 1994).

The aim of the present work was to study the phenolic metabolites present in rosemary nectar, rosemary honey collected in the same geographical region, and in commercial rosemary honey samples produced in other areas of Spain to establish if plant phenolic metabolites could be used as markers for the floral origin of rosemary honey.

MATERIALS AND METHODS

Honey, Nectar, Pollen, and Propolis Samples. Rosemary honey samples were collected in different localities of Castilla-La Mancha (Spain) and stored at −20 °C until analyzed. The botanical origin of the samples was confirmed by pollen analysis (Louveaux et al., 1978), and all the samples contained >15% rosemary pollen, a value which is considered excellent for a monofloral rosemary honey sample to be considered of rosemary origin (Ortiz, 1992). Other commercial rosemary honey samples produced in different geographical areas in Spain were also analyzed. Rosemary nectar was obtained from honey bees collecting nectar from *Rosmarinus officinalis* flowers. Rosemary pollen was collected from bees carrying pollen from the *R. officinalis* flowers to the hive. The purity of the bee-pollen was determined by microscopic analysis by classical methods (Louveaux et al., 1978). Propolis was collected from the beehives in the same geographical area in which rosemary honey and nectar were collected.

Extraction of Flavonoids from Nectar. The bees were trapped into a test tube with dried CO_2 and stored at −20 °C until analyzed. Bees were thawed, the honey stomach was separated from the bee with a knife and forceps, and the stomach content was collected with a capillary. The honey stomach liquids were joined (350 μL), diluted with distilled water (1 mL), and centrifuged in an Eppendorf test tube. The pellet was examined with a microscope to evaluate the pollen composition (Louveaux et al., 1978) and ~55% of rosemary pollen was found. The supernatant was then diluted with 5 mL of distilled water and filtered through a solid-phase extraction cartridge (Sep-Pak RP-18, Waters) to retain phenolic compounds and discard sugars and other polar compounds that eluted with water. The phenolic compounds were then eluted with methanol, concentrated under reduced pressure (40 °C), and redissolved in 300 μL of methanol:water (1:1 v/v). Then 20 μL were analyzed by reversed-phase HPLC.

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Table 1. Flavonoids Detected in Rosemary Honey

no.	name	structure
1	pinobanksin	3,5,7-trihydroxyflavanone
2	quercetin	3,5,7,3′,4′-pentahydroxyflavone
3	luteolin	5,7,3′,4′-tetrahydroxyflavone
4	8-methoxykaempferol	3,5,7,4′-tetrahydroxy-8-methoxyflavone
5	kaempferol	3,5,7,4′-tetrahydroxyflavone
6	apigenin	5,7,4′-trihydroxyflavone
7	isohamnetin	3,5,7,4′-tetrahydroxy-3′-methoxyflavone
8	quercetin 3,3′-dimethyl ether	5,7,4′-trihydroxy-3,3′-dimethoxyflavone
9	pinocembrin	5,7-dihydroxyflavanone
10	quercetin 7,3′-dimethyl ether	3,5,4′-trihydroxy-7,3′-dimethoxyflavone
11	quercetin 3,7-dimethyl ether	5,3′,4′-trihydroxy-3,7-dimethoxyflavone
12	chrysin	5,7-dihydroxyflavone
13	galangin	3,5,7-trihydroxyflavone
14	unidentified flavanone	?
15	tectochrysin	5-hydroxy-7-methoxyflavone

Extraction of Flavonoids from Honey. The different honey samples (50 g each) were mixed with five parts of water (pH 2 with HCl) until completely fluid and then filtered through cotton to remove solid particles. The filtrate was then passed through a column (25 × 2 cm) of Amberlite XAD-2 (Fluka Chemie; pore size 9 nm, particle size 0.3−1.2 mm; Tomás-Barberán et al., 1992). The phenolic compounds remained in the column while sugars and other polar compounds eluted with the aqueous solvent, resulting in flavonoid recovery of >95% (Ferreres et al., 1994a; Tomás-Barberán et al., 1992). The column was washed with acid water (pH 2 with HCl, 100 mL) and subsequently with distilled water (~300 mL). The whole phenolic fraction was then eluted with methanol (~300 mL) and taken to dryness under reduced pressure (40 °C). The residue was redissolved in 5 mL of water and extracted with diethyl ether (5 mL × 3; Ferreres et al., 1994c). The ether extracts were joined, concentrated under reduced pressure, and redissolved in 0.5 mL of methanol for HPLC analysis. Samples were stored under N_2 until analyzed. Reproducibility of the HPLC analysis was ±5%.

