



Proteomic and amino acid profiling of monofloral honeys from the Thrace region: insights into composition

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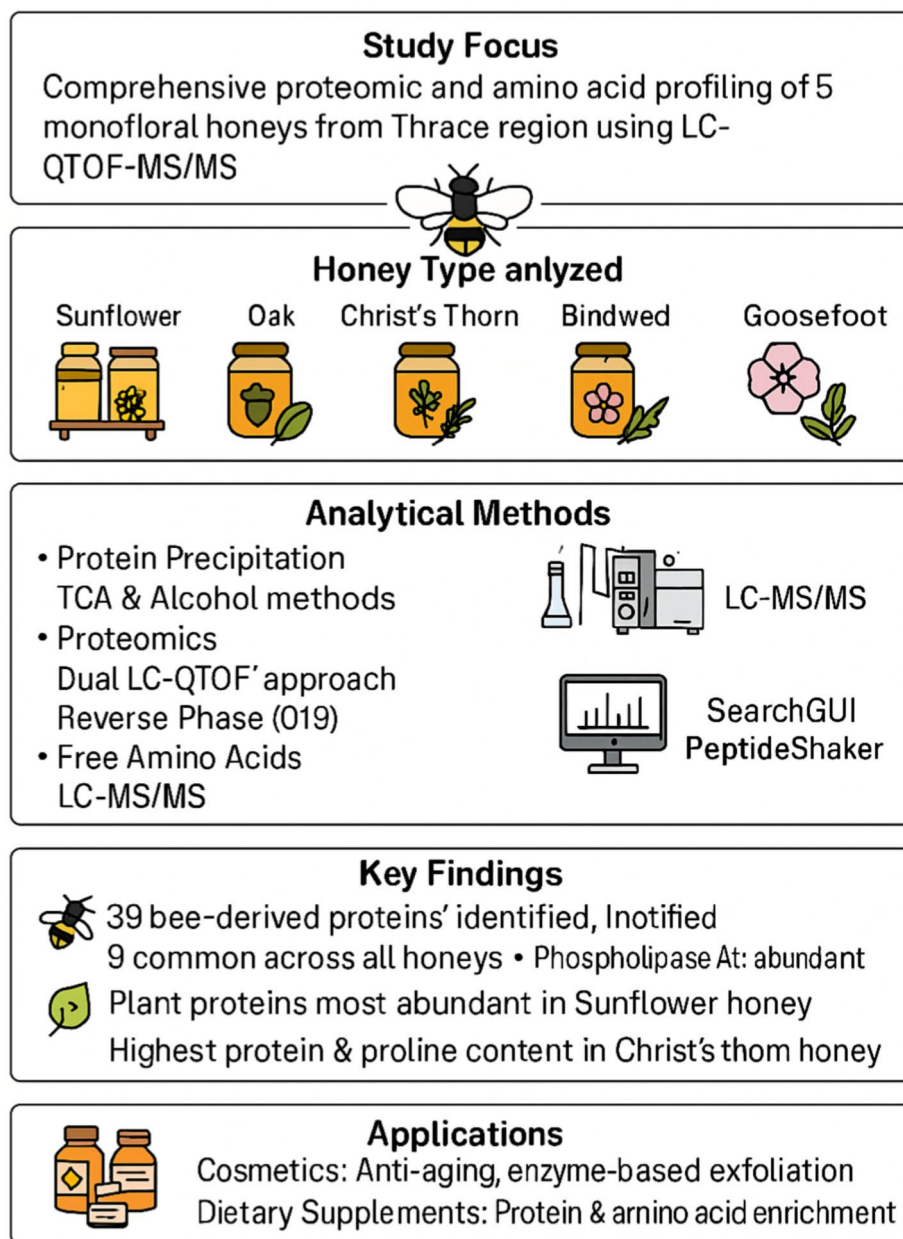
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Abstract

This study conducted a comprehensive proteomic and amino acid analysis of five monofloral honeys from the Thrace region: sunflower (*Helianthus annuus*), oak (*Quercus*), Christ's thorn (*Paliurus spina-christi*), bindweed (*Convolvulus*), and goosefoot (*Chenopodium album*). The combined use of reverse-phase and normal-phase (HILIC) LC-QTOF methods led to the identification of 39 bee-derived proteins, nine of which (including phospholipase A1 and carboxylic ester hydrolase) were common to all honeys. Plant-derived proteins were found to be most abundant in sunflower honey. NanoDrop quantification revealed protein contents ranging from 0.2% to 0.4%, with Christ's thorn honey exhibiting the highest concentration. Free amino acid profiling via LC-MS/MS identified proline as the predominant amino acid, occurring at very high levels, particularly in Christ's thorn honey (10.184 nmol/g). The findings underscore the potential of monofloral honeys for cosmetic applications (e.g., anti-aging formulations) and as dietary supplements. Furthermore, the results suggest that proteomic analysis may serve as an effective method for authenticity testing to combat honey adulteration.

