Molecular and functional differences between Kurdish honeydew and blossom honeys: a comparative study using NMR, DSC, rheology, and antioxidant assays

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Abstract

This study compared honeydew honey and blossom honey from the Kurdistan Region of Iraq, looking at their physical makeup, molecular structure, heat behavior, flow properties, and antioxidant power. The researchers used a range of methods—like electrical conductivity tests, NMR and FTIR spectroscopy, X-ray diffraction, and antioxidant assays—to get a full picture of each honey type. They found that honeydew honey had much higher mineral content and electrical conductivity than blossom honey. While blossom honey was richer in simple sugars like glucose and fructose, honeydew honey contained more complex carbohydrates. Blossom honey also showed more crystal formation due to glucose, while honeydew honey had a more disordered, amorphous structure. Honeydew honey held onto more bound water, had a stronger reaction to heat, and became runnier than blossom honey at low mixing speeds. It also contained more HMF—a freshness marker—and significantly higher antioxidant activity.In plain terms, each honey has its own personality. Blossom honey is sweeter and more prone to crystallizing, while honeydew honey is richer in minerals and antioxidants, making it potentially more valuable for health-focused uses and for verifying honey's true origin.

Keywords: Honeydew honey; Blossom honey; Kurdish honey; ^{13}C NMR; FTIR; Rheological properties; Antioxidant activity; Honey authentication; XRD; DSC.

Introduction

Honey is an ancient natural product valued for its nutritional, medical, and economic benefits. It comprises carbohydrates, amino acids, proteins, enzymes, organic acids, and other bioactive compounds. Factors such as botanical origin, geographical source, environmental conditions, and harvest practices significantly influence its physicochemical properties and functional characteristics[1,2]. Honey is primarily classified as blossom honey, derived from floral nectar, and honeydew honey, sourced from plant exudates collected by insects. These origins lead to differences in composition, crystallization, viscosity, color, conductivity, and antioxidant properties, with

honeydew typically exhibiting higher conductivity and antioxidant activity, along with darker coloration. With the growth of the honey market and rising instances of adulteration, the authentication and differentiation of honey types have become increasingly critical for quality assurance and consumer trust. Advanced analytical methods are being employed to characterize honeys and ensure their authenticity. Techniques such as nuclear magnetic resonance (NMR) assist in identifying monosaccharides and distinguishing between honey types based on carbohydrate composition. Fourier-transform infrared spectroscopy (FTIR) rapidly characterizes sugar structures, while differential scanning calorimetry (DSC) and X-ray diffraction (XRD) provide insights into thermal properties and crystallinity. Rheological analyses contribute to understanding honey's flow behavior and viscoelastic properties. Despite the recognized nutritional quality of Kurdish honey, scientific studies on its molecular structure, thermal stability, and antioxidant activity remain limited. This research aims to compare blossom and honeydew honeys from the Panjwen and Sharbazher regions of Iraq, utilizing physicochemical analysis, advanced spectroscopy, and antioxidant assays. The findings could serve as indicators of authenticity and enhance the potential use of Kurdish honey as a functional food product in the future.

Materials and Methods

Honey Sample Collection

The honey samples were collected in the spring of 2025 from two areas, on the one hand from Sharbazer and on the other hand Panjwen, in the Kurdistan Region of Iraq, blossom honey and honeydew honey, respectively. The fresh honey samples were collected directly from local beekeepers to reduce the effects of post-harvest processing as well as ensuring we have authentic honey that does not have any additives. In order to prevent moisture absorption and possible photochemical degradation, approximately 500 g of each sample of honey was placed in sterile amber glass containers and stored in the dark at room temperature.

Physicochemical Analysis

The physicochemical properties of the analyzed honey samples were determined following standard analytical procedures of AOAC [1] as electrical conductivity, ash content and moisture content. Honey samples were dissolved in distilled water and then the electrical conductivity (EC) was measured at 20°C using a conductivity meter. The ash content in the honey samples was calculated by the incineration of samples of the product in a muffle furnace at 550°C until the samples reached a constant weight. Moisture content was determined by Abbe refractometer at 20°C.

