



Article

Development of a Green Analytical Method for the Assessment of Honey Quality through Reducing Sugars, Hydroxymethylfurfural and Physicochemical Parameters

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Abstract: A low-cost and green spectrophotometric method was developed to be used as a routine quality control method of honey. The use of Carrez reagents, p-toluidine and Fehling's copper reagents was eliminated by simply filtering the solution through a membrane and measuring reducing sugars using a miniaturised alkaline hexacyanoferrate(III) reaction at 420 nm, and 5-hydroxymethylfurfural (HMF) at 284 nm against a sodium bisulfite reference. Moisture, pH, free acidity, electrical conductivity, ash and Pfund colour were measured with reagent-free or low-reagent procedures on the same samples. Under the optimum conditions (2.0 mmol L⁻¹ K₃[Fe(CN)₆], 0.20 mol L⁻¹ Na₂CO₃, 90 °C, 6 min) the sugar response was linear from 10 to 100 µg mL⁻¹ (R² = 0.9997), and the HMF response was linear from 1.0 to 20.0 mg L⁻¹ (R² = 0.9999). The limits of detection were 2.21 µg mL⁻¹ and 0.21 mg L⁻¹, respectively. Intra and Inter day RSD values were not more than 1.7% and 2.6% respectively and recovery ranged between 97.6% and 102.4%. Eighteen honeys from Iraqi markets and apiaries were analysed. Of the 15, all met the Codex Alimentarius and EU limits for all parameters, but three imported commercial blends failed the HMF limit (40 mg kg⁻¹) and two of these also failed the moisture and sucrose limits. The results were in good agreement with the Lane – Eynon method and HPLC-UV method (paired t-test, p > 0.05). The procedure achieved a score of 89 on the Analytical Eco-Scale and 0.73 on AGREE in comparison to 0.49 to 0.54 for the conventional procedures. Two components accounted for 90.7% of the variance and principal component analysis discriminated heat-damaged blends from fresh local honeys.

Keywords: Honey quality; Reducing sugars; Hydroxymethylfurfural; Green analytical chemistry; Hexacyanoferrate(III); AGREE; Chemometrics; Codex Alimentarius.

1. Introduction

1.1 Honey: composition and nutritional importance

Honey is the sweet substance that honeybees (*Apis mellifera* L.) produce from floral nectar or from secretions of living plant parts, which they collect, transform with their own enzymes, dehydrate and store in the comb [1]. It has been eaten for thousands of years and is still valued as a natural sweetener, a preservative and a traditional remedy. In Iraq, Sidr (*Ziziphus spina-christi*) honey and mountain honeys from the Kurdistan Region fetch high prices, and this price gap between local and imported products gives an obvious incentive for mislabelling and dilution.

The nutritional value of honey is primarily due to its carbohydrate content which provides approximately 300kcal/kg honey but the minor component is also important. Organic acids, amino acids, enzymes, minerals, vitamins and phenolic compounds are present in honey and these ingredients render the honey with antioxidant and antimicrobial activity [2,3]. The contents vary with the flora, climate, and the methods of extraction and storage by beekeepers and two honeys of similar sweetness may differ considerably [4].

1.2 Chemical composition of honey

Honey is a solution of sugar that is made supersaturated. It consists approximately 95% of their dry matter content and 15-20% of their total mass is generally water [5,6]. The main constituents are fructose (~38 %) and glucose (~31 %) and are followed by a cluster of disaccharides including maltose, sucrose and turanose and minor amounts of trisaccharides. The primary acid is gluconic, which is a product of the glucose and glucose oxidase. Light blossom honeys contain low amounts of mineral matter (0.1–0.3%) and some of the other types of honeys higher quantities, potassium being the predominant mineral [7]. These elements are related. Mineral content determines the electrical conductivity, the organic acids the pH and free acidity, and the sugar-to-water ratio the viscosity and crystallisation and the danger of fermentation [8]. It is for this reason that a quality assessment based on a limited number of carefully selected parameters can provide a lot of information about the origin, freshness and handling history of a sample.

1.3 Reducing sugars in honey

Glucose and fructose are reducing sugars because they contain an open-chain form containing free carbonyl group which is capable of reducing a metal ion or other oxidants. According to the Codex standard and EU Directive 2001/110/EC, blossom honey must contain at least 60 g of fructose plus glucose per 100 g and no more than 5 g of sucrose per 100 g with certain exceptions for a few unifloral honeys [1,9]. Low reducing-sugar and high apparent sucrose are classic symptoms of sugar syrup feeding by the bees and/or post harvest supplementation of syrup. Lane–Eynon titration with Fehling's solution and methylene blue is still the official procedure for these sugars [12,13].

It is accurate in the hands of the skilled chemist but requires the use of gram amounts of copper sulfate, sodium hydroxide and potassium sodium tartrate, a boiling titration and the chemist's eye at the end point. The 3,5-dinitrosalicylic acid (DNS) method is faster, but it involves phenol, sodium sulfite in a strongly alkaline solution [14]. The alkaline hexacyanoferrate(III) reaction, which was originally used for blood sugar [15,16] requires much less reagent, the yellow $[\text{Fe}(\text{CN})_6]^{3-}$ ion will be reduced to almost colourless $[\text{Fe}(\text{CN})_6]^{4-}$ and the decrease in absorbance at 420nm is proportional to the amount of reducing sugar.

1.4 Hydroxymethylfurfural as a quality indicator

5-Hydroxymethylfurfural (HMF) is a cyclic aldehyde formed when hexoses, mainly fructose, dehydrate in acid medium, and to a smaller extent through the Maillard reaction [17,18]. Fresh honey contains little or no HMF. Its concentration rises with heating, long storage and warm climates, and adulteration with acid-inverted syrups can also raise it [19–21]. The Codex standard sets a maximum of 40 mg kg⁻¹, or 80 mg kg⁻¹ for honey of declared origin from tropical regions [1]. HMF is therefore the most widely used single marker of freshness and heat damage. It has also raised toxicological interest, because its metabolite 5-sulfoxymethylfurfural showed genotoxic activity in some studies, although dietary intake from honey is usually small [18,22].

Three HMF methods are recognised by the International Honey Commission: the White UV method with bisulfite reference, the Winkler method with p-toluidine and barbituric acid, and reversed-phase HPLC [13,23,24]. The Winkler method is now discouraged because p-toluidine is toxic and a suspected carcinogen. The White method is

simpler but still clarifies the sample with Carrez I (potassium hexacyanoferrate(II)) and Carrez II (zinc acetate), which adds zinc-containing waste. HPLC is selective but expensive and slow for small laboratories [25,26].