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Graphical abstract**Highlights**

- This research is focused on the detailed accountability of cutting room wastages with respect to four categories that is, splice loss, remnant loss, buffer loss and width loss
- Comprehensive proteomic and amino acid analysis of five monofloral honeys from the Thrace region.
- Identification of 39 bee-derived proteins, with nine common across all honeys.
- Christ's thorn honey exhibited the highest protein and proline content.
- Potential applications in cosmetics (anti-aging formulations) and dietary supplements.
- Proteomics may be an effective method for honey authenticity testing.

Keywords Monofloral honey · Proteomics · Amino acid profiling · LC-QTOF · Foodomics · Honey adulteration · Proteins · Enzymes · Proline

Introduction

Amino acids play critical roles in numerous vital functions, with protein synthesis being the most prominent. These functions include enzyme synthesis, hormone production, immune system support, muscle repair and development, neurotransmitter synthesis, energy production, and nutrient transport. Of the 20 amino acids involved in protein synthesis, nine (valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, threonine, histidine, and lysine) are essential for humans and must be obtained from external sources [1]. Additionally, amino acids such as arginine, cysteine, glycine, glutamine, proline, and tyrosine, which can be synthesized in limited amounts in the human body, are classified as semi-essential or conditionally essential. Proteins, formed by the linkage of amino acids via peptide bonds, constitute the structural foundation of enzymes that catalyze biochemical reactions, are present in certain hormones, and provide structural support to the muscular and skeletal systems, making them fundamental components of bodily function and structure [2].

Honey is one of the most significant natural food substances consumed by humans throughout history. It is estimated that humans began beekeeping and producing honey around 7000 BCE. Due to its high sugar content and energy-providing properties, honey is believed to have played a role in the evolution of the human brain, with its consumption potentially contributing to brain growth [3]. Numerous ancient civilizations and religious texts have documented the benefits of honey, highlighting its various advantages [4, 5]. In traditional medicine, honey has been extensively utilized and is reported to treat a wide range of ailments. In contemporary research, one of the most widely studied properties of honey is its antimicrobial activity [6–8].

Proteomics has advanced significantly with the development of mass spectrometry. The primary objectives of proteomics include the comprehensive identification of proteins within a given matrix and the determination of their functions. By elucidating protein content, studies have indicated that purified enzymes and proteins can be utilized in various applications, such as pharmaceuticals, dietary supplements, and cosmetic products [9]. Proteomics is commonly applied in clinical settings for biomarker discovery in medical research. However, its application in the food industry has gained momentum through foodomics studies [10]. These studies focus on identifying beneficial proteins in food, detecting potentially harmful toxic peptides and proteins, developing raw materials for the cosmetic industry, and producing various dietary supplements [11, 12].

The aim of this study is to comparatively characterize the proteomic and amino acid profiles of five different monofloral honeys (sunflower, oak, Christ's thorn, bindweed,

and goosefoot) from the Thrace region. To achieve this, two complementary proteomic methods based on reverse-phase and HILIC-type normal-phase chromatography were employed to accurately identify plant- and bee-derived proteins associated with the botanical origin of the honeys. In addition, the free amino acid contents of the honeys were quantitatively determined.

Materials and methods

Reagents

Dithiothreitol (Roche, Basel, Switzerland), iodoacetamide (IAA) (Merck, Darmstadt, Germany), mass spectrometry-grade trypsin (Promega, Wisconsin, USA), CaCl₂ (Merck, Darmstadt, Germany), NaHCO₃ (Merck, Darmstadt, Germany), urea (Merck, Darmstadt, Germany), and ultrapure water (UPW) (Elga, Buckinghamshire, UK) were purchased commercially for sample preparation. Additionally, ammonium acetate (Merck, Darmstadt, Germany), formic acid (Merck, Darmstadt, Germany), methanol (Merck, Darmstadt, Germany), and acetonitrile (Merck, Darmstadt, Germany) were used for the LC-QTOF analysis and purification steps.

Sample collection

Five distinct monofloral honeys specific to the Thrace region were obtained directly from local producers, including sunflower, oak, bindweed, Christ's thorn, and goosefoot honeys. The collected samples were stored at room temperature until analysis.

Melissopalynological analysis

Qualitative and quantitative melissopalynological analysis was performed according to the methodology described by Louveaux (1978) [13]. For each monofloral honey sample, 10 g was weighed and 20 mL of ultrapure water (UPW) was added. The mixture was incubated in an ultrasonic bath at 45 °C until a homogeneous solution was obtained and was subsequently centrifuged at 3500 rpm for 30 min. The supernatant was decanted, and microscope slides were prepared from the remaining sediment using glycerin gelatin stained with basic fuchsin. The slides were then examined under a microscope. A pollen atlas was used to identify pollen and associated nectar sources. The dominant pollen contents were determined as sunflower (85%), goosefoot (60%), Christ's thorn (75%), and bindweed (55%). In addition, microscopic imaging analyses confirmed that oak honey was a honeydew honey [14].