Table (1): Physicochemical Properties of Blossom and Honeydew Honeys (Mean ± SD, n = 3)

Parameter	Honeydew Honey	Blossom Honey
Electrical Conductivity (mS cm^{-1})	1.32 ± 0.08	0.41 ± 0.05
Ash Content (%)	0.79 ± 0.06	0.22 ± 0.03
Moisture Content (%)	16.8 ± 0.5	17.5 ± 0.4

Molecular Characterization

^{13}C Nuclear Magnetic Resonance (^{13}C NMR) Analysis

The ^{13}C NMR spectra of the honey samples were examined in order to determine their molecular composition. The samples of each kind of honeys were dissolved in deuterium oxide (D_2O) and placed in small NMR tubes, with the total volume of each sample being about 0.5 g. Standard NMR spectroscopy conditions were used to record the spectra with a Bruker 400 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm). This analysis was primarily aimed at the carbohydrate region (90–105 ppm) associated with anomeric carbon atoms of glucose, fructose and oligosaccharides.

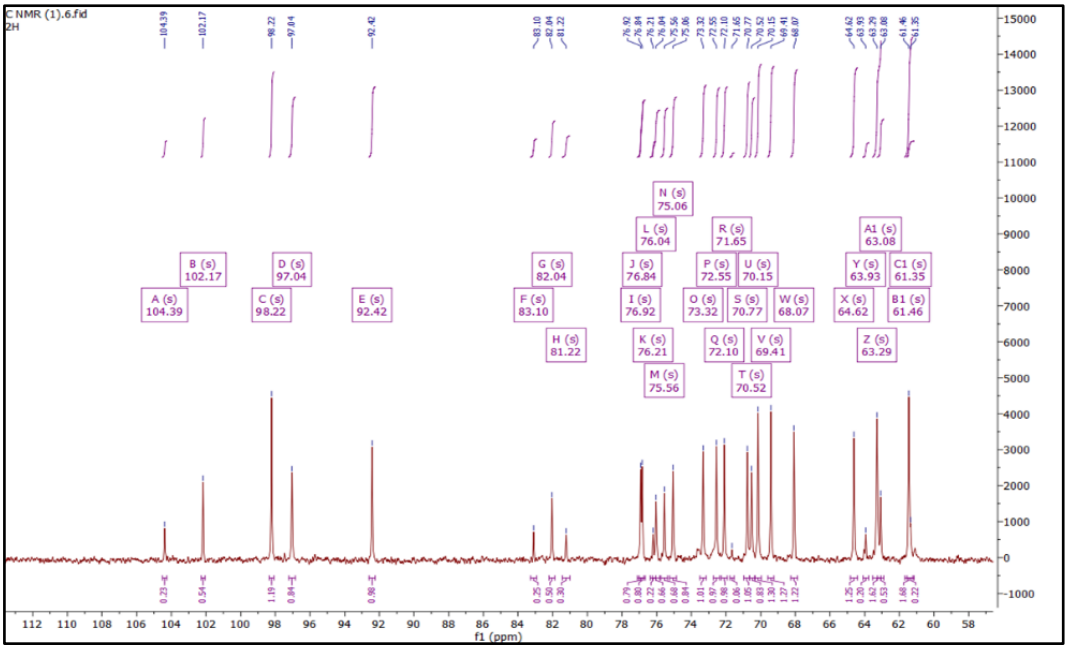

Figure (1): ^{13}C NMR spectrum of blossom honey (75 MHz, DMSO-d6) showing characteristic carbohydrate signals of glucose and fructose, with anomeric carbons at 90–105 ppm, ring carbons at 68–83 ppm, and primary alcohol carbons at 61–65 ppm.