Extraction of Flavonoids from Propolis. Propolis (~1 g) was extracted with methanol for 2 h at room temperature, and the extract was filtered through filter paper and a 0.45-μm filter before direct HPLC analysis.

HPLC Analysis of Nectar Flavonoids. All HPLC analyses were achieved with a Merck-Hitachi L-6200 liquid chromatograph with a diode array detector Merck-Hitachi L-3000 and an autosampler Merck-Hitachi A-2000A. Data were stored and processed with DAD-Manager software (Merck). The column used was a Lichrochart RP-18 (Merck, Darmstadt, Germany; 12.5 × 0.4 cm, 5-μm particle size). Elution was with water:formic acid (19:1 v:v; solvent A) and methanol (solvent B), and the flow rate was 1 mL/min. Gradient elution started with 10% B, reached 50% B at 25 min and 80% B at 35 min, and then the system became isocratic until 40 min.

HPLC Analysis of Honey and Propolis Flavonoids. This HPLC analysis was performed with the same instrument on a reversed-phase column (Lichrochart RP-18 column (Merck, Darmstadt, Germany; 12.5 × 0.4 cm, 5-μm particle size), and with water:formic acid (19:1 v:v; solvent A) and methanol (solvent B) as solvents. The elution was accomplished with a solvent flow rate of 1 mL/min. The elution gradient started with 30% methanol, remained isocratic until 15 min, reached 40% methanol at 20 min, 45% methanol at 30 min, 60% methanol at 50 min, and 80% methanol at 52 min, and then became isocratic until 60 min. The flavonoids were detected with a diode-array detector (Merck-Hitachi L-3000) to obtain the UV spectra of the different phenolic compounds, and the chromatograms were recorded at 340 and 290 nm. The structures of the different flavonoids are shown in Table 1.

Flavonoid Identification and Quantitation. The different honey and propolis flavonoids were identified by chromatographic comparisons with authentic markers (commercial or previously isolated and identified from honey; Ferreres et al., 1991, 1992) and by matching their UV spectra with those of the markers. Honey flavonoids were quantified by the absorbance of their corresponding peaks in the chromatograms as reported previously (Ferreres et al., 1994c): the flavanones as the external standard pinocembrin detected at 290 nm, the flavones with an unsubstituted ring B (chrysin, galangin and tectochrysin) as the external standard chrysin detected at 340 nm, and the rest of flavonols and flavones as the external standard quercetin detected at 340 nm.

Statistical Analysis. A Principal Component Analysis (PCA) was achieved with SPSS/PC$^+$ software. The data were treated as reported previously (Tomás-Barberán et al., 1994), and the Kaiser-Meyer-Olkin measure of sampling adequacy (= 0.13751) and the Bartlett test of sphericity (428.32969, $p < 0.001$) indicated that the PCA is only adequate to the descriptive level.

RESULTS AND DISCUSSION

The phenolic compounds present in honey can originate from flower nectar, propolis (and/or beeswax), and pollen. Those metabolites present in nectar would be the most valuable biochemical markers for floral origin because this is the main source for floral honeys. Propolis is a resin collected by bees from different plant sources that is incorporated into the hive for many functions. The chemical constituents of propolis are incorporated into beeswax and honey (Tomás-Barberán et al., 1993a). Pollen is also present in honey, although in very variable amounts, and is traditionally used in floral origin determinations. However, pollen seems to make a very small contribution to the flavonoids detected in honey (Ferreres et al., 1993).

Therefore, the study of the phenolic metabolites present in rosemary nectar and propolis is essential to understand if flavonoids could be used as biochemical markers of the floral origin of rosemary honey.