Protein quantification using NanoDrop

To optimize protein precipitation, alcohol- and acid-based precipitation methods were employed. Briefly, 5 g of sunflower honey was placed in a 50 mL Falcon tube, and 20 mL of different solutions were added, including methanol, ethanol, methanol–UPW mixtures at varying ratios, ethanol–water mixtures, 10% trichloroacetic acid (TCA), and 20% TCA solutions. The samples were vortexed for 2 min and centrifuged at 9000 rpm for 5 min. The supernatant was discarded, and the pellet was washed with 5 mL of acetone, followed by an additional centrifugation at 9000 rpm for 5 min. The pellet was then reconstituted in 10 mL of ultrapure water containing 1% formic acid for protein dissolution.

Protein quantification was performed using a NanoDrop spectrophotometer (Optizen NanoQ, Korea) [15], which indicated that the highest protein precipitation efficiency was achieved with 10% TCA in UPW. The protein content per gram of honey was determined as follows: oak (3.09 mg), Christ's thorn (3.79 mg), sunflower (2.56 mg), bindweed (2.32 mg), and goosefoot (2.28 mg).

NanoDrop was selected for protein quantification because precipitation with 10% TCA primarily isolates proteins, while most sugars, phenolic compounds, and flavonoids—potential interfering substances—remain in the solution [16].

Proteomics sample preparation

A 2 g honey sample was mixed with 10 mL of 10% TCA in ultrapure water [17]. The mixture was vortexed for 1 min and incubated in a sonicator at 45 °C for 3 min. After cooling to room temperature, the solution was centrifuged at 9000 rpm for 5 min. The supernatant was discarded, and the residue was washed twice with 2 mL of acetone.

The protein pellet was treated with a denaturing solution containing 8 M urea, 100 mM ammonium bicarbonate (ABC), and 5 mM dithiothreitol (DTT) to achieve a final protein concentration of 5 µg/µL. The mixture was incubated at 37 °C for 90 min. Following denaturation, 20 µL of 200 mM iodoacetamide (IAA) was added for alkylation, and the reaction was carried out in the dark at room temperature for 45 min. To quench unreacted IAA, 2 µL of 200 mM DTT was added. The urea concentration was then diluted to ≤ 1 M using a buffer containing 50 mM ABC and 2 mM CaCl₂.

LC–MS grade trypsin (Promega, 0.2 µg/µL) was added at a 1:50 enzyme-to-protein ratio, and the mixture was incubated overnight at 37 °C. The reaction was terminated the following morning by adding 10% formic acid. The final reaction mixture was evaporated to dryness at 35 °C and 1000 rpm [18, 19, 20].

For the purification step, a C18 cartridge (500 mg/6 mL, PromoChrom) was used. The cartridge was conditioned sequentially with 500 µL of methanol, 500 µL of 20 mM ammonium acetate (in UPW), and 500 µL of 1% formic acid in UPW. The dried reaction mixture was reconstituted in 500 µL of 1% formic acid and slowly loaded onto the conditioned cartridge. To remove salts, 500 µL of ammonium acetate followed by 1% formic acid was passed through the cartridge. Pre-prepared solutions of 10%, 30%, 60%, and 90% acetonitrile–ultrapure water (500 µL each) were subsequently applied, and each eluent was collected in separate Eppendorf tubes. The collected eluents were evaporated to dryness using a vacuum centrifuge. The residues were reconstituted in 20 µL of 1% formic acid in ultrapure water and transferred to insert vials for proteomic analysis [21].

LC-QTOF parameters

Proteomic analyses typically employ reverse-phase columns with gradient elution [7]. While reverse-phase columns (C18 or C8) are effective for analyzing longer-chain and semi-polar tryptic peptides, they may be less effective for shorter, more polar peptides containing functional groups such as phosphate, sulfate, sugars, or polar amino acids. To address this limitation, a normal-phase HILIC column was employed, and a new method was developed based on Tang et al. [22].

Analysis using the C18 reverse-phase method identified nine distinct proteins through searches against a bee taxonomy database. When the data from the HILIC column were combined with those from the C18 method, the number of confirmed proteins increased to 13. This approach resulted in a 30–40% increase in both protein identification and sequence coverage. The parameters for both methods are provided in Table 1.

Data evaluation

Data obtained from reverse-phase and normal-phase chromatography were converted from the AB Sciex data format (.wiff) to the .mgf format using MSConvert software. Protein sequences in FASTA format for the relevant taxonomies were downloaded from public databases such as UniProt and NCBI. To screen for bee-derived proteins in monofloral honeys, *Apis mellifera* protein FASTA files were obtained from these databases. For plant-derived proteins, FASTA files were retrieved for *Helianthus annuus* (sunflower), *Quercus* (oak), *Paliurus spina-christi* (Christ's thorn), *Convolvulus* (bindweed), and *Chenopodium album* (goosefoot).

