



Physicochemical properties, phytohormone profiles and quality assessment of honey from the Çoruh Valley of Türkiye

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Abstract

In this study, seventeen floral honey samples collected from Türkiye's Çoruh Valley were examined and a total of 59 parameters were analyzed to evaluate their quality attributes. Accordingly, hydroxymethylfurfural (HMF), diastase activity, fructose, glucose, sucrose, total fructose+glucose (F+G), the fructose/glucose ratio (F/G), color and $\delta^{13}\text{C}$ isotope values were determined together with amino acid profiles, organic acid composition, mineral contents, and phytohormone profiles. All chemical and biological data were evaluated using multivariate statistical approaches, including Principal Component Analysis (PCA), Pearson correlation analysis and Hierarchical Clustering Analysis (HCA), to characterize similarities and differences among the samples. The analytical results demonstrated that honeys from this region comply with established quality standards and exhibit natural purity, balanced chemical composition, and favorable nutritional properties. The detection of diverse phytohormone types and concentrations across the samples, as revealed by the analyses, indicates a rich biochemical signature originating from plant sources. This finding also highlights the functional properties, biological value, and nutritional significance of these honeys. Overall, the results confirm that the floral honeys obtained from Türkiye's Çoruh Valley are high-quality products in terms of both chemical and biological properties.

Keywords Honey · Physicochemical properties · Phytohormones · Mineral content · Amino acids · Organic acids · Quality assessment · Multivariate analysis

Introduction

Honey is a natural sweet substance produced by honeybees through the collection of plant nectar, enzymatic modification, storage in honeycomb cells, and subsequent maturation [1–3]. As a complex natural product, honey primarily contains the monosaccharides glucose and fructose, in addition to a diverse array of organic acids, minerals, vitamins, enzymes, amino acids, pollen grains, pigments, aromatic compounds, and phenolic constituents [4]. The chemical composition and quality attributes of honey, including sensory characteristics such as taste, aroma, and color, are influenced by numerous factors, including climatic conditions, floral origin, bee species, production methods, packaging, and storage duration and temperature [5].

Honey is among the most widely consumed natural sweeteners and aromatic foods worldwide. Its safe consumption and international trade require compliance with established national and international quality and authenticity standards, which provide criteria for evaluating honey quality and origin [1–3]. The quality and authenticity of honey are

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critical not only for trade purposes but also for consumer preferences, as these factors strongly influence purchasing decisions [6]. Standards classify honey according to botanical and geographical origin, production methods, and commercialization practices, and define physicochemical parameters, including compositional, sensory, and textural characteristics, for quality and authenticity assessment.

The botanical and compositional characteristics of honey provide information about its geographical origin, which significantly affects its commercial value. Therefore, determining the compositional differences among honeys produced in different regions is important, as consumers consider both the quality and chemical profile, as well as the geographic provenance, when selecting honey [7].

Carbohydrates constitute the major portion of soluble dry matter in honey, with fructose approximately 38% and glucose approximately 31%. These sugars play a central role in the nutritional and physicochemical properties of honey, influencing crystallization, viscosity, hygroscopicity, and energy content [6]. Amino acids are present at approximately 1% by weight and vary according to floral and geographical origin. Proline is the most abundant amino acid, accounting for 50 to 85% of total amino acids, and is produced by bees during the conversion of nectar into honey. Proline serves as a key indicator of honey maturity and is widely used to detect adulteration with sugar syrups [4, 8]. Studies on floral honeys have identified numerous amino acids, including proline, aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, β -alanine, α -alanine, γ -aminobutyric acid, tyrosine, valine, tryptophan, phenylalanine, isoleucine, leucine, ornithine, and lysine, with proline being the most abundant, followed by phenylalanine and tyrosine [9].

The mineral content of honey is generally low, ranging from 0.02 to 0.3% in floral honeys. Mineral composition is an important factor for evaluating honey quality and authenticity [10]. Mineral content varies according to production practices, soil characteristics, geographical conditions, and botanical origin [11]. Honey contains substantial amounts of potassium, sodium, calcium, and magnesium; lower amounts of iron, copper, manganese, and chloride; and trace amounts of boron, phosphorus, sulfur, silicon, and nickel. Potassium accounts for approximately 80% of total mineral content [6].

Honey also contains various organic acids derived from multiple sources, including acids naturally present in nectar, acids secreted by bees during processing and maturation, and compounds generated through metabolic activity within the comb [12]. Organic acids affect honey's pH, acidity, electrical conductivity, color, and flavor. In addition to contributing to the discrimination of honey according to botanical and geographical origin, these compounds influence

its antioxidant and antimicrobial properties. Among the identified organic acids in honey are oxalic, formic, malic, succinic, pyruvic, acetic, lactic, citric, and gluconic acids [13]. Analyses performed on five different types of honey revealed that gluconic acid was the most abundant organic acid, followed generally by lactic acid [12].

Phytohormones are naturally occurring organic compounds that regulate physiological processes in plants, including growth, differentiation, and development [14]. Phytohormones not only play critical roles in plant physiology but have also been reported to health benefits and medicinal properties in humans [15]. Honey is produced from the nectar of flowers rich in plant hormones, and therefore, the presence of phytohormones in honey can be anticipated. In honey, the presence of 34 phytohormones was detected, including cytokinins (CK), gibberellins (GA), indole-3-acetic acid (IAA), abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA). The kinds of honey is one of the factors that affects the type and amount of phytohormones [16].

Stable Carbon Isotope Ratio Analysis (SCIRA) is employed to assess honey quality and detect adulteration with various sugar syrups. This technique relies on the differential carbon isotope ratios that arise from distinct photosynthetic pathways in plants. Most flowering plants from which bees collect nectar utilize the Calvin cycle (C3) for sugar synthesis, exhibiting $^{13}\text{C}/^{12}\text{C}$ isotope ratios ranging from -21‰ to -32‰ . In contrast, sugars metabolically synthesized in sugarcane and maize, which follow the Hatch-Slack pathway (C4), show $^{13}\text{C}/^{12}\text{C}$ ratios between -12‰ and -19‰ . C4 plants therefore have higher $^{13}\text{C}/^{12}\text{C}$ isotope ratios compared to C3 plants. Consequently, SCIRA is a reliable method for determining honey quality and detecting adulteration with sugar syrups based on carbon isotope signatures [17, 18].