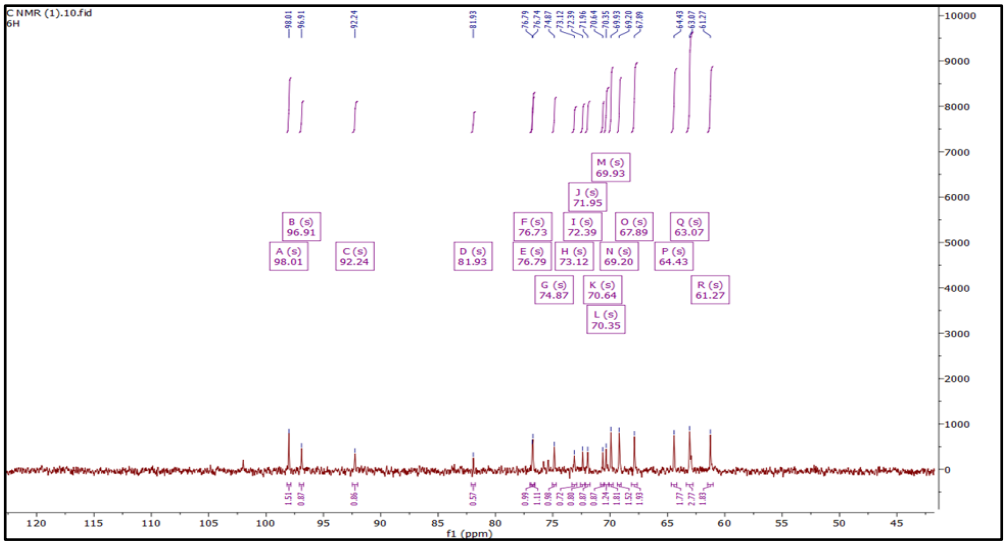

Figure (2): ^{13}C NMR spectrum of honeydew honey (75 MHz, DMSO-d_6) showing carbohydrate signals with anomeric carbons at 90–100 ppm, ring carbons at 68–83 ppm, and primary alcohol carbons at 61–65 ppm, indicating a more complex sugar composition.

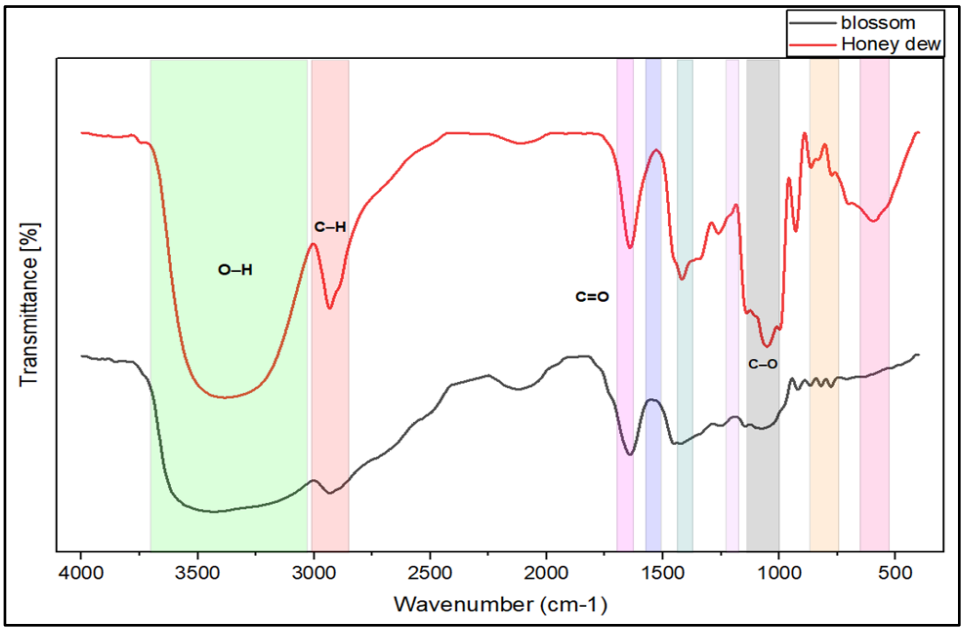

Figure (3): FTIR spectra of blossom and honeydew honey (4000–500 cm^{-1}) showing characteristic O–H, C–H, C=O, and C–O bands related to carbohydrate structures.

Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the honey samples were then taken within the frequency range of 4000–400 cm^{-1} in a Fourier-transform infrared spectrometer. There was no sample preparation for the analy-sis of honey samples directly.The direct analysis of the samples was done in the attenuated total re-flectance (ATR) mode. Special attention was paid to the carbo-hydrate fingerprint region (1200–1000 cm^{-1}) which is due primarily to the C–O and C–O–C stretching vibrations of sugars.