1.5 Physicochemical characteristics of honey

Moisture content is the main factor behind the keeping quality of honey. Above about 18–20% water, osmotolerant yeasts can grow and ferment the product, so the Codex limit is 20 g/100 g [1]. Moisture is conveniently obtained from the refractive index using the Chataway table, with no reagents [27]. Electrical conductivity (EC) reflects mineral salts, organic acids and proteins; it separates blossom honeys (below 0.8 mS cm⁻¹) from honeydew honeys and correlates closely with ash content [28,29]. Free acidity indicates fermentation, since yeasts convert sugars to alcohol and then to acetic acid, and the limit is 50 meq kg⁻¹ [9]. Colour, expressed on the Pfund scale, follows mineral and phenolic content and is used commercially to grade honey [30].

1.6 Honey quality and authenticity

Honey is one of the foods most often reported in fraud surveys. The usual practices are dilution with cheap sugar syrups, harvesting of immature honey followed by artificial drying, feeding bees with sugar during the nectar flow, and false declaration of botanical or geographical origin [10,11]. Isotope ratio mass spectrometry and NMR profiling detect many of these practices, but they are out of reach for most food control laboratories in the region. A lower-cost route combines standard quality parameters with multivariate statistics, because adulterated or overheated samples tend to fall outside the pattern formed by authentic honeys [31,32].

1.7 Analytical methods for honey analysis and the case for greener procedures

Green analytical chemistry applies the principles of green chemistry to measurement. Its aims include fewer and safer reagents, less waste, lower energy use, miniaturisation and direct analysis where possible [33–36]. Several tools now make greenness measurable. The Analytical Eco-Scale subtracts penalty points from 100 [37], GAPI uses a coloured pictogram [38], and AGREE converts the twelve principles into a single score between 0 and 1 [39,40]. Most routine honey methods were written before these tools existed, and several of them score poorly.

This work set out to replace the most hazardous steps in routine honey control while keeping the accuracy of the official methods. The specific objectives were (i) to optimise a miniaturised hexacyanoferrate(III) method for reducing sugars; (ii) to simplify the White HMF method by replacing Carrez clarification with membrane filtration; (iii) to validate both procedures following ICH Q2(R2) [41]; (iv) to apply them, together with reagent-free physicochemical tests, to 18 honeys sold or produced in Iraq; and (v) to compare the procedure with the reference methods in terms of accuracy and greenness.

2. Methodology

2.1 Materials, reagents and honey samples

D-(+)-Glucose (≥99.5%), D-(-)-fructose (≥99%), sucrose (≥99.5%) and 5-hydroxymethylfurfural (≥99%) were purchased from Sigma-Aldrich (Germany). Potassium hexacyanoferrate(III) (≥99%), anhydrous sodium carbonate (≥99.5%), sodium bisulfite (analytical grade), sodium hydroxide (≥98%), hydrochloric acid (37%) and potassium chloride were obtained from Merck (Germany). Reagents for the reference methods (Fehling A and B, methylene blue, Carrez I and II, and HPLC-grade methanol) came from the same suppliers. Deionised water (18.2 MΩ cm) was used throughout.

A mixed sugar standard was prepared to match the typical fructose to glucose ratio of honey (1.2:1, w/w). A stock solution of 1000 µg mL⁻¹ was made by dissolving 545.5 mg of fructose and 454.5 mg of glucose in 1000 mL of water, and working standards (10–100 µg

mL⁻¹) were prepared by dilution on the day of use. An HMF stock solution (100 mg L⁻¹) was prepared in water, stored at 4 °C in the dark and used within one week.

Eighteen honey samples (H1–H18) were collected between March and August 2025. Fifteen were obtained directly from beekeepers in eleven Iraqi governorates and three were imported commercial blends bought from supermarkets in Kirkuk (Table 1). Botanical origin was assigned from the beekeepers' declarations and the flora around the apiary. All samples were stored in closed glass jars at 20 ± 2 °C in the dark and analysed within four weeks of collection.

Table 1. Honey samples analysed in this study.

Cod e	Declared botanical origin	Region / source	Cod e	Declared botanical origin	Region / source
H1	Sidr	Erbil	H10	Multifloral	Erbil
H2	Sidr	Kirkuk	H11	Multifloral	Duhok
H3	Sidr	Sulaymaniyah	H12	Multifloral	Sulaymaniyah
H4	Sidr	Salah Al-Din	H13	Multifloral	Kirkuk
H5	Citrus	Diyala	H14	Clover	Nineveh
H6	Citrus	Baghdad	H15	Clover	Kirkuk
H7	Citrus	Diyala	H16	Commercial blend	Imported (retail)
H8	Eucalyptus	Babylon	H17	Commercial blend	Imported (retail)
H9	Eucalyptus	Wasit	H18	Commercial blend	Imported (retail)

2.2 Instrumentation

Absorbance was measured on a double-beam UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) with 1.0 cm quartz cells. A thermostatic water bath (±0.5 °C), an Abbe refractometer thermostated at 20 °C, a digital pH meter with a combined glass electrode, a conductivity meter with a cell constant of 1.0 cm⁻¹, an analytical balance (±0.1 mg) and 0.45 µm cellulose acetate syringe filters were also used. Reference HMF values were obtained on an HPLC system with a UV detector at 285 nm and a C18 column (250 × 4.6 mm, 5 µm), using water/methanol (90:10, v/v) at 1.0 mL min⁻¹ as described by the International Honey Commission [13].

2.3 Sample preparation

Each honey was homogenised by stirring. Crystallised samples were warmed in a closed jar at 40 °C until liquid, which is below the temperature at which HMF forms measurably over short periods [19]. Three preparations were made from each sample (Figure 1). For reducing sugars, 1.000 g of honey was dissolved in water and diluted to 100 mL, and 1.00 mL of this solution was diluted again to 100 mL, giving 100 µg of honey per mL. For HMF, 10.00 g of honey was dissolved in about 25 mL of water, made up to 50 mL and passed through a 0.45 µm membrane filter, discarding the first 2 mL. For EC and colour, a solution containing 20 g of honey dry matter in 100 mL was prepared, while moisture was read on the undiluted honey.

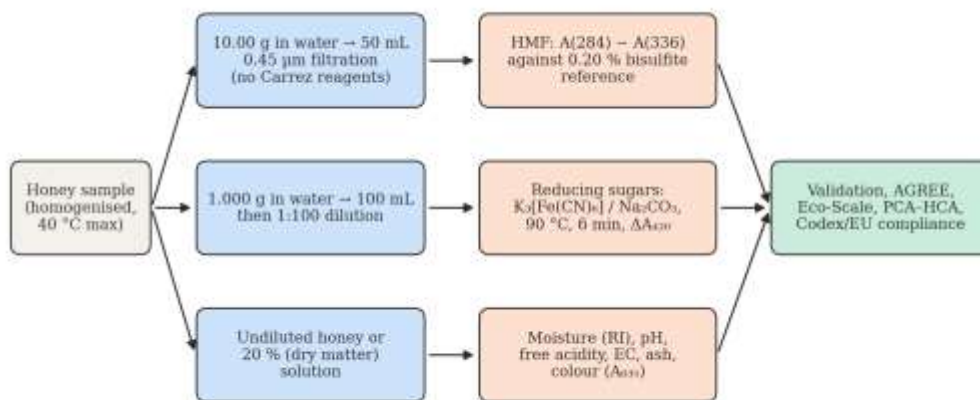
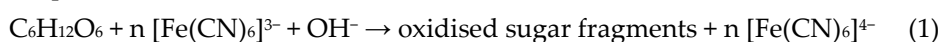


Figure 1. Workflow of the proposed green procedure for honey quality assessment.