The aim of this study is to determine the composition and quality characteristics of honey produced in the Çoruh Valley, one of Türkiye's ecologically important regions characterized by high biodiversity and rich vegetation. The Çoruh Valley, with its rich vegetation, favorable natural production conditions, and the unique compositional characteristics provided by its high biodiversity, is a high-quality and valuable honey production area in terms of both natural and functional properties. Owing to its diverse plant life, this region provides favorable natural conditions for organic beekeeping and contributes significantly to Türkiye's production of high quality honey [19]. In this context, the fundamental chemical properties of honey samples obtained from the region were determined, and their phytohormone content was analyzed to evaluate the potential effects of these compounds on key honey quality parameters. The data obtained from this study aim to contribute to current

scientific knowledge on the chemical composition of honey and to provide new insights into the relationship between phytohormones and honey quality.

Materials and methods

Honey samples

In this study, seventeen floral honey samples were collected from the North East Anatolian region of Türkiye along the Çoruh Valley (Bayburt-Aydintepe, Erzurum-İspir, Artvin-Yusufeli) (Fig. 1). The honey samples analyzed in this study are floral. The botanical origin of the samples was based on the findings of melissopalynological analyses previously performed on honey samples obtained from the same geographical region [19]. This study demonstrated that honey bees collect nectar from species belonging to different plant families, primarily *Fabaceae*, *Lamiaceae*, and *Asteraceae*, and that honey is produced within this broad botanical diversity. Palynological evaluations show that the dominant nectar sources include meadow yarrow (*Achillea millefolium* L., Asteraceae), clover (*Onobrychis viciifolia* Scop., Fabaceae), and balm (*Glechoma hederacea* L., Lamiaceae). Honey samples used in the study were taken from different producers in the region just after the production season. The samples were taken from 17 different honey producers in

the form of strained honey and were not subjected to any processing. Honey samples were placed in glass jars and kept at room temperature until analysis.

Diastase activity and hydroxymethylfurfural (HMF) analysis

Diastase activity was quantified spectrophotometrically at 660 nm following the established protocol [20]. HMF concentrations were determined at 550 nm using a UV–Vis spectrophotometer according to standard methodology [21].

Sugar analysis

The sugar composition of the samples was determined using high-performance liquid chromatography (HPLC) equipped with a refractive index detector (RID). Approximately 1 g of honey was weighed, dissolved in distilled water, and transferred to a 25 mL volumetric flask. The volume was brought to 25 mL with ultrapure water. The solution was filtered through a 0.45 µm membrane filter prior to injection. Chromatographic separation was performed using a Zorbax carbohydrate analysis column. The mobile phase consisted of acetonitrile and distilled water (80:20, v/v). Analyses were carried out with a flow rate of 1.5 mL/min using a 10 µL injection volume [22].

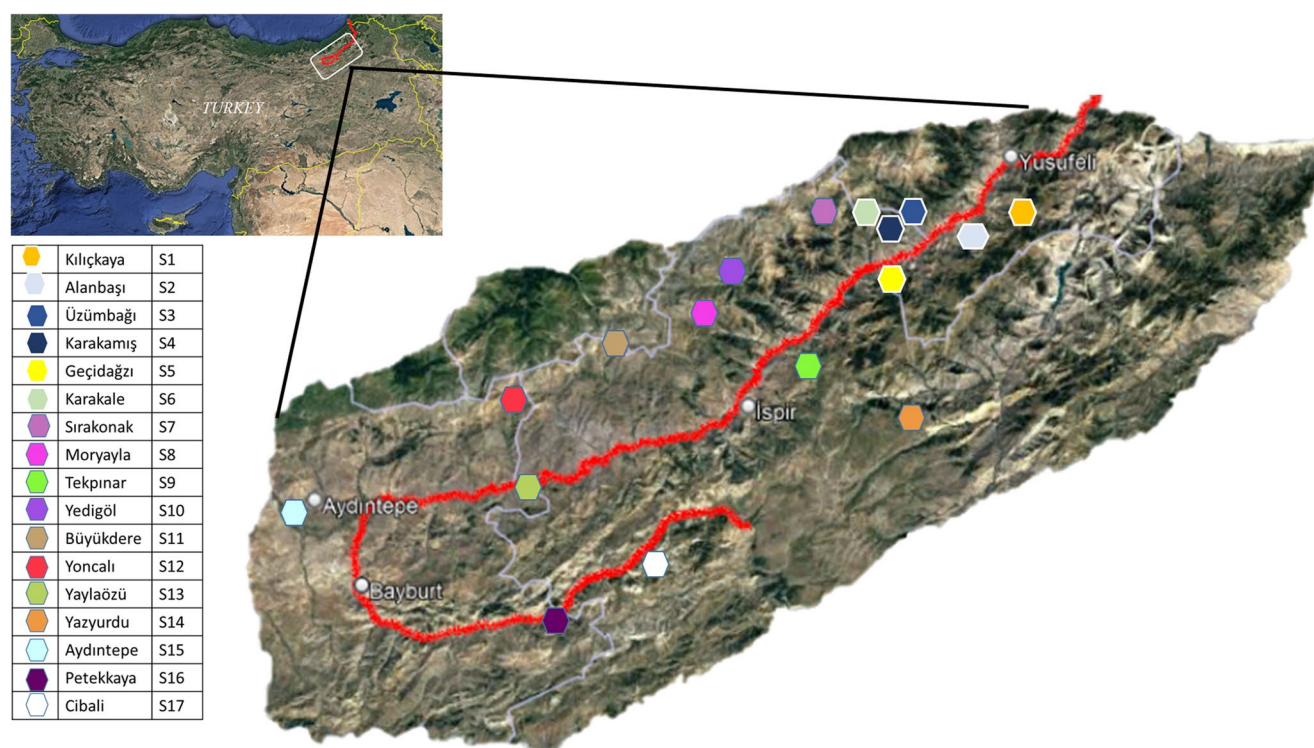


Fig. 1 Areas where honey samples were collected from producers

¹³C/ ¹²C isotope ratio analysis

The carbon isotope ratios of both bulk honey and the protein fraction were determined. Honey samples were completely combusted to generate CO₂, after which ¹³C/¹²C (δ¹³C) ratios were measured using elemental analyzer–isotope ratio mass spectrometry (EA-IRMS). The analyses were performed according to AOAC Official Method 998.12 [23].