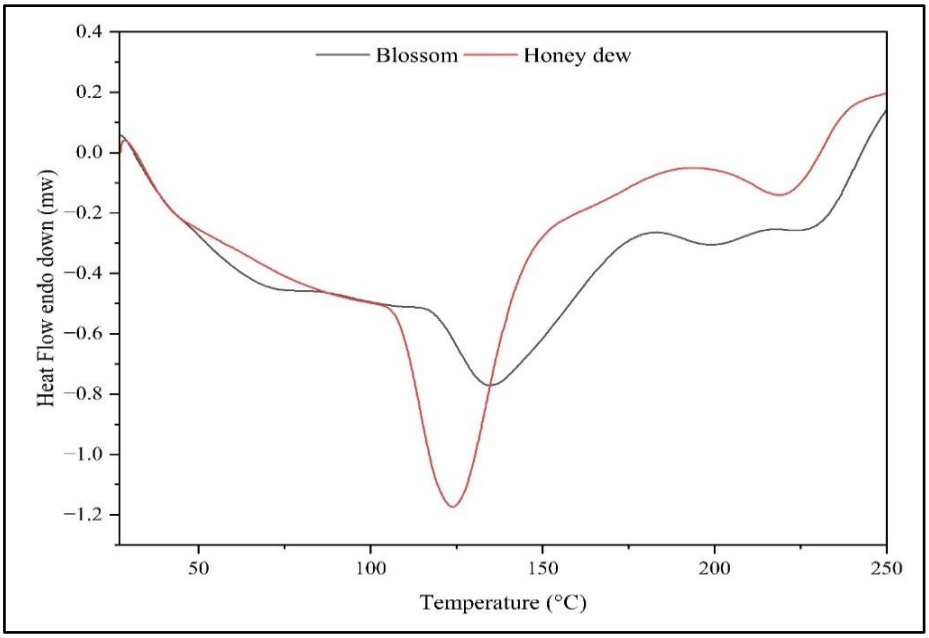

Figure (4): Differential scanning calorimetry (DSC) thermograms of both blossom and honeydew honey samples between the temperature range between 25°C and 250°C. The honeydew honey appears to have a more intense endothermic peak at approximately 120°C that would indicate it contains more water and has more amorphous carbohydrates than the blossom honey, which contains a wider thermal transition region that denotes crystalline glucose formation and dehydration.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to study the thermal properties of blossom honey and honeydew honey. About 5–10 mg of each sample of the honeys was sealed in aluminum pans and heated at a programmed rate under nitrogen atmosphere. Thermal transitions such as sugar degradation, glass transi-tion and melting behavior, and dehydration were tested across the temperature range up to about 250°C.

X-Ray Diffraction (XRD) Analysis

The crystallinity and the structure of the honey products were determined by the X-ray diffraction (XRD) technique. The diffractograms were taken with Cu-Kα radiation in the range of 2θ values of 10°–60°. Both blossom and honeydew honeys were identified for crystalline glucose structures and amorphous carbohydrate phases using dif-fraction patterns.

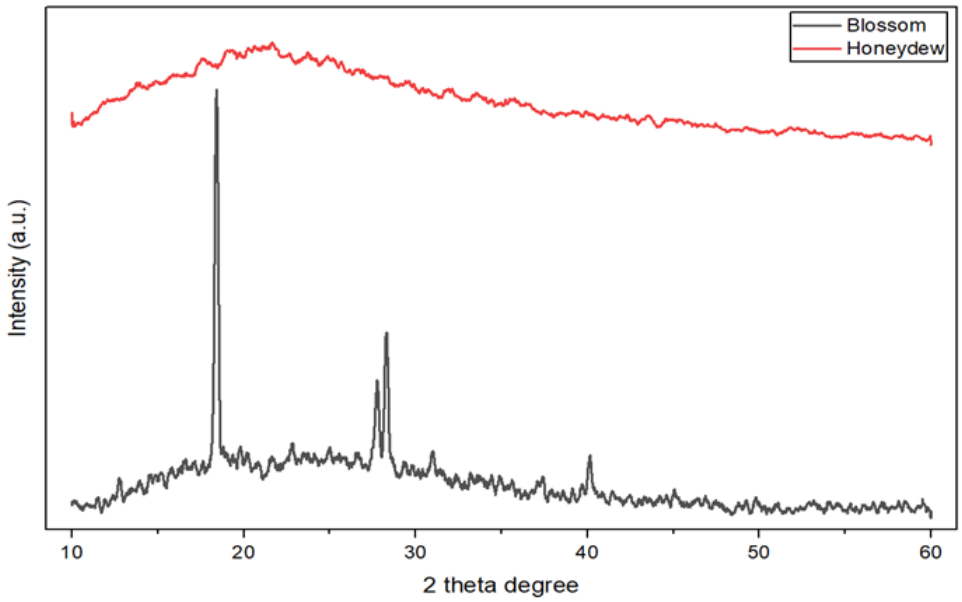

Figure (5): shows the X-ray diffraction (XRD) patterns of blossom honey and honeydew honey measured in the 2 θ range (10°-60°). It can be seen that in blossom honey, there are many crystalline peaks present between 18° and 20° indicating glucose crystal present; whereas the XRD pattern for honeydew honey has no distinct crystalline peaks (due to higher levels of oligosaccharides), thus producing a broader amorphous diffraction pattern.