2.4 Determination of reducing sugars

In a 10 mL screw-capped glass tube, 0.50 mL of standard or diluted honey solution, 1.00 mL of 5.0 mmol L⁻¹ K₃[Fe(CN)₆] and 1.00 mL of 0.50 mol L⁻¹ Na₂CO₃ were mixed. The final volume was 2.50 mL, which gives reagent concentrations of 2.0 mmol L⁻¹ hexacyanoferrate(III) and 0.20 mol L⁻¹ carbonate. The tubes were closed, heated in the water bath at 90 °C for 6 min and cooled in running water for 2 min. The absorbance at 420 nm was read against water, and the decrease in absorbance ($\Delta A_{420} = A_{\text{blank}} - A_{\text{sample}}$) relative to a reagent blank containing water instead of sugar was used as the analytical signal. The reaction consumes several moles of hexacyanoferrate(III) per mole of hexose, according to the simplified scheme:



The content of reducing-sugar (g/100 g) was determined from the calibration line and the dilution factor. A second aliquot (5 mL of the first aliquot + 0.5 mL of 6 mol L⁻¹ HCl for 10 min at 65 °C followed by neutralisation using NaOH) was subjected to mild acid hydrolysis to obtain the apparent sucrose as determined by the difference between the total sugars content (after inversion) and the reducing sugar content, multiplied by 0.95 [13].

2.5 Determination of hydroxymethylfurfural

Two 5.0 mL portions of the filtered HMF solution were transferred to test tubes. To the first tube (sample) 5.0 mL of water was added, and to the second (reference) 5.0 mL of 0.20% (w/v) sodium bisulfite was added. Bisulfite forms an addition product with the aldehyde group of HMF and removes its absorption at 284 nm, so the reference cancels the background absorbance of the honey matrix [23]. Absorbances were read at 284 and 336 nm, and the difference $A_{284} - A_{336}$ was used to correct for residual turbidity. HMF was quantified against the external calibration line and expressed as mg kg⁻¹. Solutions with A_{284} above 2.0 were diluted twice with water and the bisulfite reference was diluted in the same way. The only step removed from the IHC version of this method is the Carrez clarification; the membrane filtration takes its place.

2.6 Determination of physicochemical parameters

Moisture was obtained from the refractive index at 20 °C and converted with the Chataway table [13,27]. **pH** was measured in a 10% (w/v) honey solution. **Free acidity** was determined by titrating the same solution with 0.050 mol L⁻¹ NaOH to pH 8.30 and expressed in meq kg⁻¹. **Electrical conductivity** was measured at 20 °C in the 20% dry-matter solution and reported in mS cm⁻¹. **Ash** was calculated from EC with the relation of Piazza et al. [28]:

$$\text{Ash (\%)} = (\text{EC} - 0.143) / 1.743 \quad (2)$$

Colour was measured as the absorbance of a 50% (w/v) honey solution at 635 nm against glycerol and converted to the Pfund scale [30]:

$$\text{mm Pfund} = -38.70 + 371.39 \times A_{635} \quad (3)$$

Colour classes followed the USDA grading: white (18–34 mm), extra light amber (35–50 mm), light amber (51–85 mm), amber (86–114 mm) and dark amber (above 114 mm).

2.7 Optimization and validation of the analytical method

The sugar reaction was optimised one variable at a time with a $50 \mu\text{g mL}^{-1}$ mixed standard, varying hexacyanoferrate(III) concentration ($0.5\text{--}3.0 \text{ mmol L}^{-1}$), Na_2CO_3 concentration ($0.05\text{--}0.30 \text{ mol L}^{-1}$), temperature ($70\text{--}95^\circ\text{C}$) and heating time (2–12 min). For HMF, the bisulfite concentration in the reference (0.05–0.40%) and the need for clarification were examined. Each condition was run in triplicate.

Validation followed ICH Q2(R2) [41]. Linearity was tested with seven sugar standards and eight HMF standards in triplicate. The limits of detection and quantification were calculated as $\text{LOD} = 3.3 \sigma/S$ and $\text{LOQ} = 10 \sigma/S$, where σ is the residual standard deviation of the regression and S the slope. Precision was expressed as relative standard deviation (RSD) at three levels, on one day ($n = 6$) and on three consecutive days ($n = 9$). Accuracy was checked by spiking honey H5 with the mixed sugar standard and honey H3 with HMF at three levels, and by comparison with reference methods. Robustness was evaluated by small deliberate changes in temperature ($\pm 2^\circ\text{C}$), heating time ($\pm 0.5 \text{ min}$), carbonate concentration ($\pm 0.01 \text{ mol L}^{-1}$) and bisulfite concentration ($\pm 0.02\%$).

2.8 Statistical and chemometric analysis

All determinations were made in triplicate and results are given as mean $\pm$ standard deviation. Pearson correlation coefficients were calculated between the eight quantitative variables (reducing sugars, sucrose, HMF, moisture, pH, free acidity, EC and colour). Ash was left out because it is calculated from EC. For principal component analysis (PCA), the data matrix (18×8) was autoscaled, and components with eigenvalues above 1 were retained [42]. Hierarchical cluster analysis (HCA) used Euclidean distance with Ward's linkage [43]. Calculations were performed in Python 3.11 with NumPy and SciPy, and the significance level was set at $p < 0.05$.

2.9 Evaluation of analytical performance and comparison with conventional methods

Reducing sugars in all 18 honeys were also determined by the Lane–Eynon titration, and HMF by the IHC HPLC-UV method [12,13]. Agreement was evaluated by linear regression of proposed against reference values, by the paired t-test across samples, and by t- and F-tests on five replicate determinations of six selected samples. For the greenness assessment, the proposed procedure, the Lane–Eynon titration, the Winkler method and the HPLC-UV method were scored with the Analytical Eco-Scale [37] and with the AGREE calculator using equal weights for the twelve principles [39]. Penalty points were assigned from the GHS hazard pictograms and signal words of each reagent, the amount used per sample, energy consumption and waste volume.

2.10 Quality assessment and classification of honey samples

Each sample was compared with the Codex and EU limits: reducing sugars $\geq 60 \text{ g/100 g}$, sucrose $\leq 5 \text{ g/100 g}$, moisture $\leq 20 \text{ g/100 g}$, HMF $\leq 40 \text{ mg kg}^{-1}$, free acidity $\leq 50 \text{ meq kg}^{-1}$ and EC $\leq 0.8 \text{ mS cm}^{-1}$ for blossom honey [1,9]. To make the results easier to use for traders and inspectors, a three-level grade was defined.