Mineral analysis

Mineral quantification was conducted following microwave-assisted acid digestion. Approximately 0.5 g of honey was weighed, mixed with 9 mL of 65% nitric acid and 1 mL of 30% hydrogen peroxide, and subjected to microwave digestion. Prior to ICP-OES analysis, digested samples were diluted to 20 mL with ultrapure water. The concentrations of P, K, Ca, Mg, Na, Fe, Zn, Cu, and Mn were determined using inductively coupled plasma–optical emission spectroscopy (ICP-OES; PerkinElmer, USA) [24].

Amino acid analysis

For amino acid profiling, 1 g of honey was dissolved in 5 mL of 0.1 N HCl and homogenized for 1 min using an Ultra-Turrax. The mixture was centrifuged at 5000 rpm for 5 min, and the supernatant was filtered through a 0.22 μm membrane (Millex Millipore). The filtrates were transferred into HPLC vials for analysis. Amino acid separation was performed using HPLC (HP1100, Agilent) equipped with a diode-array detector (DAD) and a Zorbax Eclipse AAA column (4.6 mm × 150 mm, 5 μm; Agilent Technologies Inc.) in accordance with established methods [25–27].

Organic acid analysis

For organic acid determination, 1 g of honey was diluted with 10 mL of distilled water and homogenized using an Ultra-Turrax. The mixture was centrifuged at 1200 rpm for 50 min, and the supernatant was filtered through a 0.22 μm Millipore membrane filter. Analyses were conducted using an Agilent 1200 HPLC system equipped with a Zorbax Eclipse AAA column (4.6 × 250 mm, 5 μm) and a UV detector. Chromatographic separation was achieved at a flow rate of 1 mL/min and a column temperature of 25 °C [26, 28].

Phytohormones analysis

For phytohormone extraction, 1 g of honey sample was accurately weighed and mixed with chilled 80% methanol (−40 °C), followed by thorough homogenization and incubation in the dark for 24 h. After incubation, the extract was evaporated to

dryness at 35 °C using a rotary evaporator, and the resulting residue was reconstituted in 0.1 M KH₂PO₄ buffer (pH 8.0). The reconstituted solution was then subjected to solid-phase extraction using a Sep-Pak C18 cartridge (Waters) for cleanup, and the phytohormone fractions retained on the cartridge were eluted with 80% methanol. The purified extracts were analyzed by high-performance liquid chromatography (HPLC) equipped with a UV detector (Agilent 1200 system) using a Zorbax Eclipse-AAA C18 analytical column. Chromatographic separation was achieved with a mobile phase consisting of 13% acetonitrile adjusted to pH 4.98, at a flow rate of 1.2 mL/min, with the column temperature maintained at 25 °C [29–31].

Color analysis

Color analysis of honey samples was performed using a Minolta CR-200 (Japan) colorimeter. The L*, a*, and b* color parameters of the samples were determined from the measurements. The L* value represents the brightness level of the sample, with 0 representing black and 100 representing white. The a* value indicates a positive red (+a*) and negative green (−a*) color component; the b* value indicates a positive yellow (+b*) and negative blue (−b*) color component. Color measurements were performed in triplicate for each sample, and the average of the obtained results was used for evaluation.

Statistical Analysis

All analyses were performed in triplicate to ensure analytical reliability. Seventeen honey samples were analyzed, and the results are reported as mean ± standard deviation. To evaluate compositional patterns and classify the honey samples, multivariate statistical analyses, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), were conducted. In addition, the relationship between the variables was determined using the Pearson correlation coefficient (r). In the HCA, hierarchical relationships among the samples were determined using Euclidean distance and the Ward linkage method. PCA and HCA computations were performed using XLSTAT software (Premium trial version, Addinsoft, New York, USA) [32]. These analyses provided insights into sample grouping based on their chemical composition and allowed for the identification of potential patterns related to botanical and geographical origin.

Results and discussion

Some chemical analysis results

Hydroxymethylfurfural (HMF) is a critical quality indicator used to assess both the freshness and potential adulteration

of honey, and it also provides valuable information regarding thermal treatments applied during processing. Fresh honey contains little to no HMF; therefore, its concentration is widely accepted as an essential parameter for evaluating honey freshness and overall quality [6]. In this study, HMF levels in the honey samples ranged from 11.80 to 31.00 mg/kg, with a mean value of 21.57 ± 6.30 mg/kg (Table 1), all of which were below the maximum allowable limit of 40 mg/kg established for floral honeys [1, 3]. HMF formation increases substantially under prolonged heating, elevated processing temperatures, or extended storage periods due to monosaccharide degradation and the Maillard reaction, while adulteration with various sugar syrups can also lead to significantly elevated levels [4]. The mean HMF concentration obtained in this study aligns with previously reported values, such as the range of 1.26–26.28 mg/kg documented in a study examining twelve different honey samples [33]. Overall, the HMF concentrations determined in the analyzed samples indicate that the honeys exhibit acceptable quality characteristics, comply with established regulatory limits, and do not reflect signs of excessive heat treatment or adulteration.

Diastase activity is a key enzymatic parameter originating from honeybees, playing a role in starch degradation, and is widely recognized as an indicator of honey freshness, heat exposure, and storage stability, as the enzyme is sensitive to thermal denaturation and decreases progressively with elevated processing temperatures and prolonged storage periods [17]. Fresh honeys typically exhibit high diastase activity; however, the activity diminishes depending on both the duration and temperature of heat treatment as well as storage conditions [34]. In this study, the diastase activity levels of the honey samples ranged from 8.3 to 23.0, with an average value of 14.6 ± 3.9 (Table 1). These results

align with the values reported by Pasiadis et al. (2017), who determined that diastase activity in floral honeys generally varies between 7.0 and 22.0 [35]. The findings from the present work indicate that the enzymatic structure of the samples was largely preserved, suggesting that the honeys were not exposed to detrimental thermal processing or long-term storage, and therefore meet the internationally recognized quality criteria.

