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When Floral Availability Fails to Predict Bee Foraging: Melissopalynological, Physicochemical, Phenolic, and Volatile Characterization of a Misclassified 'Onion Honey' Sample

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Abstract: Honey composition reflects floral origin, but crop abundance may not predict bee foraging. This study characterized a honey sample from apiaries adjacent to flowering onion (*Allium cepa* L.) fields using melissopalynological, physicochemical, antioxidant, phenolic, and volatile analyses. The pollen spectrum was dominated by *Sinapis* (28%) and *Sanguisorba* (14%), whereas *Allium* represented 5%. Moisture (20%), pH (4.15), electrical conductivity (357 µS/cm), HMF (6.21 mg/kg), proline (473 mg/kg), and sugars met accepted quality criteria. Total phenolic and flavonoid contents were low, and antioxidant activity was moderate. Phenolic profiling identified p-hydroxybenzoic and protocatechuic acids, quercetin, chrysin, and pinocembrin. Onion flowers showed a sulfur-rich volatile profile dominated by dipropyl disulfide, but major onion-specific volatiles were absent from honey; only dimethyl trisulfide occurred in both matrices. The sample should therefore be classified as multifloral rather than monofloral onion honey. Combining pollen and chemical fingerprints improved botanical-origin assessment.

Keywords: Antioxidant activity, honey, melissopalynology, onion.

Çiçeklerin Bulunabilirliğinin Arıların Besin Arama Davranışını Tahmin Etmede Başarısız Olduğu Durumlar: Yanlış Sınıflandırılmış Bir 'Soğan Balı' Örneğinin Melissopalynolojik, Fizikokimyasal, Fenolik ve Aroma Karakterizasyonu

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Öz: Balın bileşimi botanik kökeni yansıtsa da, çevredeki ürün bolluğu arıların besin arama davranışını her zaman belirlemez. Bu çalışmada, çiçeklenen soğan (*Allium cepa* L.) tarlalarına bitişik kovanlardan alınan bir bal örneği melissopalynolojik, fizikokimyasal, antioksidan, fenolik ve uçucu bileşik analizleriyle karakterize edilmiştir. Polen spektrumunda *Sinapis* (%28) ve *Sanguisorba* (%14) öne çıkarken, *Allium* oranı %5'tir. Nem (%20), pH (4,15), elektriksel iletkenlik (357 µS/cm), HMF (6,21 mg/kg), prolin (473 mg/kg) ve şekerler kabul edilen kalite ölçütlerini karşılamıştır. Toplam fenolik ve flavonoid içerikleri düşük, antioksidan aktivite ise orta düzeydedir. Fenolik profilde *p*-hidroksibenzoik ve protokateşik asitler, kuersetin, krizin ve pinosembrin belirlenmiştir. Soğan çiçekleri dipropil disülfidin baskın olduğu kükürtçe zengin bir uçucu profil göstermiş; başlıca soğana özgü uçucular balda bulunmamış ve iki matriste yalnızca dimetil trisülfid ortak belirlenmiştir. Bu nedenle örnek monofloral soğan balı değil, multifloral bal olarak sınıflandırılmalıdır. Polen ve kimyasal parmak izlerinin birlikte kullanılması botanik köken değerlendirmesini güçlendirmiştir.

Anahtar kelimeler: Antioksidan aktivite, bal, melissopalinoloji, soğan.

INTRODUCTION

Honey is a natural sweet substance produced by *Apis mellifera* from plant nectar or honeydew and is widely valued for its nutritional, sensory, and functional properties (Al et al., 2009; Alvarez-Suarez et al., 2010). Its composition includes sugars, organic acids, amino acids, enzymes, volatile compounds, phenolic acids, and flavonoids. Because floral origin strongly affects these constituents, botanical authentication is essential for quality evaluation and accurate market labeling (Oddo et al., 2004).

Onion (*Allium cepa* L.) is an important crop in the Amaryllidaceae family. Although its flowers provide nectar, *Allium* pollen may be underrepresented in honey because of pollen morphology and limited pollen collection by bees (Louveaux et al., 1978). Consequently, honey produced in onion-dominated landscapes may contain little onion pollen, making classification based solely on melissopalynology uncertain.