Study of Phenolic Compounds from Rosemary Nectar. Direct collection of rosemary nectar from flowers is a very difficult task, because of the small flower size and to the very small volume of nectar produced. Therefore, collection of rosemary nectar with the help of bees was achieved. The floral origin of nectar was confirmed by pollen analysis that indicated that the percentage of rosemary pollen was 55%, which is an excellent value taking into account that pollen production in rosemary is very small (Ortiz, 1992). In spite of the very small amount of nectar collected (~350 μL), solid-phase extraction allowed the sample preparation to be analyzed by HPLC (Figure 1). The HPLC chromatograms recorded at different wavelengths with a diode array detector showed that a major UV absorbing compound (**B**) was detected (93% of the total absorbance of the chromatogram at 340 nm together with trace amounts of a second compound (**A**; 7% of the total absorbance). The presence of other phenolic compounds, such as rosmarinic acid, a phenolic acid derivative which is characteristic of rosemary leaves and which is also

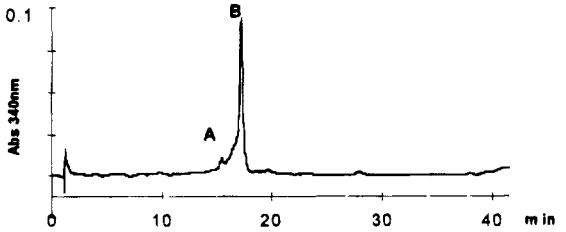

Figure 1. HPLC chromatogram of rosemary nectar phenolics, with detection at 340 nm: (A) quercetin 3-sophoroside; (B) kaempferol 3-sophoroside.

present in related genera, was also studied. However, rosmarinic acid was not detected in the nectar chromatograms. The UV spectrum of the main compound (**B**; 265, 349 nm) suggested that this was a kaempferol derivative glycosylated on the hydroxyl at the 3-position (Mabry et al., 1970). Nectar was hydrolyzed with 2 N HCl at 80 °C (30 min), extracted with ethyl acetate; and concentrated to dryness and redissolved with methanol for HPLC analysis. The aglycon was identified as kaempferol (3,5,7,4′-tetrahydroxyflavone) by its UV spectrum, recorded with the diode array detector (265, 375 nm), and by cochromatographic comparison with an authentic marker (Roth, Germany). Compound **B** coincided chromatographically with a flavonoid glycoside detected previously in rosemary bee pollen, that had been identified as kaempferol 3-sophoroside (Ferreres et al., 1992). The UV spectrum of **A** indicated that this was a quercetin 3-glycoside (254, 265 shoulder, 355; Mabry et al., 1970). This coincided chromatographically with the other main flavonoid present in rosemary pollen that was identified as quercetin 3-sophoroside. Previous studies indicated that both **A** and **B** were present in rosemary bee pollen in similar proportions (Ferreres et al., 1992), whereas in nectar, **B** is the main phenolic metabolite present (Figure 1).

Flavonoid glycosides present in nectar are hydrolyzed to give the corresponding aglycons by the glycosidases of bee salivary glands (Sabatier et al., 1992) and therefore only the aglycons are detected in honey, as recently shown in a study on citrus nectar and honey (Ferreres et al., 1993). Thus, in the case of rosemary honey, it is expected that the aglycon of **B**, which is kaempferol, is present as a relevant constituent of the flavonoid profile.

Study of Flavonoids from Propolis. The main phenolic metabolites present in honey are those derived from propolis, which are incorporated into beeswax and honey (Bogdanov, 1989; Tomás-Baberán et al., 1993a) and originate from poplar (*Populus* sp.) bud exudates in temperate areas (Tomás-Barberán et al., 1993b; Greenaway et al., 1990). When poplars are available, this is the source preferred by bees for propolis collection (García-Viguera et al., 1992). Propolis samples from bee hives located in the same production area where rosemary nectar and honey were collected were analyzed as described previously (Ferreres et al., 1992), and the main flavonoids detected are shown in the chromatogram of Figure 2. Flavanones, flavones, and flavonols are detected, as well as caffeic acid derivatives, such as dimethyl allyl caffeate and phenyl ethyl caffeate, which are characteristic of propolis. These results coincide with those reported previously that suggest that poplar bud exudates are the main source for phenolic compounds present in propolis. The study of propolis flavonoids is useful because it shows which flavonoids, among those detected in rosemary honey, are incorporated from propolis and which originate from