Sugars constitute the majority of the water-soluble dry matter in floral honey, with fructose consistently representing the predominant monosaccharide, followed by glucose. In the present study, the concentrations of fructose (37.40 ± 3.45), glucose (31.18 ± 3.28), and sucrose (1.43 ± 0.89) were quantified (Table 1). The carbohydrate composition of honey is known to vary depending on geographical factors and botanical origin [36]. The mean combined fructose and glucose content was 68.58 ± 5.65 , meeting the minimum quality requirement of 60% established for floral honeys, while sucrose levels in all samples remained below the maximum permissible limit of 5%. The fructose-to-glucose ratio is a key parameter influencing the crystallization tendency of honey and is also widely used to detect adulteration with various sugar syrups [37]. According to the Turkish Food Codex (2020), this ratio should range between 0.9 and 1.4 for floral honeys [3]; the mean ratio obtained in this study (1.20 ± 0.12) fell entirely within these regulatory limits. In a study conducted to determine the physicochemical properties and $^{13}\text{C}/^{12}\text{C}$ isotope ratios of nineteen different floral honey samples, glucose (30.2 ± 1.7), fructose (38.8 ± 1.8), and F/G (1.29 ± 0.5) values were determined [17]. The obtained glucose, fructose, and F/G ratio results were found to be consistent with the findings determined within the scope of this study. Overall, the sugar profile of honey plays a critical role in evaluating product quality and serves as an essential analytical tool for identifying potential adulteration practices.

Table 1 Some chemical parameters and stable carbon isotope ratios of honey sample

Parameters	Mean $\pm$ SD	Minimum	Maximum
Hydroxymethylfurfural (HMF), mg/kg	21.57 ± 6.30	11.80	31.00
Diastase Activity	14.6 ± 3.9	8.3	23.0
Fructose (F), g/100 g	37.40 ± 3.45	28.40	43.40
Glucose (G), g/100 g	31.18 ± 3.28	23.60	36.50
Sucrose (S) g/100 g	1.43 ± 0.89	0.30	3.20
Sum of fructose and glucose (F + G), g/100 g	68.58 ± 5.65	52.00	75.70
Ratio of fructose and glucose (F/G)	1.20 ± 0.12	0.97	1.40
$\delta^{13}\text{C}_\text{p}$ (‰) Protein	-25.64 ± 0.47	-26.50	-25.00
$\delta^{13}\text{C}_\text{H}$ (‰) Honey	-25.62 ± 0.26	-26.10	-25.20
$\delta^{13}\text{C}$ (‰) Differences Protein-Honey	-0.01 ± 0.61	-1.00	0.90
Adulteration(C4 ratio, %)	1.02 ± 0.85	0.1	3.5

SD – standard deviation. $n = 17$

$^{13}\text{C}/^{12}\text{C}$ analysis of honey samples

Stable Carbon Isotope Ratio Analysis (SCIRA) is commonly employed to detect adulteration of honey with various sugar syrups. In this study, the $\delta^{13}\text{C}$ values of honey samples ($\delta^{13}\text{C}_\text{H}$) and the protein fractions extracted from honey ($\delta^{13}\text{C}_\text{p}$) were determined. The $\delta^{13}\text{C}_\text{H}$ values ranged from -26.10‰ to -25.20‰ , with an average of $-25.62 \pm 0.26\text{‰}$, while the $\delta^{13}\text{C}_\text{p}$ values varied between -26.50‰ and -25.00‰ , with a mean of $-25.64 \pm 0.47\text{‰}$ (Table 1). According to AOAC Official Method 978.17, authentic honey should exhibit a $\delta^{13}\text{C}_\text{H}$ value more negative than -23.5‰ , indicating the absence of sugar syrup adulteration [21]. In addition, the C4 sugar content of each sample was calculated using $\delta^{13}\text{C}_\text{H}$ and $\delta^{13}\text{C}_\text{p}$ values in accordance with

AOAC Method 998.12, which defines honey containing less than 7% C4 sugars as authentic [23]. All honey samples in this study exhibited $\delta^{13}\text{C}$ values between -26.10% and -25.20% and contained less than 7% C4 sugars, demonstrating that the samples were genuine and free from adulteration with C4 sugar syrups such as corn or cane syrups. The use of these analytical methods is highly important for reliably detecting adulteration practices involving C4 sugar syrups in honey and plays a critical role in safeguarding product authenticity and consumer safety.

Mineral substance content

The mineral profile of floral honey exhibits substantial variability, primarily influenced by soil composition, geographical and ecological conditions, the botanical diversity of nectar-source plants, and climatic factors [11]. Mineral elements serve as robust chemical markers for discriminating the botanical and geographical origin of honey and play a critical role in authenticity assessment [38]. Moreover, the mineral composition is recognized as a fundamental quality parameter, contributing significantly to the nutritional and functional attributes of honey [39]. In the present study, the concentrations of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) in the seventeen honey samples are presented in Fig. 2, revealing pronounced elemental variability among samples. Among all quantified minerals, K was identified as the predominant macroelement, with an average concentration of $523.08 \pm$

65.70 mg/kg, representing the major contributor to the mineral framework of the samples. Mg ranked second with an average level of 84.21 ± 13.54 mg/kg, while Na and Ca were detected at 66.00 ± 13.80 mg/kg and 61.12 ± 8.25 mg/kg, respectively. The concentration ranges obtained in this study demonstrated strong agreement with previously reported values for multifloral honeys from Türkiye (K: 483.7–3644 mg/kg, Ca: 38.4–179.5 mg/kg, Na: 14.9–83.8 mg/kg, Mg: 16.9–109.5 mg/kg, Zn: 14.29–22.16 mg/kg, Fe: 0.86–5.45 mg/kg, Mn: 0.11–2.64 mg/kg). This consistency indicates that the analyzed samples fall within natural variability limits and exhibit the characteristic macro- and micro-element composition typical of multifloral honeys [40]. Overall, the mineral composition of the samples not only provides a distinctive chemical signature for origin verification but also represents a decisive contributor to the nutritional capacity, quality attributes, and functional properties of honey.