Complementary tools such as gas chromatography–mass spectrometry (GC–MS), antioxidant assays, and chromatographic phenolic profiling can strengthen

botanical-origin assessment (Pichichero et al., 2009; Escriche et al., 2023). For honeys associated with underrepresented pollen types, agreement between pollen data and chemical fingerprints provides more reliable evidence than either approach alone.

This study was designed as a single-sample case study to characterize a honey produced adjacent to flowering onion fields by integrating melissopalynological, physicochemical, antioxidant, phenolic, and volatile analyses and by comparing its volatile profile with that of onion flowers. The study tested whether the abundance of a crop in the surrounding landscape was reflected in bee foraging and in the final composition of honey. Given the case-study design, the findings are intended to provide an initial characterization of this particular honey-landscape context rather than broadly generalizable conclusions regarding onion-associated honeys.

MATERIAL AND METHOD

Sample Collection

A honey sample was collected from beehives located adjacent to *Allium cepa* cultivation fields in Balıkesir, Türkiye, during peak flowering. Fresh onion flowers were collected from the same area for comparative volatile analysis. The honey was stored in a clean, airtight container at room temperature and protected from direct sunlight until analysis.

Figure 1. Beehives beside flowering onion fields in Balıkesir, Türkiye.

Melissopalynological Analysis: The botanical origin of the honey was evaluated according to the melissopalynological procedure described by Louveaux et al. (1978). Slides were prepared after acetolysis, and at least 500 pollen grains were counted under light microscopy. Pollen grains were identified using reference collections and pollen morphology atlases, and their relative frequencies were calculated.

Physicochemical Analysis: Moisture, pH, electrical conductivity, CIELAB (L*, a*, b*) color values, hydroxymethylfurfural (HMF), proline, and sugar composition were determined using standardized honey-analysis procedures. Moisture content was determined refractometrically at 20 °C according to the harmonized methods of the International Honey Commission (IHC, 2009). The pH was measured in an aqueous honey solution using a calibrated pH meter, while electrical conductivity was determined at 20 °C in a honey solution corresponding to 20 g dry matter per 100 mL of distilled water, following the IHC procedure. CIELAB color parameters (L*, a*, and b*) were measured using a (CR-400, Minolta, Osaka, Japan) colorimeter. Proline content was determined by the ninhydrin-based spectrophotometric method described by the IHC (2009), with absorbance measured at approximately 510 nm. HMF and sugar composition were determined as described below. All physicochemical measurements were performed in triplicate using separate aliquots of the same honey sample; thus, the replicates represent analytical rather than independent biological replicates.

Fructose, glucose, and sucrose were quantified by high-performance liquid chromatography with refractive-index detection (HPLC–RID). Standard solutions (1.25–40 mg/mL) were filtered through 0.45 µm membranes and used to construct calibration curves. Separation was performed on an amino-bonded column (250 × 4 mm) with acetonitrile–water (80:20, v/v) as the isocratic mobile phase at 1.5 mL/min, a 20 µL injection volume, and 30 °C (Alparslan et al., 2026).

For sugar analysis, 1 g of honey was mixed with 10 mL of 80% ethanol and filtered. The filtrate was evaporated to dryness, reconstituted in ultrapure water, filtered, and injected into the HPLC system.

HMF was determined by reversed-phase HPLC with diode-array detection following the harmonised method of the International Honey Commission (IHC, 2009). Honey (1 g) was diluted to 50 mL with distilled water, filtered through a 0.45 µm membrane, and injected after column equilibration. HMF was identified by comparison with the retention time and spectral characteristics of an authentic standard (Yetkin et al., 2023).

Total Phenolic and Flavonoid Contents: Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method. Briefly, 20 µL of the honey sample solution was mixed with 400 µL of 0.2 N Folin–Ciocalteu reagent and distilled water to a volume of 680 µL. After incubation for 3 min at room temperature, 400 µL of 7.5% Na_2CO_3 solution was added, and the mixture was incubated for 2 h at room temperature. Absorbance was measured at 760 nm. Gallic acid was used for calibration, and TPC was expressed as mg gallic acid equivalents per gram of honey (mg GAE/g).