3. Results and Discussion

3.1 Analytical method optimization

Hexacyanoferrate(III) shows a broad band with a maximum at 420 nm, and this band falls in proportion to the amount of reducing sugar added (Figure 2a). The reduced form, $[\text{Fe}(\text{CN})_6]^{4-}$, does not absorb at this wavelength, so no second reagent is needed to develop colour. HMF gives a sharp maximum at 284 nm with a shoulder near 230 nm, and the absorbance at 336 nm is close to zero (Figure 2b). Measuring at 284 nm against the bisulfite reference and subtracting the 336 nm reading therefore removes both the honey background and any light scattering.

The effects of the four reaction variables are shown in Figure 3. The signal increased with hexacyanoferrate(III) concentration up to 1.5 mmol L^{-1} and then levelled off. A concentration of 2.0 mmol L^{-1} was chosen, because it keeps the initial absorbance near 1.0 and leaves enough oxidant for the highest standard. The carbonate concentration had a similar effect: the reaction was incomplete below 0.15 mol L^{-1} and the signal fell slightly above 0.25 mol L^{-1} , probably because the stronger alkali degrades the sugars through side reactions that do not reduce the oxidant in the same ratio. The response rose steeply with temperature between 70 and 90°C and changed little above 90°C . At 90°C the reaction was complete after 6 min, and longer heating gave no gain. These conditions shorten the procedure compared with the boiling-water methods, which usually need 10–15 min [14,16].

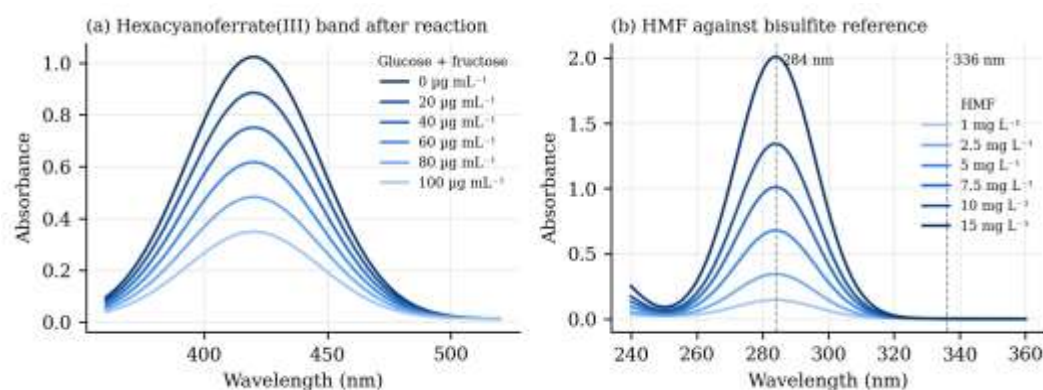


Figure 2. UV-Vis spectra of (a) the hexacyanoferrate(III) reagent after reaction with increasing concentrations of mixed glucose/fructose standard and (b) HMF standards measured against the bisulfite reference.

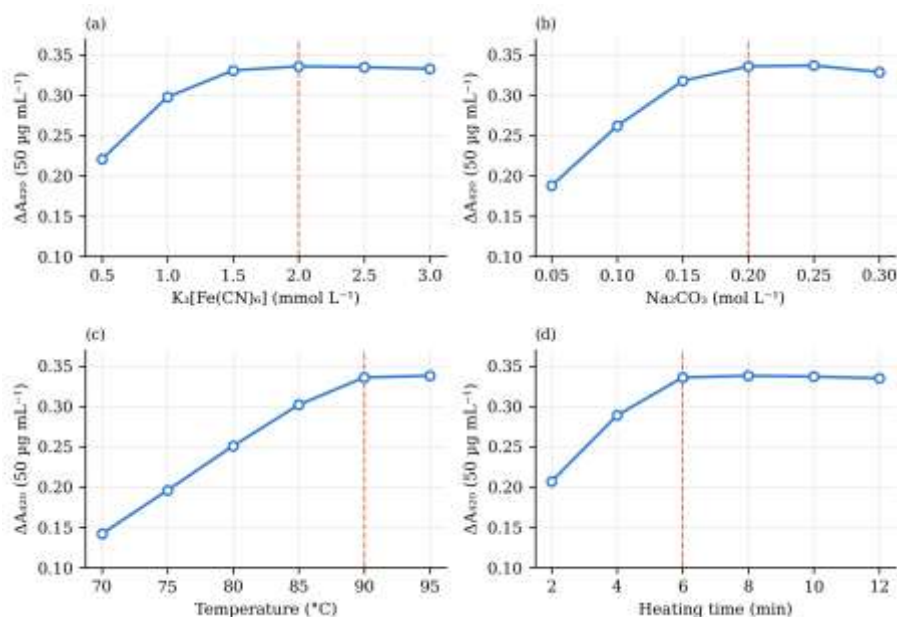


Figure 3. Optimization of the reducing-sugar reaction: effect of (a) $K_3[Fe(CN)_6]$ concentration, (b) Na_2CO_3 concentration, (c) temperature and (d) heating time on ΔA_{420} of a $50 \mu g mL^{-1}$ standard (mean $\pm$ SD, $n = 3$). Dashed lines mark the selected values.

For HMF, a bisulfite concentration of 0.10% in the final reference solution removed more than 99% of the HMF absorbance at 284 nm, while higher amounts gave no further change. The effect of dropping Carrez clarification was tested on six samples covering the whole colour range. The filtered and clarified extracts gave HMF values that differed by 0.4–2.1%, with no systematic bias (paired $t = 1.12$, $p = 0.31$). Colloids and proteins that Carrez reagents precipitate absorb similarly in the sample and reference cells, and the 336 nm correction deals with the remaining turbidity. The selected conditions are summarised in Table 2.

Table 2. Optimization of the proposed procedure.

