



Original Article

Sidr *Ziziphus spina-christi* Honey Quality Across Western Libya: A Preliminary Physicochemical and Pollen-Based Study

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Abstract

Background: Sidr honey is a valuable natural product with recognized nutritional and bioactive properties, yet information on its physicochemical quality in Libya remains scarce. This study aimed to characterize selected quality parameters of Sidr honey from Western Libya and provide baseline data for future nutritional and therapeutic investigations. **Methods:** Honey samples were collected from four regions (Zawia, Tarhuna, Sabratha, Qasr Khair). Two beekeepers per region were sampled during two harvesting periods separated by 7–14 days. Each beekeeper contributed three jars per period, yielding 48 jars in total. Jars from each period were pooled into composite samples ($n = 2$ per region), homogenized, and analyzed in triplicate. Botanical origin was verified by sensory indicators and confirmed by melissopalynological analysis, with *Ziziphus spina-christi* pollen predominating (60–67%). Regional differences were assessed using one-way ANOVA ($df = 3, 20$) followed by Tukey's HSD. Given the small sample size, parameters near the 0.05 threshold were interpreted cautiously. **Results:** Regional variation was observed in moisture, viscosity, electrical conductivity, total soluble solids, pH, free acidity, lactone, total acidity, fructose, glucose, sucrose, maltose, and the glucose/water ratio ($p \leq 0.020$), though these findings should be interpreted as exploratory given the analytical design ($n=6$ replicates per region, derived from 2 composite samples measured in triplicate). Moisture ranged from $17.40 \pm 0.49\%$ (Tarhuna) to $19.16 \pm 0.54\%$ (Sabratha), while electrical conductivity varied from 0.015 ± 0.003 S/cm (Zawia) to 0.025 ± 0.005 S/cm (Qasr Khair). pH increased significantly across regions ($4.20\text{--}5.08$; $p = 0.0016$), with Tarhuna, Sabratha, and Qasr Khair means exceeding the upper bound (4.5) typically reported for genuine honey, a finding that warrants further verification. Fructose ranged from 44.02 ± 0.95 g/100 g (Tarhuna) to 49.62 ± 0.55 g/100 g (Zawia). Specific gravity and the fructose/glucose ratio (1.51–1.69) did not differ significantly among regions ($p \geq 0.238$). **Conclusion:** This study provides a preliminary baseline characterisation of Libyan Sidr honey, melissopalynologically supported as monofloral. Given the limited sample size, the observed regional patterns should be regarded as preliminary indications rather than confirmed geographic differences, and this dataset establishes a foundation for broader investigations into nutritional composition, bioactive compounds, antioxidant activity, and therapeutic potential.

Keywords: Sidr honey; *Ziziphus spina-christi*; melissopalynology; physicochemical properties; electrical conductivity; geographical origin; sugar composition; Western Libya; Tukey HSD.

Introduction

Honey is a highly valued natural food with a complex composition. This composition is shaped by botanical origin, geographical provenance, and climatic conditions. It is further influenced by soil characteristics, nectar availability, harvesting practices, and storage conditions. Although sugars and water constitute its major components, honey also contains organic acids, minerals, enzymes, proteins, and other minor constituents. These minor constituents contribute to its quality, stability, and sensory characteristics. Consequently, honey produced in different geographical regions may exhibit measurable differences in quality parameters, even when marketed under the same botanical designation [13, 12, 17]. Sidr honey, generally associated with nectar collected from *Ziziphus* species, is among the most valued monofloral

honey in the Middle East and North Africa. Its reputation and commercial value are tied to its distinctive characteristics and traditional significance. However, the designation “Sidr honey” does not necessarily indicate a chemically homogeneous product. Geographical location,

environmental conditions, surrounding vegetation, climatic variation, and the availability of alternative floral resources may influence the composition of honey produced from *Ziziphus* trees. A comprehensive study of 794 Sidr honey samples from 12 geographical origins demonstrated substantial variability in physicochemical and biochemical characteristics, highlighting the importance of geographical provenance in the characterisation of Sidr honey [12]. Selected quality parameters provide useful information for characterising honey and assessing differences among production regions. Moisture is a key quality indicator because it is



associated with viscosity, storage stability, susceptibility to fermentation, and shelf life. Specific gravity and viscosity provide complementary information about the physical characteristics of honey and are influenced by its water content and the concentration of soluble solids [17]. Total soluble solids (TSS) further reflect the concentration of dissolved components, primarily sugars, while electrical conductivity (EC) is influenced by ionisable substances, including mineral salts and organic acids, and has been widely investigated as a parameter associated with botanical and geographical origin [13].