Total flavonoid content (TFC) was determined by the aluminum-based colorimetric method. The honey sample solution was mixed with 50 µL of 10% aluminum nitrate [$Al(NO_3)_3$] and 50 µL of 1 M ammonium acetate, and the final volume was adjusted to 3 mL with methanol. After incubation for 40 min at room temperature, absorbance was

measured at 415 nm against the corresponding blank. Quercetin was used as the calibration standard, and the results were expressed as mg quercetin equivalents per gram of honey (mg QE/g). All measurements were performed in triplicate.

Antioxidant Activity: Antioxidant activity was evaluated using the ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay (Brand-Williams et al., 1995). Measurements were performed in triplicate. FRAP was expressed as µmol $FeSO_4 \cdot 7H_2O$/g, and DPPH activity was reported as the concentration required to scavenge 50% of the radical (SC_{50}, mg/mL).

Volatile Compound Analysis: Volatile compounds were isolated by headspace solid-phase microextraction (HS–SPME) using a divinylbenzene/carboxen/polydimethylsiloxane fiber. Honey or fresh onion flowers were placed in 40 mL vials and equilibrated at 50 °C for 45 min with magnetic stirring (Demir Kanbur et al., 2021). Volatile compounds were tentatively identified by comparison of their mass spectra with those available in the NIST and Wiley mass-spectral libraries. Retention index (RI) values were also used as complementary criteria to support compound identification. The experimentally obtained RI values are provided in Tables 4 and 5 together with the corresponding chromatographic and spectral information.

Phenolic Compound Analysis: Individual phenolic compounds were determined by reversed-phase high-performance liquid chromatography coupled with a photodiode-array detector (RP-HPLC-PDA), following the method described by Kara et al. (2022) with minor modifications. Chromatographic analysis was performed using a Shimadzu LC-20AT HPLC system equipped with a PDA detector and a Fortis C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of (A) 2% acetic acid in water and (B) acetonitrile–water (70:30, v/v), applied under programmed gradient conditions. The flow rate was 1.0 mL/min, the injection volume was 20 µL, and the column temperature was maintained at 30 °C. Phenolic compounds were identified by comparing their retention times and UV–Vis spectral characteristics with those of authentic standards. Quantification was performed using external calibration curves prepared from the corresponding phenolic standards, and chromatograms were evaluated at 250, 280, 320, or 360 nm according to the maximum absorbance of each compound. Calibration curves showed good linearity, with correlation coefficients ranging from 0.998 to 1.000. The concentrations of individual phenolic compounds were expressed as µg/g honey.

Statistical Analysis: Analytical measurements were performed in triplicate and are presented as mean ± standard deviation where applicable. Because the study evaluated one honey sample and one composite onion-flower sample, no inferential statistical tests were applied. Volatile compounds were reported as relative peak-area percentages.

RESULTS

Melissopalynological analysis identified 18 pollen taxa (Table 1). *Sinapis* (28%) was the most abundant taxon and was classified as secondary pollen. *Sanguisorba* (14%) and Cistaceae (13%) were the next most abundant taxa, while *Allium* represented 5% of the pollen spectrum. No dominant pollen taxon (≥45%) was detected.

Table 1. Percentages and frequency classes of pollen taxa identified in the honey sample.

Taxon	Percentage (%)	Frequency class
Sinapis	28	**secondary**
Salix	2	rare
Trifolium	3	rare
Sanguisorba	14	minor
Ranunculaceae	4	minor
Cistaceae	13	minor
Rhamnaceae	3	minor
Papaver	2	rare
Pinus	1	rare
Rumex	2	rare
Asteraceae	4	minor
Apiaceae	1	rare
Echium	1	rare
Allium	5	minor
Plantago	2	rare
Campanula	2	rare
Rosaceae	3	rare
Poaceae	3	rare
Undefined	3	-

Frequency classes: dominant pollen (≥45%), secondary pollen (16–44%), minor pollen (3–15%), and rare pollen (<3%) (Louveaux et al., 1978; Sorkun, 2008).