The .mgf data were analyzed using the open-source SearchGUI software, which employed multiple peptide search engines, including X!Tandem, Comet, MyriMatch,

Table 1 LC-QTOF methods parameters

LC Parameters:	High-performance liquid chromatography parameters for proteomics method1: Reverse Phase	High-performance liquid chromatography parameters for proteomics method2: HILIC-type Normal Phase
System:	Agilent 1260 (Autosampler, Binary pump, Column Oven, Solvent Cabin)	Agilent 1260 (Autosampler, Binary pump, Column Oven, Solvent Cabin)
Column:	Agilent Zorbax 300SB-C18 column 1.0 mmx50 mm, 3.5 µm particle size	Agilent Zorbax HILIC Plus 2.1 × 100 mm, 3.5 µm particle size
Mobile phase A:	%0.1 formic acid in ultra pure water	20 mM ammonium formate and % 2 formic acid in ultra pure water
Mobile phase B:	%0.1 formic acid in acetonitril	Acetonitril
Autosampler temperature:	4 °C	4 °C
Flow rate:	0.12 mL·min ⁻¹	0.2 mL·min ⁻¹
Gradient:	0 min. %5 B, 8.0 min. %5 B, 40 min. %40 B, 41 min. %80 B, 55 min. %80 B, 56 min. %5 B, 75 min. %5 B	0 min. %90 B, 3.0 min. %90 B, 15 min. %70 B, 30 min. %40 B, 33 min. %40 B, 35 min. %90 B, 45 min. %90 B
Column temperature:	35 °C	30 °C
Injection volume:	5 µl	5 µl
Total run time:	75 min	45 min
QTOF Parameters:	High resolution mass spectrometry parameters for proteomics method1	High resolution mass spectrometry parameters for proteomics method2
Instrument:	AB-Sciex 4600 QTOF	AB-Sciex 4600 QTOF
Ionization mode	Electrospray ionization positive (+ESI)	Electrospray ionization positive (+ESI)
Ion source gas 1	35 psi	30 psi
Ion source gas 2	45 psi	40 psi
Curtain gas	25 psi	30 psi
Temperature	350 °C	400 °C
Ion spray voltage (ISFV):	5000 Volt	5000 Volt
Declustering potential	120 Volt	100 Volt
Collision energy	10 eV	10 eV
TOFMs	IDA experiment, 300–2000 mass range	IDA experiment, 100–1500 mass range
TOFMsMs	50–1750 mass range, 35 eV collision energy	40–1000 mass range, 35 eV collision energy

Andromeda, and MS-GF+, to identify peptides and proteins from the mass spectra [23]. A 1% false discovery rate (FDR) threshold was applied during the search. The identified peptides were further validated using PeptideShaker software. Qualitative and quantitative amino acid analyses were performed using Agilent MassHunter software.

Free amino acid profile

A 2 g honey sample was weighed using a precision balance, and 10 mL of 1% formic acid solution was added. The mixture was thoroughly vortexed and centrifuged at 5000 rpm for 5 min. From the clear supernatant, 50 µL was collected and mixed with 50 µL of internal standard (IS). Subsequently, 900 µL of an acetonitrile:water mixture (6:1, v/v) was added. The resulting solution was transferred to a vial and analyzed using the method and instrument parameters described by Bayram et al. [24] to determine the amino acid profile.

The internal standard mixture consisted of amino acids labeled with ¹³C, ¹⁵N, and deuterium and was purchased from Cambridge Isotope Laboratories. Amino acid calibration standards were obtained from Jasem Laboratory (Istanbul, Türkiye). All method parameters are provided in Table 2 [25].

Discussion and results

NanoDrop analysis revealed that monofloral honeys contain approximately 0.2–0.4% protein. The highest protein content was observed in Christ's thorn honey (approximately

Table 2 LC-MsMs methods parameters

High-performance liquid chromatography parameters for free amino acids analysis	
Column	Jasem amino acid column
Mobile phase A	25 mM ammonium formate, 2% formic acid in UPW
Mobile phase B	Acetonitrile
Autosampler temperature	4 °C
Flow rate Gradient:0 min. %78 B, 1.0 min. %78 B, 4.0 min. %300 B, 4.5 min. %30 B, 5.5 min %78 B, 10 min. %78 B	0.7 mL·min ⁻¹
Column temperature	30 °C
Injection volume	5 µl
Total run time	10 min
Mass spectrometry (Agilent 6460 LC-MsMs) parameters for free amino acids analysis	
Ionization mode	Electrospray ionization positive and negative
Gas temperature	150 °C
Gas flow	10 L·min ⁻¹
Nebulizer	40 Psi
Sheath gas temperature	275 °C
Sheath gas flow	10 L·min ⁻¹
Capillary voltage	2000 V positive, 0 V negative
Nozzle voltage	0 V
Scan type	Multiple Reaction Monitoring