Acidity is another important component of the honey quality profile. pH reflects hydrogen-ion activity, whereas free acidity represents titratable acidic components, and lactones contribute to the overall acid balance; the combined assessment of pH, free acidity, lactone, and total acidity can therefore provide useful information for characterizing honey and identifying potential differences among regional products [12,13]. The carbohydrate fraction of honey is dominated by fructose and glucose, with smaller amounts of sucrose, maltose, and other sugars; the fructose-to-glucose ratio is frequently considered in honey characterization because the greater solubility of fructose relative to glucose affects the tendency of honey to crystallize, and the glucose-to-water ratio provides complementary information on this tendency [12].

The relationship between honey composition and geographical origin has been demonstrated in several regional studies, including Kenyan [17], Ethiopian [18], Turkish [16], and Brazilian [15] honeys, collectively supporting the relevance of regional comparisons for developing geographical reference profiles.

Despite the recognized value and commercial reputation of Sidr honey, scientific information on the quality characteristics of Sidr honey produced in Libya, and comparative information on regional differences within Western Libya specifically, remains limited. Establishing comparative baseline data is therefore important for documenting the characteristics of locally produced Sidr honey and evaluating the extent of regional variability.

Accordingly, the present study investigated regional variation in selected quality parameters — moisture, specific gravity, viscosity, electrical conductivity, total soluble solids, pH, free acidity, lactone, total acidity, and selected carbohydrate-related indices — of Sidr honey produced in Zawia, Tarhuna, Sabratha, and Qasr Khair, confirming the monofloral botanical origin of the samples by melissopalynological analysis, and provides preliminary baseline evidence for the scientific characterization, quality assessment, and future geographical traceability of Libyan Sidr honey.

Materials and Methods

Study Area and Honey Sample Collection

A comparative analytical study was conducted to investigate regional variation in selected quality parameters of Sidr honey produced in Western Libya. Honey samples were collected from four geographically distinct production regions: Zawia, Tarhuna, Sabratha, and Qasr Khair. In each region, two well-known and trusted local beekeepers were identified. Apiaries and the surrounding forage areas of each beekeeper were monitored during two harvesting periods, separated by approximately 7–14 days, to verify the presence and flowering of abundant Sidr (*Ziziphus spina-christi*) trees. The harvesting and extraction processes were observed directly, and aroma and taste were used as additional sensory indicators of botanical source. In each harvesting period, each beekeeper contributed three 0.5-kg jars of honey. This gave six jars per beekeeper across the two periods, and 12 jars per region (2 beekeepers × 6 jars). Across the four regions, this yielded a total of 48 jars.

For each harvesting period in each region, the jars from the two beekeepers were pooled and thoroughly homogenized to produce one composite sample representing that period. This gave two independent composite samples per region and eight composite samples in total across the four regions. Each composite sample was analyzed in triplicate, so that each region contributed 2 composite samples × 3 analytical replicates = 6 analytical measurements per parameter. The composite sample — not the individual analytical replicate — constitutes the experimental unit for statistical comparison.

Samples were collected in clean, dry, tightly sealed containers, labeled according to their geographical origin and harvesting period, transported to the laboratory, and stored under suitable conditions until analysis. Before each analytical determination, samples were thoroughly homogenized to ensure uniformity and representative measurement.

Melissopalynological Confirmation of Botanical Origin

To confirm the monofloral botanical origin of the honey samples, pollen analysis (melissopalynology) was performed following standard procedures [14]. Pollen grains were isolated from each composite honey sample, mounted on glass slides using Basic Fuchsin stain in a glycerin jelly medium, and examined by compound light microscopy at ×400 and ×1000 magnification. Pollen types were identified by comparison with reference pollen morphology, and pollen density was estimated as grains per 10 g of honey. *Ziziphus spina-christi* (Sidr) pollen was found to predominate in all analyzed samples, accounting for 60–67% of the total pollen spectrum, corresponding to approximately 6,500–9,200 grains per 10 g of honey,



consistent with the classification of the samples as monofloral Sidr honey.

Determination of Moisture Content and Total Soluble Solids

Moisture content and total soluble solids (TSS) were determined using a digital refractometer (PAL-22S, ATAGO Co., Ltd., Tokyo, Japan). Moisture content was obtained from the refractive index according to the standard conversion procedure applicable to honey, and TSS was recorded as a percentage (%). Each homogenized sample was measured after stabilization under controlled laboratory conditions, and the prism was cleaned between samples to prevent cross-contamination.

Determination of Specific Gravity

Specific gravity was determined gravimetrically from the ratio between the mass of a defined volume of honey and the mass of an equivalent volume of water under controlled temperature conditions, using a clean, dry vessel of known volume.

Determination of Viscosity

Honey viscosity was determined using a rotational viscometer (DV2T, AMETEK Brookfield, Middleboro, MA, USA), with samples homogenized and equilibrated to a controlled laboratory temperature before measurement. Viscosity was recorded after stabilization and expressed in Poise (P).