The FRAP value was 4.507 ± 0.040 µmol $FeSO_4 \cdot 7H_2O$/g, and the DPPH SC_{50} value was 33.550 ± 0.950 mg/mL.

TPC and TFC were 0.148 ± 0.060 mg GAE/g and 0.002 ± 0.001 mg QE/g, respectively. The physicochemical, antioxidant, and sugar-composition results are summarized in Table 2.

Table 2. Physicochemical properties, antioxidant activity, and sugar composition of the honey sample.

Parameter	Unit	Result
Electrical conductivity	µS/cm	357
Moisture	%	20.0
pH	-	4.15
Color, L*	-	60.11
Color, a*	-	25.35
Color, b*	-	86.08
Total phenolic content	mg GAE/g	0.148 ± 0.060
Total flavonoid content	mg QE/g	0.002 ± 0.001
FRAP	µmol $FeSO_4 \cdot 7H_2O$/g	4.507 ± 0.040
DPPH SC_{50}	mg/mL	33.550 ± 0.950
HMF	mg/kg	6.21
Proline	mg/kg	473.53 ± 19.57
Fructose	%	31.16
Glucose	%	30.90
Sucrose	%	2.06
Fructose + glucose	%	62.06
Fructose/glucose ratio	-	1.01

The phenolic profile was characterized mainly by p-hydroxybenzoic acid (3.16 µg/g), protocatechuic acid (1.84 µg/g), quercetin (1.61 µg/g), pinocembrin (1.36 µg/g), and chrysin (1.09 µg/g). p-Coumaric acid, trans-cinnamic acid, and gallic acid were also detected at lower concentrations (Table 3).

Table 3. Phenolic composition of the honey sample.

Class	Compound	Concentration (µg/g)
Phenolic acids—Hydroxybenzoic acids		
	p-Hydroxybenzoic acid	3.16
	Protocatechuic acid	1.84
	Gallic acid	0.21
	Chlorogenic acid	ND
	Syringic acid	ND
	Ellagic acid	ND
	Vanillic acid	ND
Phenolic acids—Hydroxycinnamic acids		
	trans-Cinnamic acid	0.44
	Ferulic acid	ND
	p-Coumaric acid	0.72
	Caffeic acid	ND
	Caffeic acid phenethyl ester (CAPE)	ND
Flavonoids—Flavonols		
	Rhamnetin	ND
	Quercetin	1.61
	Rutin	ND
	Myricetin	ND
	Galangin	ND
Flavonoids—Flavan-3-ols		
	Epicatechin	ND
	Catechin hydrate	ND
Flavonoids—Flavones		
	Chrysin	1.09
	Daidzein	ND
	Apigenin	ND
	Luteolin	ND
Flavonoids—Flavanones		
	Pinocembrin	1.36
	Hesperetin	ND
	Naringenin	ND

ND: not detected.

Fifteen chromatographic peaks were recorded in the honey (Table 4). Palmitic acid (36.98% combined area), 1,2-ethanedithiol (12.95%), and decyl alcohol (11.09%) were the principal contributors. Dimethyl trisulfide, the only volatile also identified in onion flowers, accounted for 3.29%.

Twenty-eight volatile compounds were identified in onion flowers, accounting for 100% of the total peak area. The profile was dominated by sulfur-containing compounds and short-chain aldehydes, particularly dipropyl disulfide (25.03%), capronaldehyde (16.01%), 1,3-dimercaptopropane (14.88%), and 3-mercapto-2-butanol (6.20%) (Table 5).

Table 4. Volatile compounds identified in the honey sample.