Table 3 Proteins transferred from honeybee to monofloral honey

	Protein Inference	Main Accession	Description	<i>Helianthus Annuus</i> (Sunflower Honey)	<i>Quercus</i> (Oak Honey)	<i>Paliurus Spina-christi</i> (Christ's thorn Honey)	<i>Convolvulus</i> (Bindweed Honey)	<i>Chenopodium Album</i> (Goosefoot Honey)
1	Single Protein	A0A7M7MR23	Phospholipase A1 (A0A7M7MR23_APIME)	✓	✓	✓	✓	✓
2	Single Protein	A0A7M7IPH8	Carboxylic ester hydrolase (A0A7M7IPH8_APIME)	✓	✓	✓	✓	✓
3	Single Protein	D3JZ08	Bee-milk protein (D3JZ08_APIME)	✓	✓	☒	✓	✓
4	Single Protein	A0A7M7MM52	Uncharacterized protein LOC413627 (A0A7M7MM52_APIME)	✓	✓	✓	☒	☒
5	Single Protein	O18330	Major royal jelly protein 1 (MRJP1_APIME)	✓	✓	✓	✓	✓
6	Related Proteins	A0A7M6W5F9	Bee-milk protein (A0A7M6W5F9_APIME)	✓	✓	✓	✓	✓
7	Single Protein	O77061	Major royal jelly protein 2 (MRJP2_APIME)	✓	✓	✓	✓	✓
8	Related Proteins	Q17061	Major royal jelly protein 4 (MRJP4_APIME)	✓	☒	☒	✓	✓
9	Single Protein	A0A7M7R8W1	Uncharacterized protein LOC408608 (A0A7M7R8W1_APIME)	✓	✓	✓	✓	✓
10	Single Protein	Q17058	Alpha-glucosidase (MAL1_APIME)	✓	✓	✓	✓	✓
11	Related Proteins	A0A7M7H1V4	Transmembrane channel-like protein 3 isoform X1 (A0A7M7H1V4_APIME)	✓	☒	☒	☒	☒
12	Single Protein	A0A7M7R5K0	Glucosylceramidase (A0A7M7R5K0_APIME)	✓	☒	☒	☒	✓
13	Related Proteins	Q17060	Major royal jelly protein 3 (MRJP3_APIME)	✓	✓	✓	✓	☒
14	Related Proteins	A0A7M7IGF5	Uncharacterized protein LOC102656661 isoform X2 (A0A7M7IGF5_APIME)	✓	☒	☒	☒	☒
15	Single Protein	Q3L632	Bee-milk protein (Q3L632_APICA)	✓	☒	☒	☒	✓

Table 3 (continued)

16	Single Protein	Q8N0N7	Alpha-amylase (Q8N0N7_APIME)	✓	✓	✓	☒	☒
17	Related Proteins	A0A7M7I Q52	Phospholipase A1 (A0A7M7IQ52_APIME)	✓	✓	☒	☒	☒
18	Single Protein	A0A7M7G M85	CLIP domain-containing serine protease (A0A7M7GM85_APIME)	✓	✓	✓	☒	☒
19	Related Proteins	A0A8U0W Q84	Bee-milk protein (A0A8U0WQ84_APIME)	✓	✓	✓	✓	✓
20	Related Proteins	A0A7M7H 2Y6	Uncharacterized protein LOC100578893 isoform X2 (A0A7M7H2Y6_APIME)	✓	☒	☒	☒	☒
21	Related Proteins	A0A8U0W Q67	Glucose oxidase (A0A8U0WQ67_APIME)	✓	✓	✓	✓	✓
22	Related Proteins	A0A7M6W 876	Alpha-amylase (A0A7M6W876_APIME)	✓	✓	✓	☒	✓
23	Related Proteins	A0A7M7I Q91	Protein roadkill isoform X4 (A0A7M7IQ91_APIME)	✓	☒	☒	☒	☒
24	Unrelated Proteins	A0A7M7G C96	Glucose dehydrogenase [FAD, quinone] (A0A7M7GC96_APIME)	✓	✓	✓	☒	✓
25	Single Protein	A0A7M7R C42	Laccase-5 (A0A7M7RC42_APIME)	✓	✓	☒	☒	✓
26	Single Protein	A0A7M7I Z4	Uncharacterized protein LOC107964884 (A0A7M7IIZ4_APIME)	☒	✓	☒	☒	☒
27	Related Proteins	A0A7M7L 3M5	Cilia- and flagella-associated protein 58 isoform X1 (A0A7M7L3M5_APIME)	☒	✓	☒	☒	☒
28	Single Protein	A0A7M7L 8U7	LOW QUALITY PROTEIN: formin-2 (A0A7M7L8U7_APIME)	☒	✓	☒	☒	☒
29	Single Protein	A0A7M7S QQ4	Bee-milk protein (A0A7M7SQQ4_APIME)	☒	✓	✓	☒	☒
30	Single Protein	A0A7M7M KV5	Negative elongation factor B (A0A7M7MKV5_APIME)	☒	✓	☒	☒	☒
31	Related Proteins	A0A7M7I 65	Radial spoke head protein 3 homolog isoform X2 (A0A7M7I65_APIME)	☒	✓	☒	☒	☒