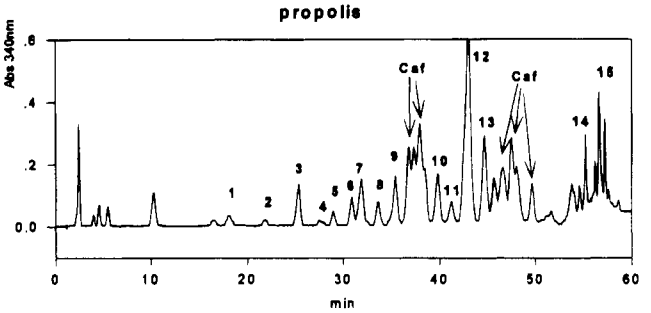

Figure 2. HPLC chomatogram of propolis phenolics: (**1**) pinobanksin; (**2**) quercetin; (**3**) luteolin; (**4**) 8-methoxykaempferol; (**5**) kaempferol; (**6**) apigenin; (**7**) isorhamnetin; (**8**) quercetin 3,3′-dimethyl ether; (**9**) pinocembrin; (**10**) quercetin 7,3′-dimethyl ether; (**11**) quercetin 3,7-dimethyl ether; (**12**) chrysin; (**13**) galangin; (**14**) unidentified flavanone; (**15**) tectochrysin; (**Caf**) caffeic acid esthers (among them phenylethyl caffeate and dimethylallyl caffeate).

nectar and could be useful markers of the floral origin. Unfortunately, kaempferol, the expected marker for rosemary nectar, is also present in propolis (**5**), although is detected as a minoritary constituent (Figure 2).

Flavonoids from Rosemary Honey. The flavonoids present in rosemary honey samples produced in different regions in Spain were studied. Honey samples produced in the same geographical region where nectar was gathered (Castilla-La Mancha) and commercial samples from other regions were analyzed. Pollen composition was analyzed in those samples collected in Castilla-La Mancha (Table 2) and the results obtained indicate that these samples could be considered as excellent monofloral rosemary honeys (the percentage of rosemary pollen was in general between 25 and 59%, and only one sample contained 15%). All samples showed a common flavonoid profile, as illustrated in Figure 3. When comparing these flavonoid profiles with that of propolis (Figures 2 and 3), the main difference is that kaempferol (**5**) is much more relevant in the chromatograms obtained from honey than in that of propolis. In Tables 2 and 3, the amounts of the different flavonoids present in the analyzed samples are shown. These results indicate that kaempferol (**5**) is a preeminent flavonoid in the different samples analyzed, whereas the glycoside (**B**), which was the main constituent in nectar, was not detected at all. In honey samples collected in Castilla-La Mancha, the total amount of flavonoids ranged between 1.6 and 16.1 μg of flavonoids/g of honey. This high variability is not unexpected if we take into account that most of the flavonoids present in honey originate from propolis and its content depends on the propolis contamination of beeswax and other parts of the hive. Therefore, those flavonoids coming from nectar should be less variable in honey than those coming from propolis. When the coefficient of variation (CV = standard deviation/mean • 100) was calculated for the individual flavonoids, the smallest value was found for kaempferol (26.0); this result supports the floral origin of this flavonoid (Table 2). Another compound with a small CV was quercetin (**2**), which is the aglycone of the other minor constituent of rosemary nectar (**A**). However, the rest of flavonoids that come from propolis and/or beeswax, and are incorporated into honey to different extents without any relationship with the floral origin of honey [i.e. pinobanksin (**1**), pinocembrin (**9**), chrysin (**12**), galangin (**13**) and tectochrysin (**15**)], were present in very variable amounts (for instance from 0.1−3.3 μg/g of honey in the case of pinobanksin) and were responsible for the