Peak no.	RI (Retention Index)	RT (min)	Area (%)	Compound	Functional group
1	736	3.232	15.08	1,2-Ethanedithiol	Sulfur-containing compound
2	760	3.680	2.96	Phenethyl alcohol	Aromatic alcohol
3	775	4.449	3.42	Capronaldehyde	Aldehyde
4	962	9.681	3.88	Benzaldehyde	Aromatic aldehyde
5	970	9.965	3.83	Dimethyl trisulfide	Polysulfide
6	996	10.957	3.13	Sorbic aldehyde	Conjugated aldehyde
7	1036	12.479	5.93	2-Ethyl-1-hexanol	Aliphatic alcohol
8	1107	15.326	6.19	Pelargonaldehyde (nonanal)	Aliphatic aldehyde
9	1187	15.365	3.12	Butyrone	Ketone (tentative)
10	1339	23.684	4.15	Butyroin	Hydroxy ketone
11	1648	33.527	12.91	Decyl alcohol	Long-chain alcohol
12	1915	40.970	5.56	2-Methylundecanal	Aldehyde
13	1955	42.404	26.65	Palmitic acid	Fatty acid
15	2319	50.338	3.19	Ethyl n-propyl ketone	Ketone
Total			**100.00**		

Table 5. Volatile compounds identified in onion flowers.

Peak no.	RI (Retention Index)	RT (min)	Area (%)	Compound	Functional group
1	704	2.634	1.85	Pent-2(E)-enal	Aldehyde
2	775	3.963	1.87	Capronaldehyde	Aldehyde
3	831	4.915	1.64	2-Methyl-3-ethylacrolein	Aldehyde
4	852	5.984	2.25	Hex-2(E)-enal	Aldehyde
5	906	7.637	2.38	2,5-Dimethylthiophene	Sulfur heterocycle
6	926	8.356	2.01	2,3-Dimercaptohexane	Thiol
7	934	8.666	0.86	Ethylene glycol monoethyl ether	Ether
8	941	8.904	1.89	Ethyl lactate	Ester
9	970	9.974	0.42	Dimethyl trisulfide	Polysulfide
10	992	10.795	0.41	cis-Limonene oxide	Epoxide
11	1036	12.474	0.47	2-Ethyl-1-hexanol	Aliphatic alcohol
12	1063	13.517	0.27	Hept-2(E)-enal	Aldehyde
13	1111	15.331	16.01	Capronaldehyde	Aldehyde
14	1112	15.395	25.03	Dipropyl disulfide	Disulfide
15	1119	15.652	6.20	3-Mercapto-2-butanol	Thio-alcohol
16	1121	15.710	14.88	1,3-Dimercaptopropane	Thiol
17	1135	16.254	0.89	2,3-Dimethyl-2-methylfuran	Heterocycle
18	1155	17.018	4.23	Isopropyl mercaptan	Thiol
19	1165	17.395	1.27	Butyl lactate	Ester
20	1179	17.904	0.83	2,5-Dimethylthiophene	Sulfur heterocycle
21	1213	19.175	1.04	Caprylaldehyde	Aldehyde
22	1279	21.565	0.88	(E)-Cinnamaldehyde	Aromatic aldehyde
23	1334	23.514	4.98	4-Methylthio-2-butanone	Thio-ketone
24	1344	23.857	1.23	Pelargonic acid	Fatty acid
25	1349	24.016	3.32	Allyl disulfide	Disulfide
26	1579	32.006	1.17	Diallyl tetrasulfide	Tetrasulfide
27	1955	42.410	0.93	Palmitic acid	Fatty acid
28	2301	49.953	0.79	Terpin-3-en-1-ol	Monoterpene alcohol
Total			**100.00**		

DISCUSSION

The absence of a dominant pollen type and the presence of 18 taxa demonstrate that the sample is multifloral. Although the apiary was adjacent to flowering onion fields, *Allium* pollen represented only 5% of the spectrum, whereas *Sinapis* reached 28%. In the present sample, proximity to flowering onion fields did not result in a high proportion of Allium pollen in the honey. This observation also illustrates the difficulty of authenticating honeys associated with potentially underrepresented pollen types by melissopalynology alone. (Louveaux et al., 1978; Escriche et al., 2023).

The electrical conductivity of 357 µS/cm is consistent with a blossom honey of moderate mineral content. Moisture was 20%, at the upper quality limit for blossom honey, while pH 4.15 indicated the acidic character typical of honey. Tananaki et al. (2025) reported comparable mean moisture (19.2 ± 1.9%) and pH (4.03 ± 0.16) for strawberry-tree honey. Accordingly, appropriate storage remains important for minimizing fermentation risk, particularly at the observed moisture level.