Table 3 (continued)

32	Single Protein	A0A7M7LRW3	Glucose dehydrogenase [FAD, quinone] (A0A7M7LRW3_APIME)	☒	✓	✓	☒	☒
33	Single Protein	A0A7M7H2Q2	Uncharacterized protein LOC724133 (A0A7M7H2Q2_APIME)	☒	☒	✓	☒	☒
34	Single Protein	A0A7M7MNX1	Uncharacterized protein LOC411330 (A0A7M7MNX1_APIME)	☒	☒	☒	☒	✓
35	Single Protein	A0AA47GHV0	Uncharacterized protein (A0AA47GHV0_9LACO)	☒	☒	☒	☒	✓
36	Single Protein	A0A7M7MWS9	Mismatch repair endonuclease PMS2 (A0A7M7MWS9_APIME)	☒	☒	☒	☒	✓
37	Single Protein	A0A7M7SRP5	Uncharacterized protein LOC411486 (A0A7M7SRP5_APIME)	☒	☒	☒	☒	✓
38	Related Proteins	A0A8U1GQ41	Venom serine protease 34 (A0A8U1GQ41_APIME)	☒	☒	☒	☒	✓
39	Related Proteins	A0A7M7GVJ0	Glucose-methanol-choline oxidoreductase N-terminal domain-containing protein (A0A7M7GVJ0_APIME)	☒	☒	☒	☒	✓

0.38%). Proteomic analysis of the monofloral honeys, based on searches against a bee taxonomy database, identified 39 distinct proteins, nine of which were consistently detected across all samples. Relative concentration assessments based on peak intensities indicated that phospholipase A1 and carboxylic ester hydrolase were present in higher abundances.

Monofloral honey is defined as honey derived predominantly from a single floral source, with pollen or nectar content exceeding 45% [26]. In the Thrace region, monofloral varieties such as sunflower, Christ's thorn, bindweed, oak, and goosefoot honeys are commonly produced. Previous proteomic studies on monofloral honeys have aimed to determine botanical origin and identify specific markers, however, these efforts have had limited success [27]. Proteomic analyses of commercially important manuka honey have identified 17 distinct plant-derived proteins [28]. Recent studies using QTOF, Orbitrap, and MALDI-TOF have detected royal

jelly peptides, although the complete protein profile has not yet been fully elucidated [29]. Similarly, MALDI-TOF studies on acacia and canola monofloral honeys attempted to identify marker proteins, despite statistically significant mass differences, the protein profiles could not be fully characterized [30].

In the present study, a detailed proteomic profile of monofloral honeys was established. Using an LC-QTOF-based proteomic approach and two complementary chromatographic methods, proteins of both bee and plant origin were identified. Sunflower honey contained 25 bee-derived and 14 plant-derived proteins. The proteins identified in the monofloral honeys, including those consistently detected across samples, are presented in Table 3.

Taxonomic analysis of plant-derived proteins in monofloral honeys showed that sunflower honey contained the highest number of plant-derived proteins. Overall, plant-derived proteins were less numerous than bee-derived proteins. The plant-derived proteins identified in the monofloral

honey is presented in Table 4. These proteins are likely to originate from pollen.

The amino acid contents of sunflower (*Helianthus annuus*), oak (*Quercus*), Christ's thorn (*Paliurus spina-christi*), bindweed (*Convolvulus*), and goosefoot (*Chenopodium album*) honeys were determined using LC–MS/MS. Notably, Christ's thorn honey exhibited a high proline content. Previous studies have also reported elevated proline levels in certain monofloral honeys. For example, proline concentrations in black cumin and thyme honeys were reported to range from 1500–2000 mg/kg and 800–1400 mg/kg, respectively.

These variations have been attributed to factors such as plant morphology, climate, and harvest period. Christ's thorn is a plant species that thrives in arid and hot regions.

To cope with drought and heat stress, it may accumulate high levels of proline in its nectar, which may explain the elevated proline concentration (10,185 nmol/g, approximately 1172 mg/kg) observed in its honey. The proline values in the other honeys were consistent with previously reported data, particularly for oak and sunflower honeys [31–35]. The amino acids and their quantities in all honeys are presented in Table 5.