Determination of Electrical Conductivity

Electrical conductivity (EC) was measured using a conductivity meter (HI98311 DiST 5, Hanna Instruments, Woonsocket, RI, USA) on aqueous honey solutions prepared according to the standard procedure for honey analysis, after calibration with an appropriate standard solution. EC was expressed in S/cm.

Determination of pH

The pH of the honey samples was determined using a calibrated digital pH meter on solutions of homogenized honey diluted with distilled water according to the standard procedure for honey analysis. The electrode was rinsed with distilled water between measurements.

Determination of Free Acidity, Lactone, and Total Acidity

Standard titrimetric procedures for honey analysis determined free acidity, lactone, and total acidity. A known amount of homogenized honey was dissolved in distilled water and titrated with standardized sodium hydroxide solution; free acidity was recorded to the specified endpoint and expressed as milliequivalents per kilogram (meq/kg). Lactone content was determined by the subsequent titration step and expressed in meq/kg. Total acidity was calculated as the sum of free acidity and lactone (Total Acidity = Free Acidity + Lactone) for each analytical replicate.

Determination of Sugar Composition

Fructose, glucose, sucrose, and maltose concentrations were determined by high-performance liquid chromatography (HPLC) using a Prominence-i LC-2030C system (Shimadzu Corporation, Kyoto, Japan) equipped with a refractive index detector (RID-20A) and a Rezex RCM-Monosaccharide Ca²⁺ column. Homogenized honey was dissolved in distilled/deionized water, diluted, and filtered before injection; individual sugars were identified by retention time against analytical standards, and quantified using calibration curves. Concentrations were expressed as g/100 g of honey. The fructose-to-glucose (F/G) ratio was calculated as: F/G ratio = Fructose (g/100 g) / Glucose (g/100 g). The glucose-to-water (G/W) ratio was calculated as: G/W ratio = Glucose (g/100 g) / Moisture (%).

Preliminary validation of the HPLC method was performed to ensure the reliability of the sugar composition data. The limits of detection (LOD) and quantification (LOQ) were estimated from signal-to-noise ratios (S/N = 3 and 10, respectively). Recovery tests (n = 2) yielded acceptable values within 95–102%, and repeatability, expressed as relative standard deviation (RSD, n = 3), was below 5% for all quantified sugars.

Analytical Quality Assurance

All laboratory instruments were calibrated before analysis. Analytical procedures adhered to established principles and standard methods for honey quality assessment, and instruments were operated in accordance with manufacturers' specifications. Representative homogenized samples were used throughout, and precautions were taken to minimise contamination and measurement variability.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA) and cross-checked in Python (SciPy/statsmodels). For each parameter, the three analytical replicates of each composite sample were averaged, so that each region was represented by two independent values (one per composite sample); this composite-sample mean, not the individual analytical replicate, was treated as the unit of statistical analysis. Descriptive statistics (mean, standard deviation, coefficient of variation, minimum, and maximum) were calculated for each region from these two composite-level values.

Differences among the four regions were evaluated by one-way analysis of variance (ANOVA) with 3 and 20 degrees of freedom (between and within groups, respectively), treating the six analytical replicates per region (2 composite samples × 3 replicates) as the unit of analysis. Because these replicates are nested within only 2 independent composite samples per region, this design carries a risk of pseudoreplication that may inflate apparent



statistical significance relative to a model using composite-sample means alone; this is addressed further in the Limitations (Section 4.1). Where ANOVA indicated a significant regional effect ($p < 0.05$), Tukey's honestly significant difference (HSD) test was used for pairwise comparisons and to assign compact-letter groupings.

The statistical analysis treats the six analytical replicates per region (three replicate measurements from each of two independent composite samples) as the unit of analysis, yielding an ANOVA structure of $F(3, 20)$. This substantially increases the nominal degrees of freedom relative to an analysis based on composite-sample means alone, which would instead yield $F(3, 4)$. However, because the true number of independent biological/production units per region remains two composite samples, the six replicates are technical (analytical) replicates nested within those composite samples rather than fully independent observations. Treating them as independent in the ANOVA carries a risk of pseudoreplication, which can artificially inflate apparent statistical power and narrow confidence intervals, potentially overstating the significance of regional differences reported here.

A related methodological limitation must be stated explicitly: because the underlying biological replication remains two composite samples per region, the effective degrees of freedom for detecting true regional differences are lower than the nominal $df = 3, 20$ suggests, for the pseudoreplication reasons noted above. Formal tests of the normality and homogeneity-of-variance assumptions underlying ANOVA (e.g., Shapiro–Wilk, Levene's test) were not performed in this analysis and are not reported. For these reasons, the ANOVA and Tukey HSD results reported here — including for parameters reaching conventional statistical significance ($p < 0.05$) — should be interpreted as preliminary indications of regional differences rather than definitive statistical evidence, and should ideally be confirmed in future work using a substantially larger number of independent composite samples per region as the unit of analysis. Statistical significance was set at $p < 0.05$ for two-tailed tests.