Fructose plus glucose accounted for 62.06%, while sucrose was 2.06%, values consistent with mature blossom honey (Codex Alimentarius Commission, 2001; Machado et al., 2022). The low HMF concentration (6.21 mg/kg) indicates limited heat exposure and storage-related deterioration (Halouzka et al., 2016), and the proline content (473.53 mg/kg) supports the natural contribution of bee-derived enzymatic processes (da Silva et al., 2016). The results also complied with the relevant limits of the Turkish Food Codex Honey Communiqué (2020/7).

The relatively low TPC and TFC values were accompanied by assay-dependent antioxidant responses. Compared with lavender honey reported by Kolayli et al. (2024), the sample contained less total phenolic and flavonoid material, yet its FRAP value was higher and its DPPH SC_{50} fell within the range reported for lavender honeys. This divergence emphasizes that antioxidant behavior cannot be inferred solely from total phenolic or flavonoid measurements and may depend on the identities and interactions of individual compounds (Tananaki et al., 2024).

The phenolic fingerprint was dominated by p-hydroxybenzoic and protocatechuic acids, together with quercetin, chrysin, and pinocembrin. These compounds are frequently reported among honey polyphenols and possess recognized antioxidant and anti-inflammatory activities (Chagas et al., 2022). Nevertheless, their concentrations were modest, and the profile should be interpreted primarily as a compositional fingerprint rather than direct evidence of a health effect.

Onion flowers exhibited a sulfur-rich volatile profile dominated by dipropyl disulfide, 1,3-dimercaptopropane, 3-mercapto-2-butanol, and isopropyl mercaptan. This agrees with the characteristic *Allium* chemotype described by Yuasa et al. (2022), in which organosulfur compounds are major contributors to the pungent aroma of onion tissues.

In contrast, the honey profile was dominated by fatty acids, alcohols, and aldehydes, and none of the major onion-flower sulfur markers was detected. Dimethyl trisulfide was the only compound shared by the two matrices; its presence may reflect limited nectar contact or environmental proximity rather than a defining onion contribution. The combined pollen and volatile evidence therefore supports classification of the sample as multifloral honey produced in an onion-dominated landscape, not as monofloral onion honey. Because this is a single-sample case study, the proposed chemical distinctions should be validated using larger, multi-season datasets.

CONCLUSION

This single-sample case study provides an integrated characterization of a honey produced in the vicinity of onion (*Allium cepa* L.) fields based on melissopalynological, physicochemical, antioxidant, phenolic, and volatile analyses. Although the hives were located adjacent to flowering onion fields, *Allium* pollen accounted for only 5% of the pollen spectrum, indicating that proximity to onion cultivation did not result in a high proportion of Allium pollen in this particular honey sample. The sample fulfilled the evaluated quality criteria for moisture, pH, electrical conductivity, HMF, proline, and sugar composition and contained several phenolic compounds, including p-hydroxybenzoic acid, protocatechuic acid, quercetin, chrysin, and pinocembrin.

Comparison of the volatile profiles of honey and onion flowers revealed marked compositional differences. Onion flowers exhibited a sulfur-rich volatile profile characterized by dipropyl disulfide and other organosulfur compounds, whereas these characteristic compounds were largely absent from the honey. Dimethyl trisulfide was the only compound detected in both matrices. Taken together, the pollen and volatile profiles obtained in the present study did not provide sufficient support to classify this sample as monofloral onion honey and instead were more consistent with a multifloral origin. These findings suggest that combining melissopalynological data with complementary chemical profiling may improve the assessment of honeys associated with pollen types that may be underrepresented.

However, because the study was based on a single honey sample and one composite onion-flower sample, the findings should be interpreted as case-specific and should not be generalized to onion-associated honeys without validation using larger, multi-season, and geographically diverse sample sets. Future studies may help determine whether the characteristic volatile compounds observed in onion flowers can serve as potentially informative indicators in the authentication of Allium-associated honeys.

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