Amino acid composition

Amino acid composition provides important information regarding both the origin and authenticity of honey [41]. Numerous studies have investigated the determination of botanical and geographical origin based on the amino acid profile of honey. Pollen is the most significant source of amino acids present in honey; therefore, the amino acid content is a key characteristic in determining its botanical and geographical origin [41, 42]. In the honey samples analyzed, the most abundant amino acids were proline, phenylalanine, lysine, histidine, hydroxyproline, serine, arginine, glutamic

Fig. 2 Mineral profile of honey samples

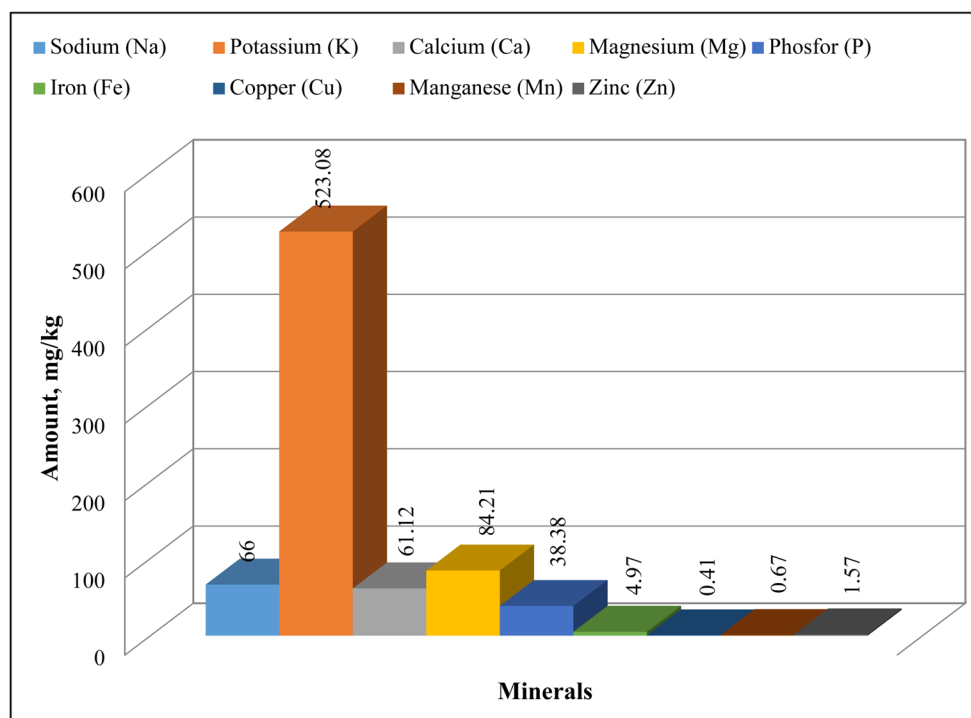


Table 2 Amino acid profile of honey samples(mg/100 g)

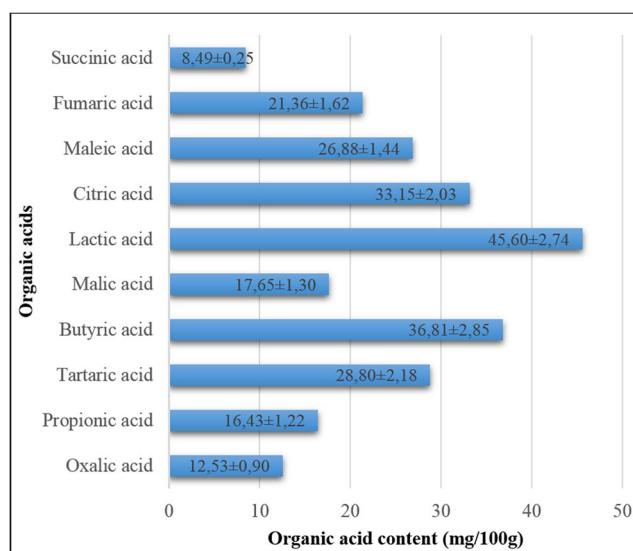
Parameters	Mean±SD	Minimum	Maximum
Proline	33.235±62.28	18.5	44.3
Aspartic acid	11.84±0.71	10.20	13.20
Glutamic acid	15.28±0.99	13.60	17.50
Asparagine	12.73±0.81	11.80	14.50
Serine	16.07±1.80	13.60	19.50
Glutamine	9.44±0.70	8.40	11.30
Histidine	21.92±2.28	18.60	27.40
Glycine	5.11±0.67	4.20	6.60
Threonine	7.95±0.81	6.40	9.10
Arginine	15.98±0.79	14.50	17.50
Alanine	11.62±0.59	10.20	13.00
Tyrosine	12.57±0.94	10.80	14.20
Cystine	4.10±0.67	3.20	5.20
Valine	13.17±0.79	11.50	14.40
Methionine	4.58±0.39	3.90	5.40
Tryptophan	8.67±0.59	7.50	9.60
Phenylalanine	34.54±3.09	30.20	41.20
Isoleucine	11.66±0.97	10.20	13.60
Leucine	9.58±0.93	7.50	11.20
Lysine	22.13±1.04	20.30	24.40
Hidroksi prolin	17.69±1.17	14.20	19.20

SD – standard deviation. *n* = 17

acid, valine, asparagine, tyrosine, aspartic acid, isoleucine, and alanine. Leucine, glutamine, tryptophan, threonine, and glycine were detected in lower amounts, whereas the sulfur-containing amino acids methionine and cysteine were present at the lowest levels (Table 2). Proline is an endogenous amino acid produced in the salivary secretions of honey bees (*Apis mellifera* L.) during the transformation of nectar into honey, and its concentration varies depending on the floral source. The proline content of honey is an indicator of maturity and serves as an important criterion for detecting adulteration with certain sugar syrups, thereby contributing to the assessment of honey purity [17, 43–45]. Other amino acids found in honey originate from nectar, pollen, and bee-derived sources [45]. The number of amino acids present in honey typically ranges from 11 to 21. Although proline is the predominant amino acid in honey, glutamic acid, alanine, phenylalanine, tyrosine, leucine, and isoleucine are also among the most frequently detected amino acids [4].