Results

Descriptive statistics (means, standard deviations, and coefficients of variation across $n = 6$ analytical replicates per region: 2 composite samples $\times$ 3 replicates) are presented in Table 1; one-way ANOVA results ($df = 3, 20$) are presented in Table 2; and Tukey HSD mean groupings are presented in Table 3. Graphical summaries are shown in Figures 1 and 2.

Moisture content ranged from 17.40% in Tarhuna to 19.16% in Sabratha honey ($F = 20.94$, $p < 0.001$), while

specific gravity did not differ significantly among regions (1.347–1.360; $F = 0.06$, $p = 0.980$). Viscosity ranged from 68.13 Poise (Sabratha) to 70.59 Poise (Tarhuna), and this difference reached statistical significance ($F = 12.34$, $p < 0.001$). Electrical conductivity (EC) was lowest in Zawia honey (0.015 S/cm) and highest in Qasr Khair honey (0.025 S/cm; $F = 8.46$, $p < 0.001$), while total soluble solids (TSS) ranged from 79.58% (Sabratha) to 82.53% (Tarhuna; $F = 10.96$, $p < 0.001$).

The pH of the studied honeys ranged from 4.20 (Zawia) to 5.08 (Qasr Khair), and this difference was statistically significant ($F = 7.41$, $p = 0.0016$); the regional means for Tarhuna (4.82), Sabratha (4.62), and Qasr Khair (5.08) exceeded the upper bound (4.5) typically reported for genuine honey [7], a finding discussed further below. Free acidity varied from 38.37 meq/kg (Qasr Khair) to 47.87 meq/kg (Sabratha; $F = 32.03$, $p < 0.001$), while lactone content ranged from 9.74 meq/kg (Tarhuna) to 12.58 meq/kg (Zawia; $F = 7.35$, $p = 0.0016$). Total acidity, calculated as the sum of free acidity and lactone for each analytical replicate before averaging, ranged from 49.20 meq/kg (Qasr Khair) to 60.08 meq/kg (Sabratha), and differed significantly among regions ($F = 35.44$, $p < 0.001$). Regarding sugar composition, fructose content ranged from 44.02 g/100g (Tarhuna) to 49.62 g/100g (Zawia; $F = 30.07$, $p < 0.001$), while glucose ranged from 29.97 g/100g (Zawia) to 31.88 g/100g (Sabratha), and this difference also reached statistical significance ($F = 8.23$, $p < 0.001$). The fructose/glucose ratio did not differ significantly among regions (1.51–1.69; $F = 1.53$, $p = 0.238$). Sucrose ranged from 5.10 g/100g (Qasr Khair) to 5.78 g/100g (Tarhuna; $F = 7.26$, $p = 0.0018$), while maltose ranged from 5.85 g/100g (Qasr Khair) to 6.68 g/100g (Tarhuna; $F = 10.32$, $p < 0.001$). The glucose/water ratio ranged from 1.53 (Zawia) to 1.81 (Sabratha), and this difference was also statistically significant ($F = 4.14$, $p = 0.0195$).

Coefficients of variation (CV%) among the six analytical replicates per region were generally below 10% for most parameters, but were markedly higher for electrical conductivity (13.1–21.3% across regions), and, to a lesser extent, for the fructose/glucose ratio (7.4–14.1%, exceeding 10% in Tarhuna and Qasr Khair), pH (5.0–8.7%), lactone (4.9–15.5%), and the glucose/water ratio in Qasr Khair (16.7%), reflecting greater within-region variability for these parameters (Table 1). Statistical comparisons for all measured parameters are presented in Table 2 (one-way ANOVA) and Table 3 (Tukey HSD grouping), and the corresponding graphical patterns are shown in Figures 1 and 2. Given that each region is represented by only two independent composite samples, these statistical comparisons are best read as an early indication rather than a definitive regional comparison.

**Table 1:** Descriptive Statistics (Mean $\pm$ SD, CV%, Min–Max) of Sidr Honey Quality Parameters by Region (n = 6 analytical replicates per region; 2 composite samples $\times$ 3 replicates)