Table 2. Flavonoids in Rosemary Honey Samples Collected in the Same Geographical Region as Nectar[a]

honey samples	% rosemary pollen	flavonoids											
		1	2	3	4	5	6	7	9	12	13	15	total
G-332	50	0.18	0.11	0.03	0.20	0.52	0.12	0.19	0.36	1.27	0.14	0.04	3.26
G.293	35	2.58	0.28	0.61	0.34	0.72	0.57	0.67	1.44	2.89	1.25	0.24	11.59
G-328	59	0.10	0.13	0.09	0.03	0.44	0.03	0.06	0.12	0.48	0.11	0.04	1.63
G-337	25	0.96	0.11	0.28	0.06	0.44	0.27	0.55	0.98	1.00	0.46	0.13	6.24
G-338	15	0.57	0.28	0.23	0.20	0.70	0.24	0.39	0.75	2.20	0.47	0.18	6.21
G-334	55	3.30	0.30	0.88	0.23	0.85	1.02	0.97	2.56	3.89	1.40	0.20	16.14
G-329	51	0.14	0.31	0.04	0.10	0.76	0.03	0.15	0.32	0.75	0.14	0.10	2.84
mean		1.12	0.22	0.31	0.17	0.63	0.33	0.43	0.93	1.93	0.58	0.13	6.84
sd		1.30	0.09	0.32	0.11	0.16	0.36	0.33	0.85	1.21	0.53	0.08	5.26
CV[b]		116.0	43.7	104.3	65.5	26.0	110.0	76.6	90.8	62.8	90.9	58.7	76.80

[a] Values are μg/g of honey. (1) pinobanksin; (2) quercetin; (3) luteolin; (4) 8-methoxykaempferol; (5) kaempferol; (6) apigenin; (7) isorhamnetin; (9) pinocembrin; (12) chrysin; (13) galangin; (15) tectochrysin. [b] Coefficient of variation (% of rosemary pollen analysis in accordance to Louveaux et al. (1978)).

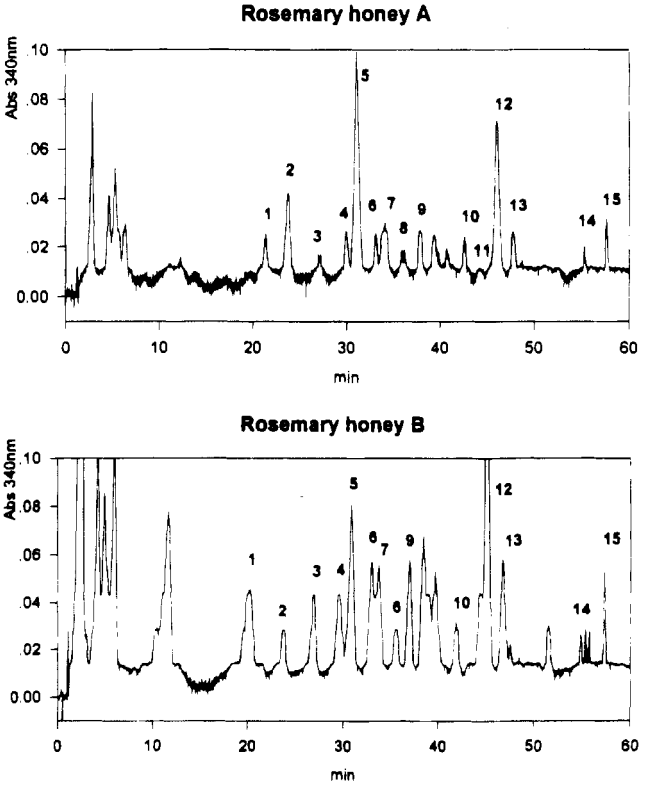

Figure 3. HPLC chromatograms of rosemary honey flavonoids (samples G-329 **A** and R-18 **B**), with detection at 340 nm: (**1**) pinobanksin; (**2**) quercetin; (**3**) luteolin; (**4**) 8-methoxykaempferol; (**5**) kaempferol; (**6**) apigenin; (**7**) isorhamnetin; (**8**) quercetin 3,3′-dimethyl ether; (**9**) pinocembrin; (**10**) quercetin 7,3′-dimethyl ether; (**11**) quercetin 3,7-dimethyl ether; (**12**) chrysin; (**13**) galangin; (**14**) unidentified flavanone; (**15**) tectochrysin.

variations in the total flavonoid observed in the different honey samples analyzed. The differences in the amount of flavonoids present in the rosemary honey samples analyzed are clearly observed in Figure 3. The content of kaempferol ranged between 0.44 and 0.85 μg/g of honey, and its content has no relationship with the percentage of rosemary pollen present in the different honey samples (Table 2). These results are in agreement with those previously found for citrus honey (Ferreres et al., 1993).