Organic acid content

The amounts of oxalic, propionic, tartaric, butyric, malic, lactic, citric, maleic, fumaric, and succinic acids in the honey samples were quantified, and the results are presented in Fig. 3. The organic acid profile and levels in honey are mainly derived from nectar or honeydew; they are also formed during fermentation through the metabolic conversion of glucose and fructose [12]. The type and concentration of organic acids in honey are important criteria

**Fig. 3** Organic acid profile of honey samples, SD – standard deviation. *n* = 17

for determining and distinguishing honey varieties, as well as for assessing honey quality and authenticity [12, 46]. In the analyzed samples, lactic acid (45.60 ± 2.74 mg/100 g), butyric acid (36.81 ± 2.85 mg/100 g), citric acid (33.15 ± 2.03 mg/100 g), tartaric acid (28.80 ± 2.18 mg/100 g), maleic acid (26.88 ± 1.44 mg/100 g), fumaric acid (21.36 ± 1.62 mg/100 g), malic acid (17.65 ± 1.30 mg/100 g), propionic acid (16.43 ± 1.22 mg/100 g), oxalic acid (12.53 ± 0.90 mg/100 g), and succinic acid (8.49 ± 0.25 mg/100 g) were determined. Various organic acids were detected in honey samples from different botanical origins in Spain; gluconic acid was found in high concentration, followed by lactic and formic acids [46]. Succinic, lactic, malic, and oxalic acids were found to be within the range specified in this study, while citric acid was found to be slightly lower. The eight organic acids analyzed in this study were also evaluated in a previous study conducted on Brazilian flower honey samples [47]. Succinic, citric, and malic acids were at concentrations lower than those specified in this study, fumaric, maleic, and tartaric acids were at high concentrations, while lactic and propionic acids were within the specified range. Another study reported gluconic acid as the major organic acid detected in all samples, while formic, malic, citric, and succinic acids were found at minor levels, and oxalic acid was not detected [48].

Phytohormones analysis results

Pollen is highly enriched in phytohormones, and because floral honey is produced by bees from plant nectar, the presence of various phytohormones in honey is anticipated [16]. Salicylic acid has been reported at different

concentrations in several monofloral honeys [49, 50] and abscisic acid has been detected in Serbian multifloral honeys [51]. In the present study, six key phytohormones gibberellic acid (GA), salicylic acid (SA), indole-3-acetic acid (IAA), abscisic acid (ABA), cytokinin (CK), and jasmonic acid (JA) were quantified in the honey samples (Table 3). Among these compounds, GA (385 ± 91 pg/g) exhibited the lowest concentration, while ABA ($544,810 \pm 194,355$ pg/g) represented the most abundant phytohormone. The average concentrations of the detected phytohormones were as follows: GA 385 ± 91 pg/g, SA $3,058 \pm 752$ pg/g, IAA 855 ± 221 pg/g, ABA $544,810 \pm 194,355$ pg/g, CK $2,021 \pm 513$ pg/g, and JA $3,168 \pm 934$ pg/g. Previous reports on Robinia raw honey documented IAA, ABA, SA, and JA within comparable ranges, and the results obtained in this study are largely consistent with those findings. Overall, the detection of multiple phytohormones in diverse concentrations demonstrates that honey possesses a complex plant-derived biochemical profile. This richness in phytohormones enhances both the functional value and nutritional importance of honey, reinforcing its role as a naturally bioactive food component within a healthy diet.

Color analysis results

Honey color is one of the most defining sensory factors for consumers. This characteristic generally varies depending on the honey's botanical origin, nectar source, pigment content, and pollen composition. Changes in honey color can occur slowly over time under storage conditions or rapidly when exposed to heat treatment [52, 53]. The results regarding the color values of the honey samples are presented in Fig. 4. The L^* color parameter of the samples varied between 30.30 and 50.80, with an average value of 43.51 ± 5.08 . The highest L^* value was found in sample number 13. This indicates that this sample has a lighter color compared to the other samples. The a^* color parameter was determined to have positive values in all samples, indicating the presence of red tones and the absence of green tones. a^* values ranged from 9.40 to 20.70, with an average of 14.76 ± 3.22 . The b^* color parameter varied between 18.50 and 45.10, with an average value of 37.11 ± 7.33 . These results show that the samples have a significant color intensity in terms of yellow tones.

Correlation between certain parameters

In this study, the relationships between variables were evaluated using the Pearson correlation coefficient, with r values ranging from -1 to $+1$; positive values indicate positive correlations, and negative values indicate negative correlations. Due to the large number of parameters, only relationships

Table 3 Phytohormone content of honey samples(pg/g)

Parameters	Mean $\pm$ SD	Minimum	Maximum
Gibberellic Acid (GA)	385 ± 91	309	663
Salicylic Acid (SA)	3058 ± 752	1671	4152
Indole-3-acetic acid (IAA)	855 ± 221	430	1213
Absciscic acid (ABA)	$544,810 \pm 194,355$	271,935	962,499
Cytokinin (CK)	2021 ± 513	1119	3032
Jasmonic acid (JA)	3168 ± 934	2371	6132

SD – standard deviation. $n = 17$

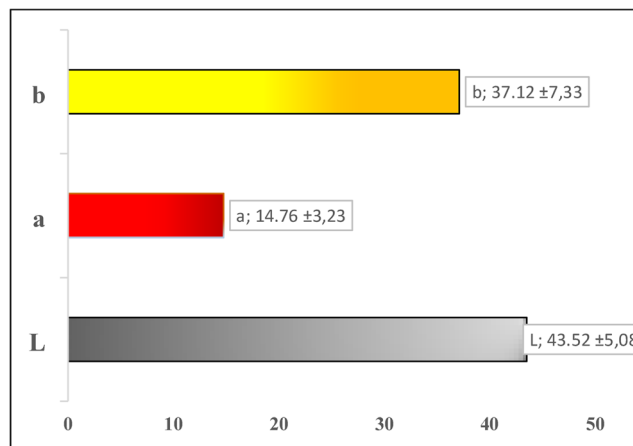


Fig. 4 Color values of honey samples (L, a, b)