In conclusion, palynological analyses were performed on five different monofloral honeys obtained from local producers in the Thrace region. The total protein contents of the honeys were determined using NanoDrop, and their free amino acid profiles and individual amino acid quantities were characterized through chromatographic analyses. Proteomic analysis enabled the identification of both

Table 4 Plant derived proteins in monofloral honeys

<i>Helianthus Annuus (Sunflower Plant)</i>			
	Protein Inference	Main Accession	Description
1	Single Protein	A0A251U2C2	Putative berberine/berberine-like, FAD-binding, type 2 (A0A251U2C2_HELAN)
2	Unrelated Proteins	A0A251SI56	Putative pectinesterase, catalytic (A0A251SI56_HELAN)
3	Related Proteins	A0A251UKI3	Gnk2-like domain-containing protein (A0A251UKI3_HELAN)
4	Related Proteins	A0A251RN28	Pectate lyase (A0A251RN28_HELAN)
5	Single Protein	A0A251SC22	Putative SGNH hydrolase-type esterase domain-containing protein (A0A251SC22_HELAN)
6	Related Proteins	A0A251SRR2	Plant basic secretory protein (BSP) family protein (A0A251SRR2_HELAN)
7	Single Protein	A0A251RMM7	Dirigent protein (A0A251RMM7_HELAN)
8	Single Protein	A0A251UU03	ANTH domain-containing protein (A0A251UU03_HELAN)
9	Single Protein	A0A251UJI1	Putative pentatricopeptide repeat-containing protein (A0A251UJI1_HELAN)
10	Single Protein	A0A251UEC5	Glycosidase (A0A251UEC5_HELAN)
11	Single Protein	A0A251RXI5	Uncharacterized protein (A0A251RXI5_HELAN)
12	Single Protein	A0A251TPJ1	Desiccation-related protein PCC13-62 (A0A251TPJ1_HELAN)
13	Related Proteins	A0A251TS73	Plant basic secretory protein (BSP) family protein (A0A251TS73_HELAN)
14	Single Protein	A0A251U8T0	Gnk2-like domain-containing protein (A0A251U8T0_HELAN)
<i>Quercus (Oak plant)</i>			
	Protein Inference	Main Accession	Description
1	Single Protein	A0A7N2MZX4	Peptidyl-prolyl cis–trans isomerase (A0A7N2MZX4_QUELO)
<i>Paliurus Spina-christi (Christ's Thorn Plant)</i>			
	Protein Inference	Main Accession	Description
1	Unrelated Proteins	A0A6P3ZHR5	Metalloendoproteinase 2-MMP-like (A0A6P3ZHR5_ZIZJJ)
2	Single Protein	A0A6P4ALA6	Endochitinase-like (A0A6P4ALA6_ZIZJJ)
<i>Convolvulus (Bindweed Plant)</i>			
	Protein Inference	Main Accession	Description
1	Single Protein	A0A0K1NZX4	MutL-like protein 1 (Fragment) (A0A0K1NZX4_9APIA)
<i>Chenopodium Album (Goosefoot Plant)</i>			
	Protein Inference	Main Accession	Description
1	Single Protein	L0P183	Maturase K (L0P183_9CARY)

bee- and plant-derived proteins. To improve the detection of polar peptides, a normal-phase proteomic method based on a HILIC column was developed. Data generated from both reverse-phase and normal-phase methods were combined and analyzed using SearchGUI software, and the identified peptides were subsequently validated using PeptideShaker software.

Conclusion

Proteomic analysis of monofloral honeys identified proteins originating from both bees and floral sources. Nine bee-derived proteins were consistently detected across all monofloral honeys. Among these, the enzymes phospholipase A1 and carboxylic ester hydrolase are particularly noteworthy.