In the available commercial samples, the total amount of flavonoids ranged between 7 and 20 μg/g of honey (Table 3), and the kaempferol content ranged between 0.62 and 1.18 μg/g of honey. As observed in the honey samples from Castilla-La Mancha, the CV for kaempferol was again the smallest (18.47).

Other flavonoids and phenolic acid derivatives, which are characteristic of propolis, were also observed in very different amounts in the analyzed honey samples. Thus, the flavonoids quercetin 3,3′-dimethyl ether (**8**), quercetin 7,3′-dimethyl ether (**10**), and quercetin 3,7-dimethyl ether (**11**), an unidentified lipophilic flavanone (**14**), the cinnamic acids caffeic, *p*-coumaric and ferulic, and the derivatives phenyl ethyl caffeate and dimethylallyl caffeate, were also detected in the majority of the samples. However, in some cases, these compounds were detected in trace amounts, which prevented their quantitation and, therefore, their inclusion into Tables 2 and 3. The presence of rosmarinic acid, a dimeric phenolic derivative comprised of two molecules of caffeic acid and characteristic of rosemary leaves and other related Labiatae species, was not detected in any of the different honey samples analyzed (tested by HPLC with an authentic marker, Apin Chemical Ltd., U.K.).

Principal Component Analysis. This analysis has been previously used to study the structural variability in honey chemical composition (Krauze and Zalewusky, 1991). This test was applied to the flavonoids detected in the different honey samples analyzed (Tables 2 and 3). The study showed that there were three components with eigenvalues >1, which explained 70.1% of the total variance (PC1 = 42.1%; PC2 = 17.0% and PC3 = 11.0%). In Figure 4, a representation of the loadings is shown. It is interesting to remark that **2** (quercetin) and **5** (kaempferol), which are those flavonoids originating from rosemary floral nectar, are very close in the diagram and have values for the PCA1 very close to the −1 value. On the other hand, the propolis flavanones pinobanksin (**1**) and pinocembrin (**9**) are close and located on the other side of the diagram, with PCA1 values close to 1. These results confirm that the origin of kaempferol and quercetin are closely related, as would be expected for those flavonoids coming from nectar.

CONCLUSION

The results show that the main phenolic metabolite in rosemary nectar is kaempferol 3-sophoroside, and quercetin 3-sophoroside is also present as a minor constituent. As could be expected, these glycosides are not detected in honey because they are hydrolyzed by the enzymes present in bee saliva to render the corresponding aglycones. The aglycons kaempferol and quercetin are present in rosemary honey. They originate both from propolis and nectar. Propolis analysis shows that its content of kaempferol is very small, and therefore, the kaempferol detected in rosemary honey should originate mainly from nectar. This assumption is supported by the analysis of the CV of the individual

Table 3. Flavonoids in Commercial Rosemary Honey Samples Produced in Different Geographical Areas in Spain[a]