Rheological Measurements

To assess the flow behavior and viscoelastic characteristics of the honey samples, the steady shear and oscillatory flow properties of the honey samples were measured at 25°C on a rotational remoter over a wide range of shear rates and oscillatory frequencies, respectively.

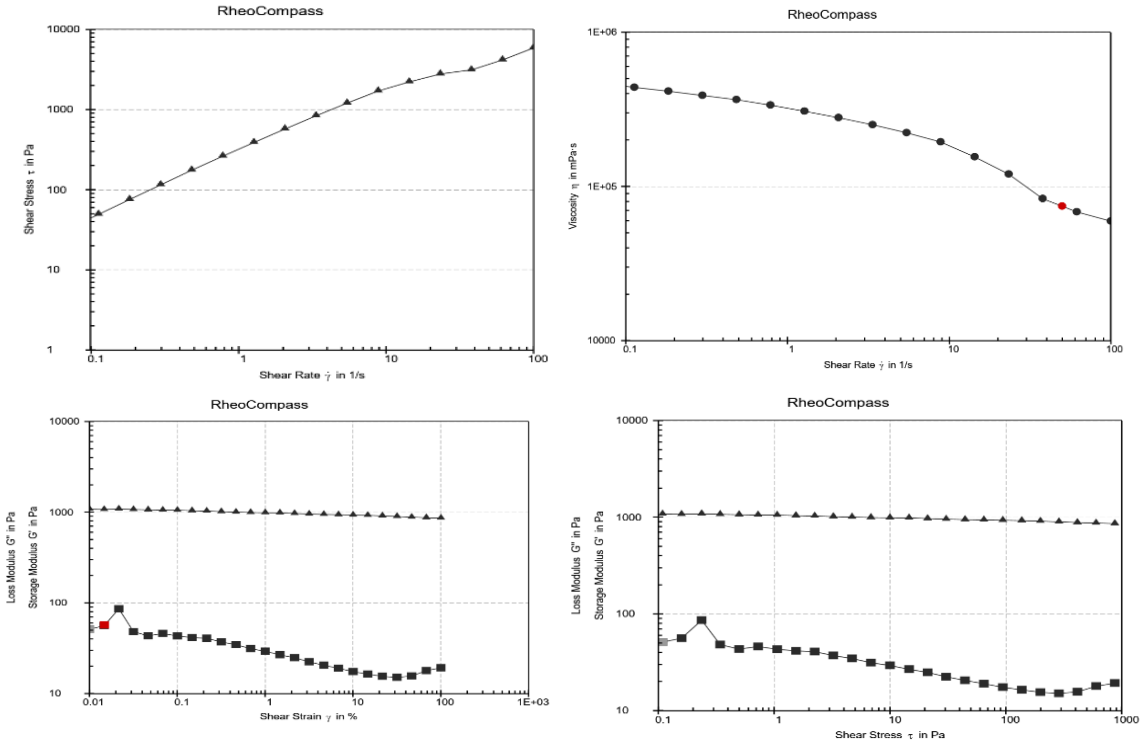

Figure (6): is shown the flow behaviour of flower honey characterised through the use of steady shear and oscillatory rheological tests. The figures illustrate how the relationship between shear stress and shear rate, viscosity versus shear rate and how storage modulus (G') and loss modulus (G") vary with both strain and stress. The data indicates weak shear-thinning behaviour has been observed and the viscous properties are significantly greater than the elastic properties (G">G').

HPLC-DAD Analysis of Hydroxymethylfurfural (HMF)

The level of hydroxymethylfurfural (HMF) was measured with high performance liquid chromatog-raphy coupled to diode-array detection (HPLC-DAD). Honey samples were dissolved in distilled water, filtered through 0.45 µm membrane filter and injected in the HPLC system. Separation was carried out under controlled mobile phase conditions and HMF was identified at its characteristic retention time (RT) of 5.2–5.3 min.

CHN Elemental Analysis

The elemental composition of the honey samples was analyzed by the CHN elemental analyzer, in which the content of carbon (C), hydrogen (H) and nitrogen (N) were measured. About 2 mg of each sample of the honeys was subjected to standard combustion in the CHN elemental analyzer.