Parameter	Zawia	Tarhuna	Sabratha	Qasr Khair
Moisture (%)	17.800 $\pm$ 0.303 (1.7%) [17.400–18.200]	17.400 $\pm$ 0.494 (2.8%) [16.700–18.000]	19.158 $\pm$ 0.535 (2.8%) [18.550–20.000]	18.867 $\pm$ 0.432 (2.3%) [18.200–19.400]
Specific gravity	1.360 $\pm$ 0.059 (4.3%) [1.260–1.430]	1.348 $\pm$ 0.057 (4.2%) [1.250–1.400]	1.347 $\pm$ 0.060 (4.5%) [1.250–1.410]	1.352 $\pm$ 0.061 (4.5%) [1.250–1.410]
Viscosity (Poise)	69.483 $\pm$ 0.649 (0.9%) [68.500–70.200]	70.592 $\pm$ 0.590 (0.8%) [69.750–71.400]	68.133 $\pm$ 1.052 (1.5%) [66.700–69.200]	70.447 $\pm$ 0.786 (1.1%) [69.550–71.500]
EC (S/cm)	0.015 $\pm$ 0.003 (19.8%) [0.011–0.018]	0.018 $\pm$ 0.002 (13.1%) [0.015–0.021]	0.024 $\pm$ 0.005 (19.4%) [0.017–0.031]	0.025 $\pm$ 0.005 (21.3%) [0.015–0.030]
TSS (%)	81.667 $\pm$ 1.211 (1.5%) [80.000–83.000]	82.533 $\pm$ 0.804 (1.0%) [81.800–84.000]	79.583 $\pm$ 0.818 (1.0%) [78.700–81.000]	81.883 $\pm$ 0.886 (1.1%) [80.500–82.800]
pH	4.200 $\pm$ 0.268 (6.4%) [3.900–4.600]	4.817 $\pm$ 0.417 (8.7%) [4.100–5.200]	4.617 $\pm$ 0.371 (8.0%) [4.000–5.000]	5.083 $\pm$ 0.256 (5.0%) [4.700–5.400]
Free Acidity (meq/kg)	39.833 $\pm$ 3.131 (7.9%) [34.000–43.000]	43.600 $\pm$ 0.812 (1.9%) [42.600–44.500]	47.867 $\pm$ 0.923 (1.9%) [46.800–48.900]	38.367 $\pm$ 1.490 (3.9%) [36.200–40.400]
Lactone (meq/kg)	12.583 $\pm$ 1.492 (11.9%) [11.000–14.400]	9.742 $\pm$ 1.514 (15.5%) [8.400–12.100]	12.217 $\pm$ 0.595 (4.9%) [11.300–13.100]	10.833 $\pm$ 0.838 (7.7%) [9.700–12.100]
Total Acidity (meq/kg)	52.417 $\pm$ 3.108 (5.9%) [48.400–57.100]	53.342 $\pm$ 0.792 (1.5%) [52.750–54.800]	60.083 $\pm$ 1.111 (1.8%) [58.900–61.900]	49.200 $\pm$ 1.626 (3.3%) [46.900–50.600]
Fructose (g/100g)	49.617 $\pm$ 0.553 (1.1%) [48.900–50.300]	44.015 $\pm$ 0.948 (2.2%) [42.750–45.110]	46.550 $\pm$ 1.355 (2.9%) [44.500–48.000]	48.117 $\pm$ 1.238 (2.6%) [46.400–49.300]
Glucose (g/100g)	29.967 $\pm$ 0.717 (2.4%) [28.800–30.600]	30.285 $\pm$ 0.713 (2.4%) [29.500–31.210]	31.882 $\pm$ 0.911 (2.9%) [30.600–33.220]	31.583 $\pm$ 0.866 (2.7%) [30.400–33.000]
Fructose/Glucose Ratio	1.508 $\pm$ 0.112 (7.4%) [1.400–1.680]	1.628 $\pm$ 0.203 (12.4%) [1.420–1.900]	1.687 $\pm$ 0.142 (8.4%) [1.520–1.880]	1.517 $\pm$ 0.213 (14.1%) [1.280–1.770]
Sucrose (g/100g)	5.633 $\pm$ 0.393 (7.0%) [5.200–6.100]	5.783 $\pm$ 0.172 (3.0%) [5.500–6.000]	5.733 $\pm$ 0.273 (4.8%) [5.400–6.100]	5.100 $\pm$ 0.261 (5.1%) [4.800–5.500]
Maltose (g/100g)	6.417 $\pm$ 0.214 (3.3%) [6.200–6.700]	6.683 $\pm$ 0.160 (2.4%) [6.500–6.900]	6.117 $\pm$ 0.319 (5.2%) [5.800–6.700]	5.850 $\pm$ 0.362 (6.2%) [5.400–6.300]
Glucose/Water Ratio	1.530 $\pm$ 0.087 (5.7%) [1.400–1.670]	1.623 $\pm$ 0.111 (6.8%) [1.450–1.750]	1.812 $\pm$ 0.064 (3.5%) [1.740–1.900]	1.562 $\pm$ 0.261 (16.7%) [1.110–1.810]

Table 2: One-Way ANOVA Results for Sidr Honey Parameters Across the Four Geographical Regions (df = 3, 20)