Variable	Range studied	Selected value	Reason
$K_3[Fe(CN)_6]$ (final)	0.5–3.0 mmol L^{-1}	2.0 mmol L^{-1}	Plateau of response; $A_0 \approx 1.0$
Na_2CO_3 (final)	0.05–0.30 mol L^{-1}	0.20 mol L^{-1}	Complete oxidation, no loss
Reaction temperature	70–95 °C	90 °C	Maximum signal, below boiling
Heating time	2–12 min	6 min	Reaction complete
Reaction volume	1.0–5.0 mL	2.5 mL	Small waste, stable reading
$NaHSO_3$ in HMF reference	0.05–0.40 % (w/v)	0.20 % (0.10 % final)	Full masking of HMF
Clarification for HMF	Carrez I/II vs 0.45 μm filter	Filtration	Same results, no Zn waste
Wavelengths	Scan 240–520 nm	420 nm; 284 and 336 nm	Band maxima; turbidity correction

3.2 Validation results

The calibration lines are shown in Figure 4 and the regression data in Table 3. For reducing sugars the line was $\Delta A_{420} = 0.00671C + 0.0026$ (C in $\mu g mL^{-1}$), with $R^2 = 0.9997$. For HMF it was $A = 0.1320C + 0.0083$ (C in $mg L^{-1}$), with $R^2 = 0.9999$. The intercepts did not differ significantly from zero at the 95% level. The HMF slope corresponds to an apparent molar absorptivity of $16,646 L mol^{-1} cm^{-1}$, close to the value of $16 830 L mol^{-1} cm^{-1}$ used in the White method [23], which confirms that the filtration step does not bias the signal.

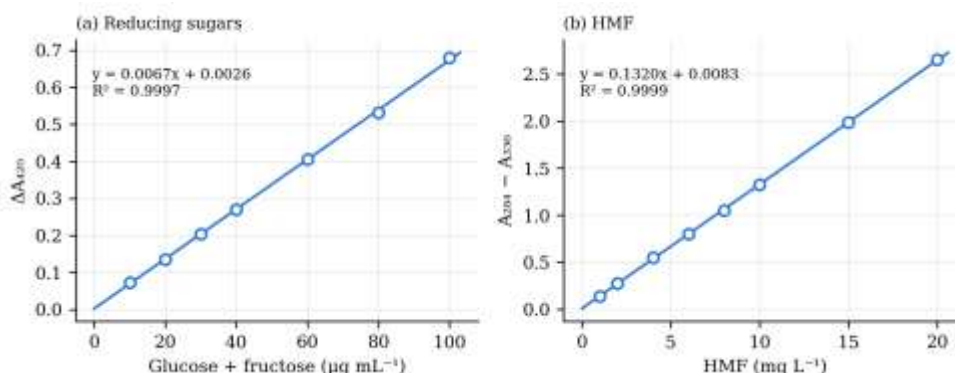


Figure 4. Calibration lines for (a) reducing sugars (glucose/fructose 1:1.2) and (b) HMF under the optimised conditions.

The LOD and LOQ for sugars were 2.21 and $6.70 \mu g mL^{-1}$. With the 1:10 000 dilution of honey, the LOQ is equivalent to 6.7 g/100 g, far below the 60 g/100 g limit, and the calibration range covers honeys with 10–100 g/100 g reducing sugars without further dilution. For HMF the LOD and LOQ were 0.21 and $0.63 mg L^{-1}$, equivalent to 1.0 and 3.1

mg kg⁻¹ in honey. This is sensitive enough for fresh honeys, which often contain less than 10 mg kg⁻¹.

Table 3. Analytical figures of merit of the proposed procedure.

Parameter	Reducing sugars	HMF
Wavelength (nm)	420 (ΔA)	284 – 336
Linear range	10–100 µg mL ⁻¹	1.0–20.0 mg L ⁻¹
Slope ± SD	0.00671 ± 0.00006	0.1320 ± 0.0005
Intercept ± SD	0.0026 ± 0.0032	0.0083 ± 0.0049
R ²	0.9997	0.9999
Residual SD (σ)	0.0045	0.0083
LOD	2.21 µg mL ⁻¹ (2.2 g/100 g)	0.21 mg L ⁻¹ (1.0 mg kg ⁻¹)
LOQ	6.70 µg mL ⁻¹ (6.7 g/100 g)	0.63 mg L ⁻¹ (3.1 mg kg ⁻¹)
Analysis time per sample	≈ 12 min	≈ 8 min

Precision and accuracy data are listed in Table 4. Intra-day RSD values ranged from 0.8% to 1.7% and inter-day values from 1.3% to 2.6%. The larger variation at the lowest level is expected, because the added amount there is only about three times the LOQ. Recoveries from spiked honeys were between 97.6% and 102.4%, within the 95–105% range usually accepted for food analysis at these concentrations. Small changes in temperature, heating time, carbonate and bisulfite concentration changed the results by less than 2% (RSD 0.9–1.8%), so the procedure is robust for routine use.

Table 4. Precision and recovery of the proposed procedure.

Analyte	Added	Found (mean ± SD)	Recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)
Reducing sugars (honey H5)	20 µg mL ⁻¹	19.52 ± 0.33	97.6	1.7	2.4
	40 µg mL ⁻¹	40.46 ± 0.49	101.2	1.2	1.8
	60 µg mL ⁻¹	60.37 ± 0.48	100.6	0.8	1.3
HMF (honey H3)	5.0 mg kg ⁻¹	4.92 ± 0.08	98.4	1.6	2.6
	10.0 mg kg ⁻¹	10.24 ± 0.14	102.4	1.4	2.1
	20.0 mg kg ⁻¹	19.81 ± 0.21	99.1	1.1	1.6

Found values are the amounts recovered after subtracting the native content of the unspiked honey; intra-day n = 6, inter-day n = 9.

3.3 Reducing sugar content

The results for all samples are given in Table 5 and Figure 5a. Reducing sugars in the fifteen local honeys ranged from 69.4 to 74.2 g/100 g. Citrus honeys (H5–H7) had the highest values, 73.2–74.2 g/100 g, and the multifloral mountain honeys the lowest, 69.4–70.8 g/100 g. These values sit in the range reported for blossom honeys from the Mediterranean area and Europe [29,31,44]. The imported blends were clearly lower: H16 contained 65.5 g/100 g, H17 61.6 g/100 g and H18 only 58.8 g/100 g, which is below the Codex minimum.

Apparent sucrose followed the opposite pattern. All local honeys contained 1.3–3.4 g/100 g, while H17 and H18 contained 6.2 and 7.7 g/100 g, above the 5 g/100 g limit. Honey with low fructose plus glucose and high sucrose is typical of feeding bees with sucrose syrup during the flow or of direct addition of partly inverted syrup [10,11]. The spectrophotometric data alone cannot tell these two causes apart, but they are enough to reject both samples.

Table 5. Reducing sugars, HMF and physicochemical parameters of the honey samples (mean $\pm$ SD, n = 3).