Table (2): Elemental Composition of Blossom and Honeydew Honeys Determined by CHN Analysis (Mean ± SD, n = 3)

Honey Type	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Total (%)
Blossom honey	34.92 ± 0.18	7.95 ± 0.07	0.02 ± 0.01	42.89 ± 0.20
Honeydew honey	33.07 ± 0.21	7.11 ± 0.05	0.03 ± 0.01	40.21 ± 0.22

Antioxidant Activity Assays

The antioxidant activity of blossom honey and honeydew honey was studied by DPPH and ABTS radical scavenging assays at various concentrations (0.2 mg mL-1 to 1.0 mg mL-1). In the DPPH assay, honey solutions were combined with the DPPH radical solution, allowed to stand in the dark for 15 minutes and then the absorbance was measured using a spectrophotometer. The ABTS assay was done in the same manner with ABTS radical cation solution. The radical scavenging activity was expressed in terms of percentage inhibition when compared with the control solution.

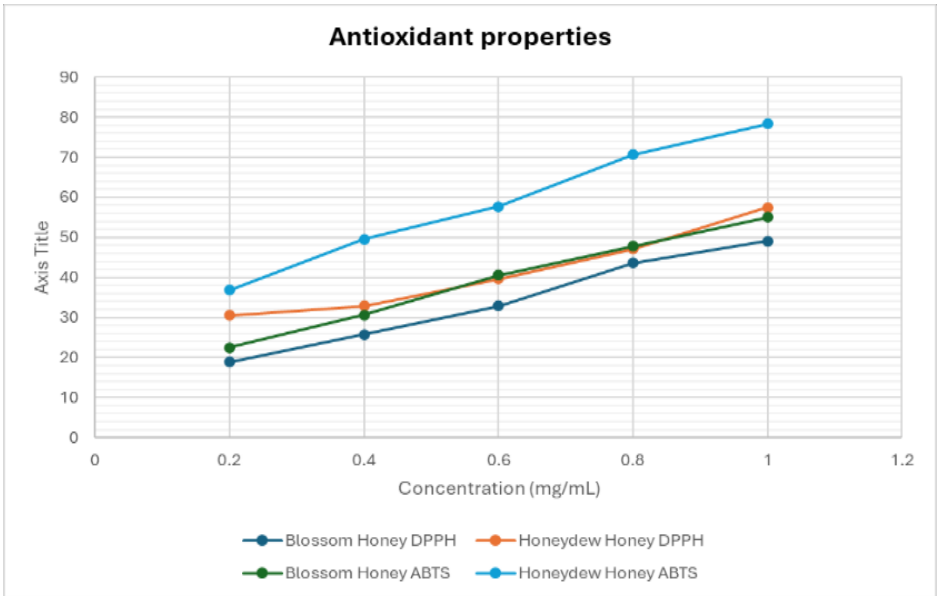

Figure (6): Antioxidant activity of blossom and honeydew honey measured by DPPH and ABTS assays at different concentrations (0.2–1 mg mL^{-1}). Honeydew honey showed higher radical scavenging activity than blossom honey.

Statistical Analysis

Each experiment was repeated three times and the results are presented as mean ± standard deviation (SD). All the data was analysed using SPSS software version 26.0. Significant differences between samples of honeys were evaluated by one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) post hoc analysis. A difference was deemed statistically significant at $P< 0.05$.

Results and Discussion

Physicochemical Properties

Physicochemical properties of honey are crucial for determining its botanical origin, mineral composition, maturity, and quality. Significant differences were observed in the electrical conductivity (EC) and ash content between honeydew and blossom honeys, while moisture percentage showed no significant difference. Honeydew honey had a higher EC (1.32 mS cm^{-1}) compared to blossom honey (0.41 mS cm^{-1}), likely due to higher mineral and organic acid content in honeydew. The ash content was also greater in honeydew (0.79%) than in blossom honey (0.22%). Overall, both types of honey met international quality standards; moisture content was 16.8% for honeydew and 17.5% for blossom honey, indicating good maturation and low fermentation tendency.

Table (3): Physicochemical Properties of Blossom and Honeydew Honeys (Mean ± SD, n = 3)

Parameter	Honeydew Honey	Blossom Honey
Electrical Conductivity (mS cm^{-1})	1.32 ± 0.08	0.41 ± 0.05
Ash Content (%)	0.79 ± 0.06	0.22 ± 0.03
Moisture Content (%)	16.8 ± 0.5	17.5 ± 0.4

Molecular Characterization

Discussion of ^{13}C NMR Results

The ^{13}C NMR spectra obtained for blossom honey and honeydew honey clearly showed differences in chemical structure and complexity of the carbohydrates from the different botanical and eco-logical origins. The signals in the region of anomeric carbon (90–105 ppm) were quite strong in the blossom honey spectrum at δ 104.39, δ 102.17, δ 98.22, δ 97.04, and δ 92.42 ppm. These peaks are assigned to the anomeric carbons of α- and β-forms of glucose and fructose, confirming that blossom honey is a simple type of saccharose, consisting of mainly simple saccharose compounds, which are principally of floral nectar origin.