Parameter	F-value	p-value	Significance
Moisture (%)	20.94	< 0.001	Significant (p<0.05)
Specific gravity	0.06	0.9802	Not significant
Viscosity (Poise)	12.34	< 0.001	Significant (p<0.05)
EC (S/cm)	8.46	< 0.001	Significant (p<0.05)
TSS (%)	10.96	< 0.001	Significant (p<0.05)
pH	7.41	0.0016	Significant (p<0.05)
Free Acidity (meq/kg)	32.03	< 0.001	Significant (p<0.05)
Lactone (meq/kg)	7.35	0.0016	Significant (p<0.05)
Total Acidity (meq/kg)	35.44	< 0.001	Significant (p<0.05)
Fructose (g/100g)	30.07	< 0.001	Significant (p<0.05)
Glucose (g/100g)	8.23	< 0.001	Significant (p<0.05)
Fructose/Glucose Ratio	1.53	0.2379	Not significant
Sucrose (g/100g)	7.26	0.0018	Significant (p<0.05)
Maltose (g/100g)	10.32	< 0.001	Significant (p<0.05)
Glucose/Water Ratio	4.14	0.0195	Significant (p<0.05)



Note: $df = 3$ (between groups), 20 (within groups), based on $n = 6$ analytical replicates per region (2 composite samples $\times$ 3 replicates). As these replicates are nested within only 2 independent composite samples per region, formal tests of ANOVA assumptions (normality, homogeneity of variance) were not performed, and results should be interpreted as preliminary given the risk of pseudoreplication.

Table 3: Mean Values with Tukey HSD Grouping Letters for Sidr Honey Parameters by Region

Parameter	Zawia	Tarhuna	Sabratha	Qasr Khair
Moisture (%)	17.80 b	17.40 b	19.16 a	18.87 a
Specific gravity	1.36 a	1.35 a	1.35 a	1.35 a
Viscosity (Poise)	69.48 a	70.59 a	68.13 b	70.45 a
EC (S/cm)	0.01 b	0.02 b	0.02 a	0.02 a
TSS (%)	81.67 a	82.53 a	79.58 b	81.88 a
pH	4.20 b	4.82 a	4.62 a	5.08 a
Free Acidity (meq/kg)	39.83 c	43.60 b	47.87 a	38.37 c
Lactone (meq/kg)	12.58 a	9.74 b	12.22 a	10.83 a
Total Acidity (meq/kg)	52.42 b	53.34 b	60.08 a	49.20 c
Fructose (g/100g)	49.62 a	44.01 c	46.55 b	48.12 a
Glucose (g/100g)	29.97 b	30.29 b	31.88 a	31.58 a
Fructose/Glucose Ratio	1.51 a	1.63 a	1.69 a	1.52 a
Sucrose (g/100g)	5.63 a	5.78 a	5.73 a	5.10 b
Maltose (g/100g)	6.42 a	6.68 a	6.12 b	5.85 b
Glucose/Water Ratio	1.53 b	1.62 a	1.81 a	1.56 b

Note: Within each row, means followed by the same letter are not significantly different according to Tukey's HSD test ($p > 0.05$). For parameters where the overall ANOVA was not significant ($p \geq 0.05$), all regions share the same letter (a) by convention.

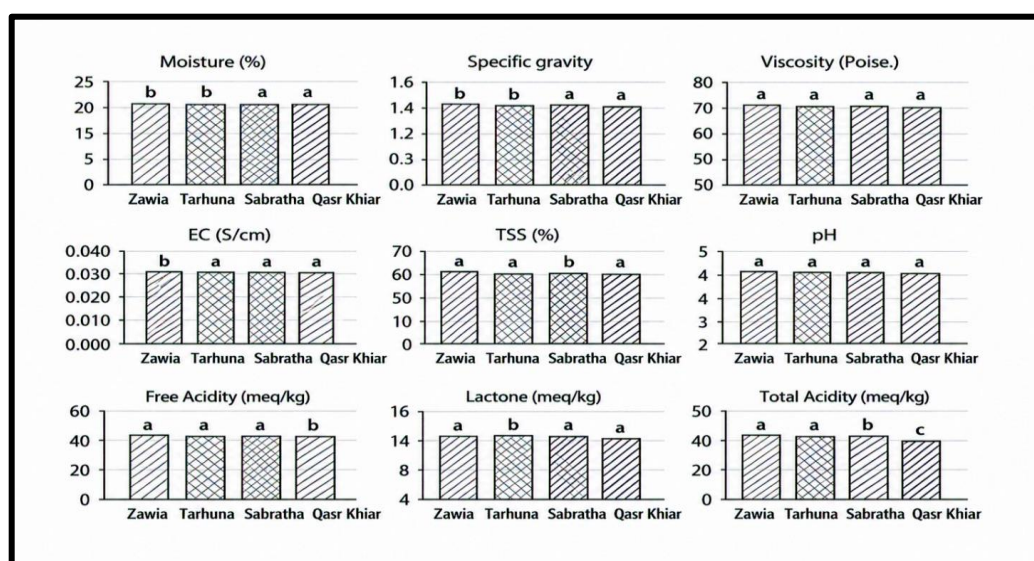


Figure 1: Mean $\pm$ SD of physicochemical and acidity parameters of Sidr honey by geographical region ($n = 6$ analytical replicates per region: 2 composite samples $\times$ 3 replicates). Letters above bars indicate Tukey HSD groupings within each panel; bars sharing a letter are not significantly different ($p > 0.05$).