with a correlation coefficient $r > 0.600$ were included in the evaluation, and the results are presented in Figure S1. According to the findings, proline showed a positive correlation with fructose ($r = 0.601$), and magnesium showed a negative correlation ($r = -0.644$); HMF showed a positive correlation with serine ($r = 0.724$), and glucose showed a negative correlation with leucine ($r = -0.677$). C13P showed positive correlations with salicylic acid ($r = 0.751$), IAA ($r = 0.753$), ABA ($r = 0.576$), and cytokinin ($r = 0.684$), and negative correlations with gibberellic acid ($r = -0.661$) and jasmonic acid ($r = -0.629$). Potassium showed positive correlations with fumaric acid ($r = 0.737$) and calcium with phenylalanine ($r = 0.668$), while negative correlations were found between calcium and glutamine ($r = -0.600$), oxalic and lactic acids showed a negative correlation with valine, while oxalic acid showed a negative correlation with lactic acid. Gibberellic acid showed negative correlations with salicylic acid, IAA, ABA, and cytokinin; and a highly positive correlation with jasmonic acid ($r = 0.985$). Salicylic acid showed a strong positive correlation with IAA ($r = 0.989$), ABA ($r = 0.795$), and cytokinin ($r = 0.985$); while IAA showed a strong positive correlation with ABA ($r = 0.700$) and cytokinin ($r = 0.961$). In terms of color parameters, the b value (yellow) showed a high positive correlation with the L

value (lightness) ($r=0.955$), while the a value (red) showed a negative correlation ($r=-0.614$).

Principal component analysis

A large dataset was generated from the analysis of multiple parameters, and Principal Component Analysis (PCA) was performed to better interpret the relationships among samples and the distribution patterns of the analyzed variables. PCA is recognized as a powerful multivariate statistical tool that extracts information from complex datasets and reduces dimensionality by transforming variables into a smaller number of components [54]. In this study, 17 honey samples and 59 variables were evaluated. PCA was applied to identify the major components explaining the variability among samples and to characterize differences in their composition. Only components with eigenvalues greater than 1 and explaining at least 10% of the total variance were retained. PC1 and PC2 accounted for 15.82% and 12.25% of the total variance, respectively, while each of the remaining components explained less than 10% of the variance. Therefore, PC1 and PC2 were used for data interpretation. The PCA score plots of the samples are presented in Fig. 5(a, b). PC1 and PC2 together explained 28.07% of the total variance.

According to PCA results, PC1 was primarily influenced by ABA, IAA, salicylic acid, cytokinin, gibberellic acid, jasmonic acid, C13P, the C13P–H difference, C4 sugar ratio, fructose, total F+G content, Ca, Cu, histidine and alanine. PC2 was largely associated with diastase activity, glucose, FG ratio, C13H, Fe, Mn, Zn, lactic acid, succinic acid, aspartic acid, serine, glutamine, tryptophan, leucine, lysine, L, a ve b.

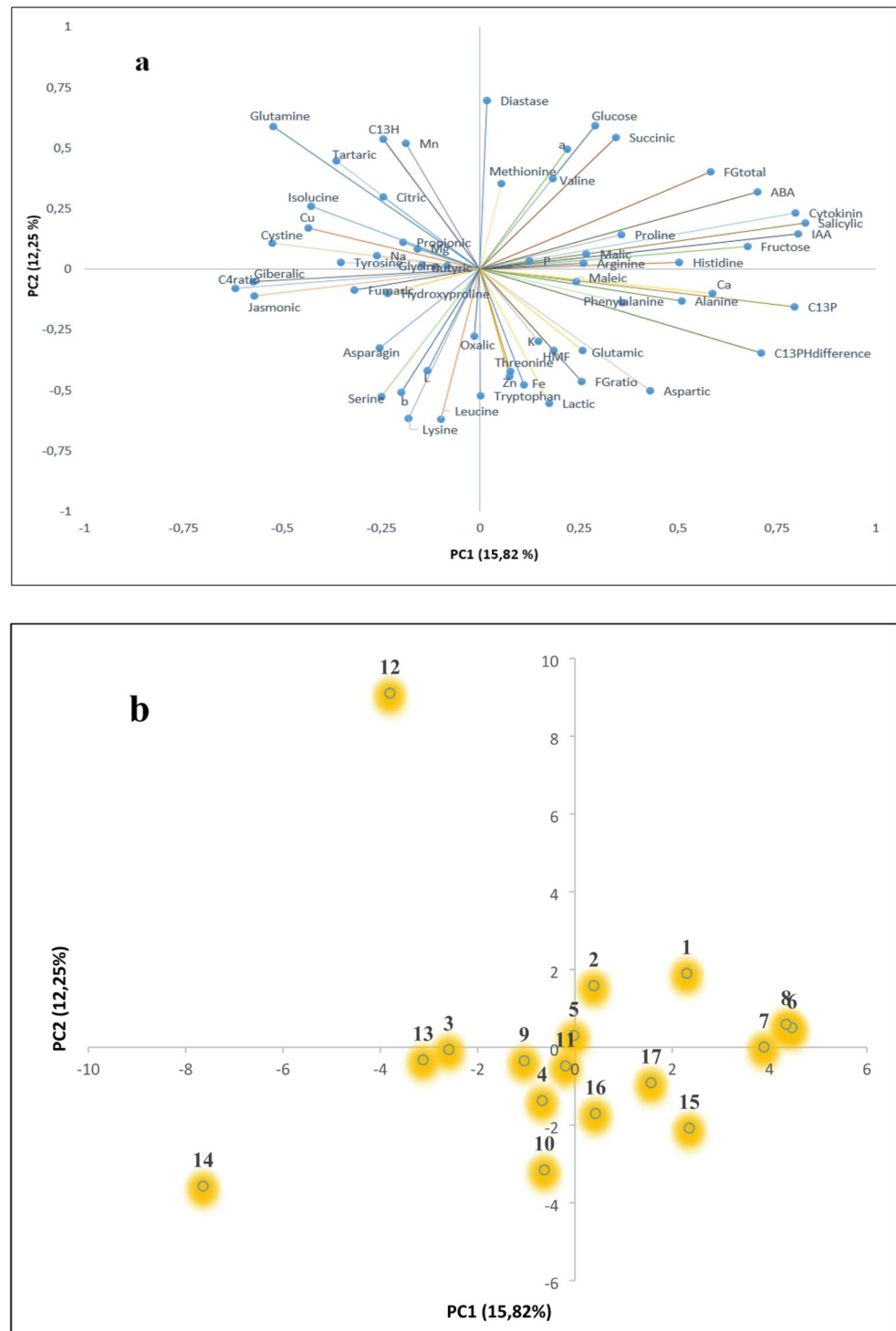
To classify the samples based on similarities among variables, hierarchical cluster analysis (HCA) was performed using Ward's method with Euclidean distance. According to HCA, the honey samples were grouped into three distinct clusters, as illustrated in the dendrogram in Fig. 5(c). Samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16, and 17 formed the first cluster; sample 12 constituted the second cluster;