Table (4): Major ^{13}C NMR Chemical Shifts Observed in Blossom Honey

Chemical Shift (ppm)	Assignment
104.39	β-fructose anomeric carbon
102.17	α-glucose anomeric carbon
98.22	β-glucose carbon
97.04	Fructose carbon
92.42	Glucose/fructose structure

FTIR Analysis

The Study revealed significant difference in FTIR spectral regions related to carbohydrates and hydroxyl functional groups between blossom honey and honeydew honey. The O–H stretch-ng vibration of water molecules and carbohydrate hydroxyl

groups showed wide absorption bands in both the honey samples between 3200–3600 cm^{-1}.

The main difference between the two samples was seen in the carbohydrate fingerprint region (1200–1000 cm^{-1}) where most of the C–O and C–O–C stretching vibrations of sugars were present. The absorption bands of the honeydew honey were stronger and more complex within this region when compared with the absorption bands from the blossom honey, indicating a higher concentration of structurally diverse carbohydrates and oligosaccharides.

Table (5): Major FTIR Absorption Regions Observed in Honey Samples

Wavenumber (cm^{-1})	Functional Group Assignment
3200–3600	O–H stretching vibrations
2900	C–H stretching
1640	Water bending vibration
1200–1000	C–O and C–O–C carbohydrate stretching

Thermal Properties (DSC)

The thermal behaviour of blossom honey and honeydew honey showed significant differences through the use of Differential scanning calorimetry (DSC) analysis. In both types of honey a major endothermic transition was observed around 100°C to 150°C, principally related to dehydration process, and relaxation of the amorphous carbohydrate phase.

The endothermic transitions in blossom honey were wider ranging between 130–140°C, which could be attributed to the increased glucose and fructose content leading to progressive dehydration and reorganization. Honeydew honey, on the other hand, had wider and sharp changes between 115–125°C, which are indicative of higher hygroscopicity and more amorphous carbohydrate structures.

In both honeys, further thermal transitions were detected between 180 and 220°C, which were related to sugar decomposition and caramelization reactions that result in the production of other thermo-formed products, such as hydroxymethylfurfural (HMF).

Table (6): Major Thermal Transitions Observed by DSC Analysis

Honey Type	Transition Range (°C)	Interpretation
Blossom honey	130–140	Dehydration and glucose reorganization
Honeydew honey	115–125	Amorphous carbohydrate relaxation
Both samples	180–220	Sugar degradation and caramelization

Structural Properties (XRD)

The X-ray diffraction analysis revealed significant difference between the crystallinity of blossom and honeydew honey. In the case of blossom honey, there were distinct peaks corresponding to the crystalline glucose domains, centered at 2θ = 18–20° and a few others secondary peaks at 26–30°, which also showed the presence of crystalline glucose domains in the honey matrix.

Honeydew honey, on the other hand had wider diffraction patterns in the ring shape with the lower intensity, suggesting mostly amorphous structures. This is thought to be due to the elevated amounts of oligosaccharides and complex carbohydrates like melezitose and erlose that inhibit the creation of clearly defined glucose crystals.

Table (7): XRD Characteristics of Blossom and Honeydew Honeys

Honey Type	Structural Characteristic	Main Diffraction Peaks
Blossom honey	Crystalline	18–20°, 26–30°
Honeydew honey	Amorphous	Broad halo pattern

Rheological Behavior

The flow behaviour and viscoelastic properties of blossom honey and honeydew honey were determined by steady shear and oscillatory rheological measurements. In both samples, the shear stress increased with the increasing shear rate, thus showing typical flow characteristics of viscous carbo-hydrate rich fluids. Near Newtonian behavior with some shear-thinning effect at high shear rates was observed for both honeys. The oscillatory measurement showed that the loss modulus (G″) was consistently higher than the storage modulus (G′) for both the samples of honey, indicating the viscous nature of the honey.