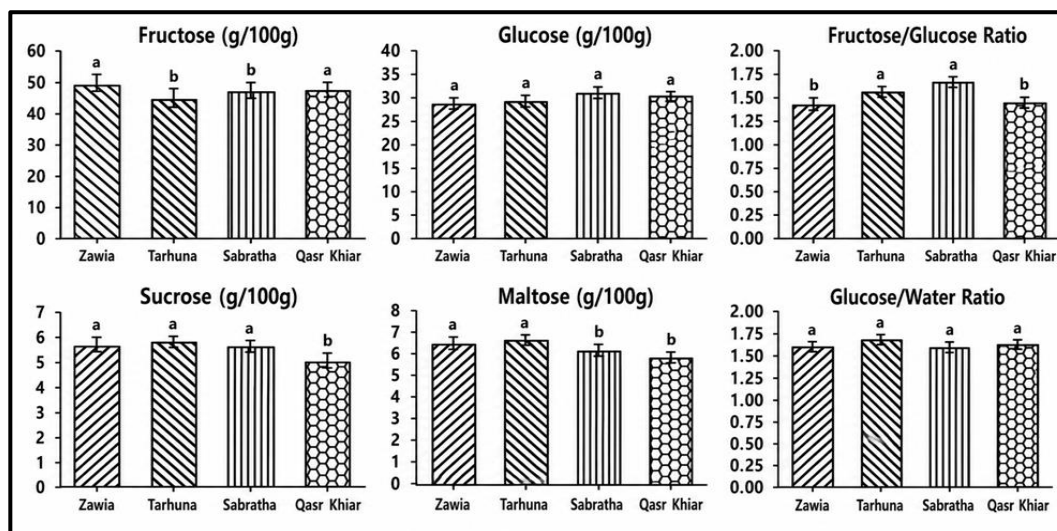


Figure 2: Mean $\pm$ SD of sugar composition and related ratios of Sidr honey by geographical region ($n = 6$ analytical replicates per region: 2 composite samples $\times$ 3 replicates). Letters above bars indicate Tukey HSD groupings within each panel; bars sharing a letter are not significantly different ($p > 0.05$).

Discussion

The moisture content recorded in the present study (17.4–19.2%) falls within the upper permissible range for honey moisture and is comparable to previous reports on Libyan honey markets [3,6], remaining below the 20% ceiling adopted by the Codex Alimentarius Commission [7], and indicating adequate maturation and a low risk of osmophilic yeast fermentation. Specific gravity and viscosity did not differ significantly among regions, consistent with the generally narrow range reported for honey of comparable moisture content, since both properties are governed primarily by the concentration of dissolved sugars and water content [17].

Electrical conductivity, which reflects the mineral (ash) and organic acid content of honey and is widely regarded as one of the most reliable indicators of botanical and geographical origin [4,19], differed significantly among the studied regions, varying more than 1.6-fold between Zawia and Qasr Khair. This pattern likely reflects differences in soil mineral composition and local floral association among the four sites, consistent with reports that EC varies with soil type and geographic zone even within honeys of similar botanical origin [1]. The recorded EC values remained broadly consistent with ranges previously reported for Sidr honey from Libya and neighboring regions [10, 12].

pH values recorded in this study (4.20–5.08, region means) differed significantly among regions ($F = 7.41$, $p = 0.0016$), with the Tarhuna (4.82), Sabratha (4.62), and Qasr Khair (5.08) means exceeding the upper bound typically reported

for genuine honey, approximately 3.2–4.5; [4,7], while only Zawia (4.20) fell within this range. This elevated and variable pH profile does not, by itself, indicate adulteration, but it is a notable deviation from the reference range that should be verified with a larger, independently replicated sample set before being interpreted as a genuine regional or botanical characteristic. Because acidity and pH are mechanistically linked to the hydrogen-peroxide-independent antibacterial activity reported for Libyan and Saudi monofloral honeys [2], the elevated pH observed in several regions may also warrant attention in future work on the bioactive potential of these honeys, though antimicrobial assays were outside the scope of the present study. Free acidity (38.4–47.9 meq/kg, region means) remained within the Codex limit of 50 meq/kg [7], and is broadly comparable to previously reported values for Libyan and regional Sidr honeys [8,9], and total acidity followed a similar pattern, remaining below levels generally associated with excessive fermentation.