Co de	RS (g/100 g)	Sucrose (g/100 g)	HMF (mg kg ⁻¹)	Moistu re (g/100 g)	pH	Free acidity (meq kg ⁻¹)	EC (mS cm ⁻¹)	As h (%)	Colo ur (mm Pfun d)
H1	72.4 $\pm$ 0.5	1.9 $\pm$ 0.11	8.2 $\pm$ 0.3	16.4 $\pm$ 0.1	4.47 $\pm$ 0.04	18.2 $\pm$ 0.5	0.68 $\pm$ 0.01	0.3 1	114
H2	70.7 $\pm$ 0.3	2.1 $\pm$ 0.13	13.1 $\pm$ 0.6	17.4 $\pm$ 0.1	4.62 $\pm$ 0.04	21.8 $\pm$ 0.7	0.72 $\pm$ 0.01	0.3 3	114
H3	72.0 $\pm$ 0.3	1.3 $\pm$ 0.05	5.6 $\pm$ 0.2	15.8 $\pm$ 0.1	4.20 $\pm$ 0.04	18.8 $\pm$ 0.4	0.55 $\pm$ 0.02	0.2 3	97
H4	70.7 $\pm$ 0.6	2.8 $\pm$ 0.13	10.8 $\pm$ 0.2	17.4 $\pm$ 0.2	4.66 $\pm$ 0.02	28.3 $\pm$ 0.5	0.67 $\pm$ 0.02	0.3 0	108
H5	73.2 $\pm$ 0.3	2.2 $\pm$ 0.09	5.8 $\pm$ 0.3	17.0 $\pm$ 0.1	3.78 $\pm$ 0.03	19.0 $\pm$ 0.3	0.19 $\pm$ 0.01	0.0 3	30
H6	73.6 $\pm$ 0.5	3.4 $\pm$ 0.11	4.8 $\pm$ 0.2	17.9 $\pm$ 0.2	3.98 $\pm$ 0.02	13.9 $\pm$ 0.3	0.30 $\pm$ 0.01	0.0 9	37
H7	74.2 $\pm$ 0.4	3.0 $\pm$ 0.17	4.4 $\pm$ 0.2	16.6 $\pm$ 0.2	3.73 $\pm$ 0.02	17.2 $\pm$ 0.6	0.20 $\pm$ 0.01	0.0 3	33
H8	70.1 $\pm$ 0.4	2.5 $\pm$ 0.16	19.9 $\pm$ 0.9	17.5 $\pm$ 0.1	4.10 $\pm$ 0.02	30.1 $\pm$ 1.1	0.57 $\pm$ 0.01	0.2 4	70
H9	70.9 $\pm$ 0.5	1.8 $\pm$ 0.08	14.3 $\pm$ 0.5	17.8 $\pm$ 0.2	4.01 $\pm$ 0.02	26.7 $\pm$ 0.6	0.55 $\pm$ 0.01	0.2 3	56
H1 0	69.4 $\pm$ 0.3	2.8 $\pm$ 0.12	13.4 $\pm$ 0.4	17.2 $\pm$ 0.1	4.18 $\pm$ 0.03	32.6 $\pm$ 0.6	0.62 $\pm$ 0.02	0.2 7	102
H1 1	70.8 $\pm$ 0.4	2.0 $\pm$ 0.11	8.5 $\pm$ 0.3	16.1 $\pm$ 0.1	4.00 $\pm$ 0.02	23.8 $\pm$ 0.8	0.48 $\pm$ 0.01	0.1 9	62
H1 2	69.6 $\pm$ 0.3	3.4 $\pm$ 0.19	16.2 $\pm$ 0.8	18.6 $\pm$ 0.2	3.93 $\pm$ 0.01	40.3 $\pm$ 1.1	0.44 $\pm$ 0.01	0.1 7	64
H1 3	69.7 $\pm$ 0.3	2.7 $\pm$ 0.11	6.9 $\pm$ 0.3	16.1 $\pm$ 0.1	4.23 $\pm$ 0.03	27.6 $\pm$ 1.0	0.70 $\pm$ 0.01	0.3 2	112
H1 4	72.4 $\pm$ 0.4	2.6 $\pm$ 0.10	10.1 $\pm$ 0.2	18.3 $\pm$ 0.2	3.79 $\pm$ 0.03	18.5 $\pm$ 0.5	0.23 $\pm$ 0.01	0.0 5	29
H1 5	71.3 $\pm$ 0.5	3.3 $\pm$ 0.21	12.7 $\pm$ 0.5	17.9 $\pm$ 0.2	3.91 $\pm$ 0.03	19.1 $\pm$ 0.4	0.29 $\pm$ 0.01	0.0 8	35
H1 6	65.5 $\pm$ 0.3	4.3 $\pm$ 0.25	48.5 $\pm$ 2.3	18.2 $\pm$ 0.1	3.68 $\pm$ 0.02	39.6 $\pm$ 1.2	0.23 $\pm$ 0.01	0.0 5	76
H1 7	61.6 $\pm$ 0.3	6.2 $\pm$ 0.34	62.7 $\pm$ 2.7	20.2 $\pm$ 0.2	3.85 $\pm$ 0.02	39.8 $\pm$ 1.3	0.47 $\pm$ 0.01	0.1 9	87
H1 8	58.8 $\pm$ 0.3	7.7 $\pm$ 0.29	93.3 $\pm$ 4.5	20.4 $\pm$ 0.2	3.84 $\pm$ 0.01	50.9 $\pm$ 1.1	0.38 $\pm$ 0.01	0.1 4	72

RS, reducing sugars (fructose + glucose); EC, electrical conductivity. Codex/EU limits: RS $\geq$ 60; sucrose $\leq$ 5; HMF $\leq$ 40; moisture $\leq$ 20; free acidity $\leq$ 50; EC $\leq$ 0.8 (blossom honey).

3.4 Hydroxymethylfurfural content

HMF was low in all local honeys, from 4.4 mg kg⁻¹ in citrus honey H7 to 19.9 mg kg⁻¹ in eucalyptus honey H8 (Figure 5b). The three citrus samples, collected in spring and

analysed within a few weeks, had the lowest values. Eucalyptus honey H8 and multiflora honey H12 reached 16–20 mg kg⁻¹. These honeys are harvested in the hot summer of central and southern Iraq, where hive temperatures above 35 °C are common, and moderate HMF values are expected under these conditions [21,45]. All local samples were well below the 40 mg kg⁻¹ limit.

The imported blends contained 48.5 (H16), 62.7 (H17) and 93.3 mg kg⁻¹ (H18). All three exceeded the general limit, and H18 exceeded even the 80 mg kg⁻¹ limit allowed for tropical honeys. Such values point to heating during processing, for example to delay crystallisation and ease bottling, to long storage in warm warehouses, or to the presence of acid-inverted syrup [19,20,26].



Figure 5. (a) Reducing sugars and apparent sucrose and (b) HMF content in the 18 honey samples, coloured by declared origin. Error bars show SD ($n = 3$); dashed lines mark Codex/EU limits.

To check how quickly HMF forms in local honey under poor storage, the citrus honey H5 (initial HMF 5.8 mg kg⁻¹) was held at 50, 60 and 70 °C for up to 48 h. HMF grew linearly with time at all three temperatures ($R^2 \geq 0.987$), with zero-order rate constants of 0.122, 0.387 and 1.22 mg kg⁻¹ h⁻¹ (Figure 6a). The Arrhenius plot gave an activation energy of 106.3 kJ mol⁻¹ (Figure 6b), in the range reported for HMF formation in other uniflora honeys [19,20]. From these constants, H5 would reach 40 mg kg⁻¹ after about 28 h at 70 °C, 88 h at 60 °C and 12 days at 50 °C. The last figure is relevant to Iraqi conditions, since honey left in a parked car or a sunlit shop window in July can reach 50 °C. The same experiment also shows that the method follows small HMF changes of 1–2 mg kg⁻¹, which is useful for shelf-life studies.