Table (8): Rheological Properties of Blossom and Honeydew Honeys

Parameter	Blossom Honey	Honeydew Honey
Flow behavior	Weak pseudoplastic	Near Newtonian
Dominant modulus	G″ > G′	G″ > G′
Viscoelastic behavior	Predominantly viscous	Predominantly viscous
Structural stability	Moderate	Higher

HPLC-DAD Analysis of HMF

HPLC-DAD chromatograms showed hydroxymethylfurfural (HMF) in all the blossom honey and honeydew honey samples. Both the samples contained the HMF compound, which appeared at the retention times of about 5.2-5.3 min. The HMF peak intensity was significantly higher in the honeydew honey than in blossom honey, suggesting that more HMF have been formed due to heating.

Table (9): HPLC-DAD Detection of HMF in Honey Samples

Honey Type	Retention Time (min)	Relative Peak Intensity
Blossom honey	5.224	Low
Honeydew honey	5.345	High

Elemental Composition (CHN Analysis)

The elemental analysis revealed obvious differences between carbon, hydrogen and nitrogen levels of blossom honey and honeydew honey, using CHN analysis.

Table (10): Elemental Composition of Blossom and Honeydew Honeys Determined by CHN Analysis (Mean ± SD, n = 3)

Honey Type	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Total (%)
Blossom honey	34.92 ± 0.18	7.95 ± 0.07	0.02 ± 0.01	42.89 ± 0.20
Honeydew honey	33.07 ± 0.21	7.11 ± 0.05	0.03 ± 0.01	40.21 ± 0.22

Antioxidant Properties

DPPH and ABTS radical scavenging assays were used to assess the antioxidant activity of blossom and honeydew honey at concentrations from 0.2-1.0mg/mL. Concentration dependent rise in antioxidant activity was observed in both the assays for both type of honey.

Table (11): DPPH Radical Scavenging Activity of Honey Samples (% Inhibition)

Concentration (mg mL^{-1})	Blossom Honey	Honeydew Honey
0.2	18.83 ± 0.31	30.57 ± 0.40
0.4	25.80 ± 0.40	32.87 ± 0.25
0.6	32.87 ± 0.25	39.63 ± 0.35
0.8	43.63 ± 0.35	47.07 ± 0.31
1.0	49.10 ± 0.30	57.43 ± 0.35

Table (12): ABTS Radical Scavenging Activity of Honey Samples (% Inhibition)

Concentration (mg mL^{-1})	Blossom Honey	Honeydew Honey
0.2	22.47 ± 0.35	36.80 ± 0.30
0.4	30.70 ± 0.30	49.53 ± 0.35
0.6	40.53 ± 0.35	57.70 ± 0.30
0.8	47.80 ± 0.30	70.63 ± 0.35
1.0	55.00 ± 0.30	78.30 ± 0.30

The present study showed that the physicochemical, structural, molecular, thermal, rheological and antioxidant properties of the honey samples collected from the Kurdish Region of Iraq (Sharbazer and Penjwen) are significantly different from each other. Honeydew honey had significantly higher electrical conductivity (EC) and ash content than blossom honey, which suggests a greater mineral content and ionic content than blossom honey. The spectral analyses based on 13C NMR and FTIR supported that significant differences were found in the carbohydrate constituents of the two kinds of honeys. The main differences between the two types of honey were that the glucose and fructose signals predominated in blossom honey, while the honeydew had more complex oligosaccharide characteristics and structurally diverse carbohydrate patterns.

The crystallinity of blossom honey was also found to be higher, and the honeydew honey had predominantly amorphous structural properties, by the XRD analysis. The thermal analysis (DSC) revealed that the endothermic transition was more prominent in the honeydew honey, indicating bound water and more structure. The honeys were shown to be viscous liquids with low shear-thinning properties by the rheological analysis but honeydew honey was more viscous stable because of its complex carbohydrate content. Hydroxymethylfurfural (HMF) peak intensities were found to be higher in the hydroxymethylfurfural (HMF) peaks detected by HPLC-DAD analysis of honeydew honey. Furthermore, antioxidant activity of both the ABTS and DPPH assays showed that the honeydew honey exhibited higher antioxidant activity, which could be due to its higher phenolic compounds, mineral content, and concentration of oligosaccharides. In general, the analytical methods used in this study were effective in differentiating the molecular composition, structural organization, physicochemical properties and functional properties of Kurdish blossom and honeydew honeys. The results obtained here will serve as valuable data for the authentication and quality evaluation of the honey and showed that Kurdish honeydew honey could be a valuable functional food with nutritional and commercial importance.

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