The fructose/glucose ratio (1.51–1.69, region means) is an important quality index related to crystallization behavior, since honeys with higher fructose relative to glucose tend to remain liquid for longer, whereas a lower ratio favors more rapid glucose crystallization [11]. The comparatively higher ratio recorded in Sabratha honey suggests a lower crystallization tendency relative to Zawia and Qasr Khair honeys. These values are broadly consistent with fructose/glucose ratios previously reported for Sidr honey from regional sources [12]. Sucrose (5.10–5.78 g/100g) and maltose (5.85–6.68 g/100g) contents remained within



ranges typically reported for genuine, unadulterated honey, in which disaccharide sugars generally represent a minor fraction of total carbohydrate content [5, 12]; the relatively low sucrose values recorded across all four regions are consistent with mature honey and do not, by themselves, indicate obvious sucrose adulteration, since elevated sucrose is often associated with premature harvesting or adulteration with sugar syrups [11].

The melissopalynological analysis performed in this study (Section 2.2) supports the classification of the samples as monofloral Sidr honey, exceeding the $\geq 45\%$ threshold conventionally used to designate a predominant pollen type [14], and addressing a limitation of purely sensory verification, and strengthening the botanical basis for the regional comparisons reported here. Preliminary HPLC method validation (recovery 95–102%, RSD < 5%) further supports the reliability of the reported sugar composition data, although formal multi-level validation with a larger number of replicates would strengthen this evidence in future work.

In Figures 1 and 2, letters above each bar correspond to the Tukey HSD grouping: bars sharing an identical letter within the same panel indicate regions whose means are not significantly different, whereas bars with different letters denote regions that differ significantly at the 5% level. Overall, the physicochemical profile of the Sidr honey samples examined largely falls within ranges established by the Codex Alimentarius Commission (2001) and International Honey Commission standards [4]. The regional patterns observed in moisture, EC, acidity, and selected sugars may reflect local variation in soil, microclimate, and floral composition, consistent with previous findings on monofloral honey [1,19], though confirmation of true geographic differences would require larger, independently replicated sample sets.

This study has one key limitation that must be considered when interpreting the results: the small number of independent composite samples per region ($n = 2$) severely limits statistical power. Several parameters that approached significance (viscosity, glucose) may reflect true regional trends that this study was underpowered to detect, or may reflect sampling variability, and neither can be distinguished with confidence at this sample size. Formal assumption testing for ANOVA (normality, homogeneity of variance) was not feasible with $n = 2$ per group, so the ANOVA and Tukey HSD findings should be read as an early signal rather than conclusive proof of regional difference. This constraint supports the need for larger follow-up studies with a greater number of independent samples per region.

Conclusion

This study provides a preliminary but scientifically grounded baseline characterization of Sidr (*Ziziphus spina-christi*) honey produced across four regions of western Libya. The high proportion of *Ziziphus* pollen (60–67%) supports the monofloral botanical origin of the samples, while moisture, pH, free acidity, and sugar composition values fell largely within internationally recognized quality standards [4,7], supporting the authenticity and market suitability of the honey examined. Despite the constraints imposed by a limited number of independent composite samples per region, this dataset establishes an important scientific reference point: to the authors' knowledge, it is among the first studies to document the physicochemical and melissopalynological profile of Libyan Sidr honey across multiple production regions within a single, standardized analytical framework. As such, this work lays a solid foundation for future research into the quality, authenticity, and, most importantly, the therapeutic potential of Libyan Sidr honey, and underscores the value of continued scientific investment in this culturally and economically significant natural product.

Recommendations

Building on the baseline dataset established here, several directions are recommended for future work. First, and most importantly, the therapeutic and bioactive properties of Libyan Sidr honey warrant dedicated investigation, including antibacterial and antioxidant activity, wound-healing potential, and its role in gastrointestinal conditions such as peptic ulcer disease, given the acidity and phenolic-related properties suggested by the present physicochemical profile. Second, follow-up studies should employ a substantially larger number of independent composite samples per region, collected across multiple harvest seasons and years, to provide adequate statistical power for confirming or refuting the regional patterns observed here. Third, formal multi-level validation of the HPLC method, including a larger number of replicates and spiked recovery experiments, would strengthen confidence in the reported sugar composition data. Fourth, complementary analyses such as mineral profiling, phenolic and flavonoid content, and antioxidant capacity assays would provide a more complete quality and authenticity profile of Sidr honey from these regions. Finally, expanding melissopalynological sampling to additional Libyan production areas would help establish more comprehensive geographical and botanical traceability for Libyan Sidr honey.



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