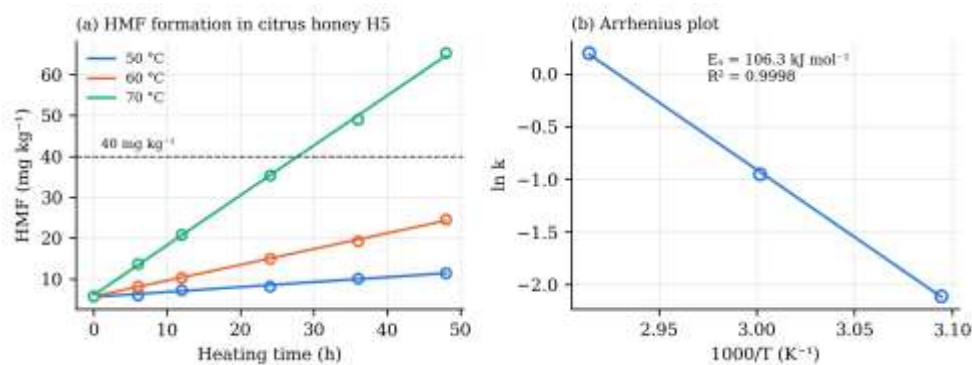


Figure 6. Thermal formation of HMF in citrus honey H5: (a) HMF against heating time at 50, 60 and 70 °C; (b) Arrhenius plot of the zero-order rate constants.

3.5 Physicochemical characteristics

Moisture ranged from 15.8 to 18.6 g/100 g in the local honeys. Sidr honeys and three of the four mountain multifloral honeys were the driest (15.8–17.4 g/100 g), in line with the long ripening period and low air humidity in their production areas. The two imported blends H17 and H18 exceeded the 20 g/100 g limit (20.2 and 20.4 g/100 g), which increases the risk of fermentation and suggests harvesting of unripe honey or dilution [1,8].

The pH values fell between 3.68 and 4.66. Sidr honeys had the highest pH (4.20–4.66), as reported for Ziziphus honeys, which are rich in minerals and have a relatively high buffering capacity. Free acidity ranged from 13.9 to 40.3 meq kg⁻¹ in the local samples, while H18 reached 50.9 meq kg⁻¹, just over the limit. High acidity in a honey that also has high moisture is consistent with the start of fermentation [31].

EC ranged from 0.19 to 0.72 mS cm⁻¹, and all samples were below the 0.8 mS cm⁻¹ limit, so all can be classed as blossom honeys [1,7]. Citrus and clover honeys gave the lowest EC (0.19–0.30 mS cm⁻¹) and ash contents (0.03–0.09%), whereas Sidr and dark mountain honeys gave the highest (0.55–0.72 mS cm⁻¹; 0.23–0.33% ash). The colour values separated the samples in the same way. Citrus and clover honeys were white to extra light amber (29–37 mm Pfund), eucalyptus honeys and multifloral H11 and H12 were light amber (56–70 mm), and Sidr honeys together with multifloral H10 and H13 were amber (97–114 mm). Dark honeys usually contain more minerals and phenolic compounds, so the colour reading provides a quick first indication of antioxidant potential [3,30].

3.6 Correlation between analytical parameters

The Pearson correlation matrix is shown in Figure 7. Two groups of variables stand out. The first group links sugars, HMF, moisture and acidity. Reducing sugars were negatively correlated with HMF ($r = -0.96$), sucrose ($r = -0.88$), free acidity ($r = -0.90$) and moisture ($r = -0.76$), while HMF was positively correlated with sucrose ($r = 0.92$) and moisture ($r = 0.82$), all at $p < 0.01$. These relations are driven mostly by the three imported blends, in which low sugar, high sucrose, high water and high HMF occur together. They therefore describe the difference between processed and fresh honey, and not a relation that holds within each honey type.

The second group links the mineral-related variables. EC correlated strongly with colour ($r = 0.88$) and with pH ($r = 0.87$), and pH with colour ($r = 0.77$). The link between EC, ash and colour is well known and reflects the fact that minerals, organic acid salts and phenolic pigments come from the same plant sources [8,28,29]. The two groups were almost independent of each other ($|r| < 0.45$ between EC or colour and the freshness variables), which means that botanical origin and processing history leave separate signatures in the data.

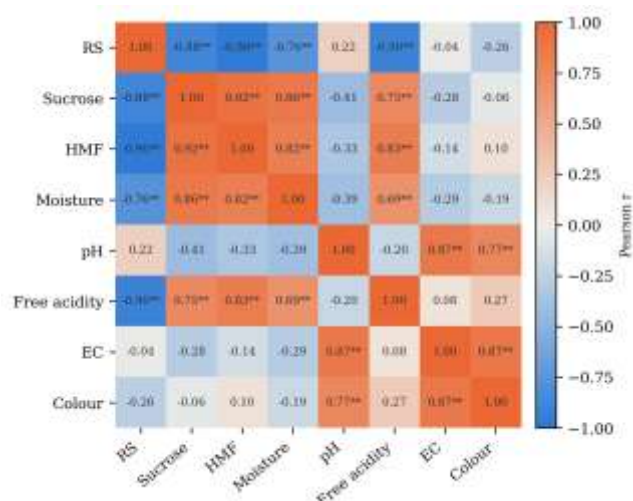


Figure 7. Pearson correlation matrix of the quality parameters (n = 18). * $p < 0.05$; ** $p < 0.01$.

3.7 Statistical classification of honey samples

PCA gave two components with eigenvalues above 1, explaining 57.0% and 33.7% of the variance (90.7% together); the third component added only 4.3%. PC1 had high positive loadings for sucrose (0.95), HMF (0.96), moisture (0.90) and free acidity (0.85) and a high negative loading for reducing sugars (-0.92), so it can be read as a freshness and authenticity axis. PC2 was dominated by colour (0.96), EC (0.92) and pH (0.80), and describes the mineral and pigment content that depends on botanical origin.

The imported blends H17 and H18 are well separated from the local honeys in the score plot (Figure 8a) to the far right along PC1, with H16 in between them and the local honeys. There is one group of honeys in the top (Sidr honeys) while the dark mountain honey H10 and H13 are in the bottom with citrus honey and clover honey in the centre and the eucalyptus honey and the rest of multifloral honeys are placed inside the frame. The same picture (Figure 8b) was returned by HCA. Cutting the dendrogram at a Ward distance of about 7 gave four clusters: (i) H17 and H18; (ii) Sidr honeys H1–H4 with multifloral H10 and H13; (iii) citrus H5–H7 with clover H14–H15; and (iv) eucalyptus H8–H9, multifloral H11–H12 and the blend H16. H16 grouped with the light-amber local honeys because its only defect is a moderate HMF excess. A classification built only on EC and colour would miss it, and this is why HMF has to be included in the model.