Table 5 Amino acid profiles and quantities of monofloral honeys

Compound	<i>Helianthus Annuus</i> (Sunflower Honey)	<i>Quercus</i> (Oak Honey)	<i>Paliurus Spina-christi</i> (Christ's Thorn Honey)	<i>Convolvulus</i> (Bindweed Honey)	<i>Chenopodium Album</i> (Goosefoot Honey)	Unit
L-Tryptophan	6.6142	20.6347	7.0492	1.7007	1.2370	nmol/g
Taurine	37.3876	16.6474	18.6419	29.6408	30.7974	nmol/g
L-Phenylalanine	144.6016	282.0224	284.4072	97.3872	124.8227	nmol/g
L-Tyrosine	52.4548	133.1425	52.8365	30.9235	33.2741	nmol/g
L-Leucine	56.8801	50.2839	73.6218	29.7208	109.2013	nmol/g
L-isoleucine	78.1589	55.3835	82.5010	32.7124	121.4644	nmol/g
L-Methionine	6.8597	7.0694	5.2994	2.4981	6.3119	nmol/g
Gamma-aminobutyric acid	65.1039	371.2799	38.6869	74.9650	73.7450	nmol/g
3-Amino isobutyric acid	nd	nd	nd	nd	nd	nmol/g
L-Norvaline	0.6246	0.5102	0.2129	nd	1.7337	nmol/g
L-Valine	176.4444	220.2617	298.0304	63.6757	294.9895	nmol/g
L-2-Aminoadipic acid	5.0986	28.8086	25.5498	58.3848	76.8580	nmol/g
L-Glutamic acid	384.3604	1146.4718	526.0650	242.4875	317.3060	nmol/g
Ethanolamine	16.9285	21.9856	9.2368	32.1611	26.5670	nmol/g
Beta-Alanine	84.5488	76.0639	86.9088	114.7019	85.8932	nmol/g
L-Aspartic acid	169.2328	1085.5809	249.0342	248.7819	370.5386	nmol/g
L-2-aminobutyric acid	nd	nd	nd	nd	nd	nmol/g
L-Threonine	119.8446	154.5286	150.4179	79.5267	219.5372	nmol/g
L-Serine	173.2862	296.5220	266.7912	97.4107	396.0965	nmol/g
L-Alanine	208.6525	578.1705	393.4423	215.9534	726.0883	nmol/g
Sarcosine	90.9557	128.7042	124.3633	117.7304	125.7005	nmol/g
L-Glycin	64.7053	131.4229	68.2358	79.4218	127.7031	nmol/g
Trans-4-hydroxy L-proline	185.4367	456.2921	466.9606	65.6852	65.8096	nmol/g
L-asparagine	248.9440	847.5135	365.6098	77.0213	136.5522	nmol/g
L-Proline	5779.9561	8870.8121	10184.9829	6338.3672	7237.0014	nmol/g
L-Glutamine	189.0382	408.8326	177.9329	68.6441	446.7494	nmol/g
L-Homocitrulline	nd	nd	nd	nd	nd	nmol/g
L-Citrulline	8.3486	119.0053	7.7847	4.9986	3.9038	nmol/g
O-Phospho-L-Serine	nd	nd	nd	nd	nd	nmol/g
O-Phosphoryl Ethanolamine	nd	nd	nd	nd	nd	nmol/g
DL-Homocystine	nd	nd	nd	nd	nd	nmol/g
L-Cystine	nd	nd	nd	2.1250	24.8962	nmol/g
L-Cystathionine	nd	nd	nd	nd	nd	nmol/g
L-Histidine	51.3816	10.6402	19.9030	33.2097	44.2600	nmol/g
L-Arginine	45.1762	144.7705	16.3516	68.1216	30.4817	nmol/g
Argininosuccinic acid	nd	nd	nd	4.4224	4.7725	nmol/g
L-ornithine	25.8618	44.2089	4.6925	14.4815	3.7352	nmol/g
DL-5-Hydroxy lysine	nd	nd	nd	0.4212	0.3634	nmol/g
L-Lysine	118.6137	81.2415	28.5120	82.6786	43.7679	nmol/g
L-Carnosine	nd	nd	nd	nd	nd	nmol/g
3-Methyl-L-Histidine	0.2463	0.1686	0.1341	0.3075	0.2378	nmol/g
1-Methyl-L-Histidine	0.6589	2.2556	0.4240	nd	nd	nmol/g
L-Anserine	nd	nd	nd	nd	nd	nmol/g

nd not detected

These enzymes hydrolyze lipids, especially triglycerides and phospholipids, in the presence of water [36, 37]. Their presence may enhance the potential of honey for cosmetic applications, particularly in anti-aging and exfoliating formulations.

Monofloral honeys with varying compositions have been reported to exhibit antifungal and antibacterial activities [38]. Although their phenolic and flavonoid contents differ, this bioactivity appears to be a common characteristic. Based on the proteomic results, the presence of phospholipase A1 and carboxylic ester hydrolase in all samples may contribute to these functional properties.

Furthermore, proteomic analysis represents a promising approach for detecting honey adulteration, a widespread issue in food products. The detection of several royal jelly-associated proteins also suggests that honey may have potential for formulation as a dietary supplement. In this study, complementary C18 and HILIC-based chromatographic methods were applied, resulting in a comprehensive evaluation of the proteome and an increase of up to 40% in the number of identified proteins.

Overall, this study comprehensively characterized both bee- and plant-derived proteins in monofloral honeys and determined their amino acid compositions. Future studies should further investigate the antimicrobial, antifungal, anti-aging, and exfoliating effects of phospholipase A1 and carboxylic ester hydrolase. The developed methods provide a robust framework for determining protein content in food products and may offer valuable applications for the food, pharmaceutical, and cosmetic industries.

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Data availability No data were used for the research described in this article.

Declarations

Competing interest The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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