and sample 14 formed the third cluster. Samples 12 and 14 exhibited significant differences in several parameters compared with the other samples. Overall, PCA and HCA demonstrated that the honey samples clustered according to their geographical and botanical origins, reflecting compositional variations among them.

Conclusion

This study provides a comprehensive evaluation of seventeen pure honey samples using fifty-seven analytical parameters, including detailed assessments of phytohormones, minerals, organic acids, and amino acids. All physicochemical indicators (HMF, diastase activity, sugar profile, F/G ratio, and $\delta^{13}\text{C}$ values) complied with international regulatory limits, confirming the authenticity and non-adulterated nature of the samples. The phytohormone composition characterized by the predominance of abscisic acid followed by jasmonic, salicylic, cytokinin, indole-3-acetic, and gibberellic acids served as a strong indicator of natural maturation processes and botanical origin. These compounds demonstrated high potential as biochemical markers for honey authentication. The mineral, organic acid, and amino acid profiles further supported the chemical integrity and nutritional richness of the samples. According to Pearson correlation analysis, significant relationships were found between the analyzed parameters. Multivariate statistical analyses (PCA and HCA) effectively discriminated among the honeys, emphasizing the relevance of phytohormones, together with mineral and amino acid data, in sample classification. Overall, the findings highlight that the evaluated honeys exhibit high quality, natural compositional characteristics, and strong authenticity signatures. The integration of phytohormone profiling into honey quality assessment frameworks may significantly enhance future authentication studies and contribute to more robust quality control strategies.

Fig. 5 Principal component analysis of the evaluated parameters. PCA according to the analyzed parameters (**a**). Distribution of honey samples according to PCA analysis results (**b**). Dendrogram determined by cluster analysis using the mean Euclidean distance and Ward's method for seventeen honey samples based on the analysis parameters evaluated (**c**). HMF(Hydroxymethylfurfural), Diastase (diastase activity), FGtotal (fruktoz+glucose), FGratio (fructose/glucose), C13P (protein fractions extracted from honey, $\delta^{13}\text{CP}$), C13H ($\delta^{13}\text{C}$ values of honey samples, $\delta^{13}\text{CH}$), C13PHdifference ($\Delta\delta^{13}\text{C}$ protein – honey), C4ratio (C4 sugar ratio, %), Na (sodium), K (potassium), Ca (calcium), Mg (magnesium), P (phosphorus), Fe (iron), Cu (copper), Mn (manganese), Zn (zinc), IAA (Indole-3-acetic acid), ABA (Absciscic acid), L, a and b (Color parameters (L^* , a^* , b^*))



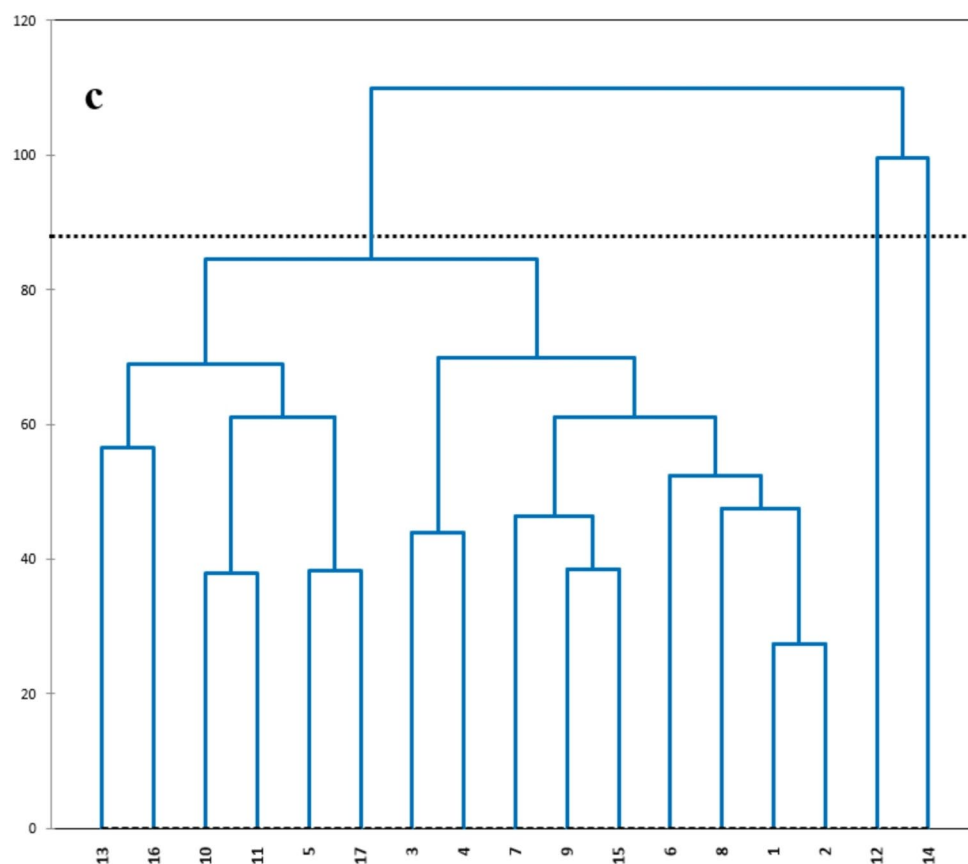


Fig. 5 (continued)

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest to disclose regarding this work.

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