honey samples	flavonoids											
	1	2	3	4	5	6	7	9	12	13	15	total
R-1	4.73	0.52	1.57	0.64	1.05	1.41	1.59	3.74	3.68	1.48	0.21	20.62
R-2	2.34	0.16	0.51	0.54	0.84	0.57	0.54	1.19	1.38	0.39	0.12	8.58
R-3	2.96	0.24	0.37	0.47	1.18	0.64	0.53	1.50	0.98	0.47	0.18	9.52
R-4	3.16	0.29	0.37	0.33	0.87	0.57	0.50	1.25	1.29	0.55	0.34	9.52
R-5	1.62	0.20	0.46	0.23	0.86	0.61	0.46	1.69	1.77	0.83	0.32	9.05
R-6	3.09	0.21	0.56	0.20	1.13	0.69	0.99	1.55	1.13	1.25	0.23	11.03
R-7	3.89	0.31	0.72	0.18	0.87	0.76	0.91	2.98	2.14	1.32	0.19	14.27
R-8	2.92	0.46	0.81	0.56	1.10	0.75	0.90	2.74	2.07	1.14	0.52	13.97
R-9	2.56	0.37	0.67	0.55	0.95	0.90	0.87	2.26	2.51	0.86	0.25	12.75
R-10	3.72	0.36	0.40	0.13	0.62	0.67	0.66	3.09	2.96	1.19	0.21	14.01
R-11	3.06	0.83	0.67	0.95	1.06	0.77	1.08	2.15	2.34	0.58	0.53	14.02
R-12	3.93	0.36	0.36	0.29	0.90	1.02	0.97	2.96	2.02	1.07	0.22	14.10
R-13	2.14	0.13	0.18	0.16	0.77	0.44	0.25	1.56	1.47	0.94	0.35	8.39
R-14	2.46	0.13	0.40	0.54	0.84	0.83	0.72	1.91	2.16	0.72	0.37	11.08
R-15	4.01	0.55	0.91	0.37	1.14	1.11	0.90	3.42	2.46	1.71	0.30	16.88
R-16	3.12	0.26	0.60	0.30	1.02	0.65	0.67	1.75	1.49	0.66	0.16	10.68
R-17	2.25	0.19	0.46	0.14	0.66	0.50	0.62	2.03	2.17	0.78	0.75	10.55
R-18	5.00	0.39	0.78	0.59	1.18	1.06	1.35	3.71	2.82	2.19	0.66	19.73
R-19	1.85	0.15	0.41	0.38	0.68	0.44	0.41	1.75	2.13	0.81	0.30	9.31
R-20	1.67	0.17	0.26	0.45	0.99	0.48	0.45	1.08	1.03	0.36	0.09	7.03
mean	3.02	0.31	0.57	0.40	0.94	0.74	0.77	2.22	2.00	0.97	0.32	12.25
sd	0.96	0.18	0.30	0.21	0.17	0.25	0.33	0.85	0.70	0.47	0.18	3.71
CV[b]	31.70	55.93	52.83	52.40	18.47	33.90	43.11	38.31	35.02	48.28	55.98	30.30

[a] Values are μg/g of honey. (1) pinobanksin; (2) quercetin; (3) luteolin; (4) 8-methoxykaempferol; (5) kaempferol; (6) apigenin; (7) isorhamnetin; (9) pinocembrin; (12) chrysin; (13) galangin; (15) tectochrysin. [b] Coefficient of variation.

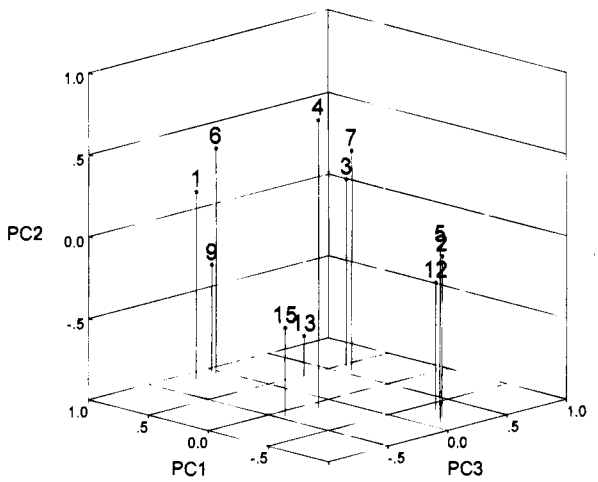

Figure 4. Principal component analysis of rosemary honey flavonoids (for flavonoid identification see Table 1).

flavonoids present in honey, which indicate that kaempferol is the less variable flavonoid in these samples. Similar results were found in rosemary honey samples collected in the same geographical area where nectar and propolis were collected as in commercial rosemary honey samples produced in Spain.

These results indicate that the presence of kaempferol in rosemary honey cannot be considered as proof of its floral origin, because this flavonol can originate from different nectars (Soler et al., 1995). However, its absence or presence in small levels (<0.3 μg/g of honey) could be considered additional evidence of a different floral origin, complementary to other analytical determinations. Analyses of flavonoids from a higher number of rosemary honey samples, produced in different geographical areas, are obviously necessary to extract definitive conclusions about the utility of flavonoid analysis in the determination of the floral origin of rosemary honey.

The results on the phenolic composition of nectar are interesting in addition, because phenolic compounds from extrafloral and floral nectars have rarely been subjected to chemical scrutiny and these substances can have a very important ecological role in plant–insect relationships (Hagler and Buchmann, 1993).

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