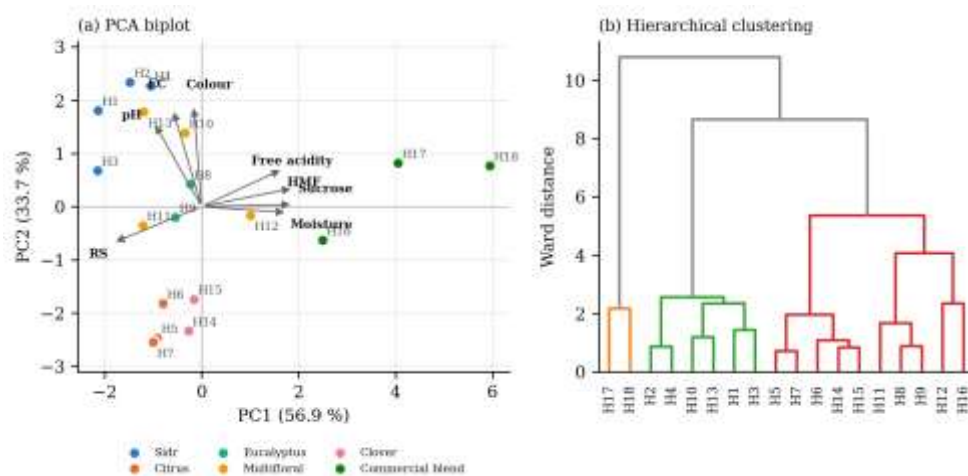


Figure 8. Chemometric classification of the honey samples: (a) PCA biplot of PC1 and PC2 (autoscaled data) and (b) HCA dendrogram (Euclidean distance, Ward linkage).

3.8 Assessment of honey quality

Agreement with reference methods. Results of the proposed procedure plotted against the reference methods fell close to the line of equality (Figure 9). For reducing sugars against Lane–Eynon, the slope was 1.018 and $R^2 = 0.9939$. For HMF against HPLC-UV, the slope was 1.051 and $R^2 = 0.9986$. Paired *t*-tests over the 18 samples found no significant difference ($t = 0.65$, $p = 0.52$ for sugars; $t = 0.18$, $p = 0.86$ for HMF; $t_{crit} = 2.110$). Replicate analyses of six samples (Table 6) gave *t* and *F* values below the critical values (2.306 and 6.39), so the proposed and reference methods have comparable accuracy and precision.

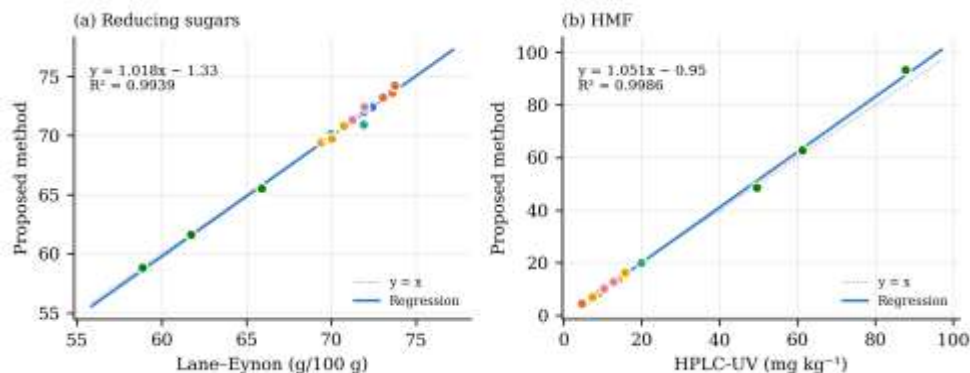


Figure 9. Comparison of the proposed procedure with (a) the Lane–Eynon titration for reducing sugars and (b) the IHC HPLC-UV method for HMF ($n = 18$). Dotted line: $y = x$.

Table 6. Statistical comparison of the proposed procedure with the reference methods (mean $\pm$ SD, $n = 5$).

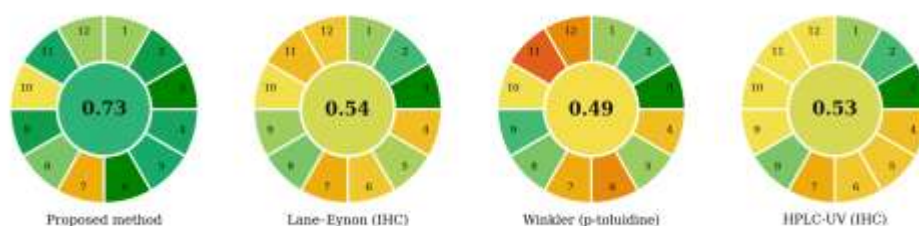
Sampl e	RS proposed (g/100 g)	RS Lane– Eynon (g/100 g)	<i>t</i>	<i>F</i>	HMF proposed (mg kg ⁻¹)	HMF HPLC- UV (mg kg ⁻¹)	<i>t</i>	<i>F</i>
H1	72.4 $\pm$ 0.50	72.4 $\pm$ 0.59	0.03	1.40	8.2 $\pm$ 0.35	8.5 $\pm$ 0.40	1.34	1.33
H5	73.2 $\pm$ 0.35	73.0 $\pm$ 0.48	0.59	1.91	5.8 $\pm$ 0.29	6.0 $\pm$ 0.32	0.86	1.21
H9	70.9 $\pm$ 0.54	71.1 $\pm$ 0.72	0.51	1.80	14.3 $\pm$ 0.51	14.9 $\pm$ 0.68	1.65	1.79
H12	69.6 $\pm$ 0.28	69.9 $\pm$ 0.33	1.34	1.42	16.2 $\pm$ 0.78	15.8 $\pm$ 0.95	0.77	1.46
H16	65.5 $\pm$ 0.29	65.9 $\pm$ 0.40	1.78	1.84	48.5 $\pm$ 2.27	49.6 $\pm$ 2.77	0.71	1.50
H18	58.8 $\pm$ 0.29	58.9 $\pm$ 0.40	0.35	1.92	93.3 $\pm$ 4.45	87.8 $\pm$ 5.97	1.64	1.79

Theoretical values at 95% confidence: $t = 2.306$ (8 degrees of freedom), $F = 6.39$ (4, 4).

Greenness. The Eco-Scale and AGREE results are compared in Table 7 and Figure 10. The proposed procedure scored 89 on the Eco-Scale, which counts as excellent green analysis (above 75), against 74 for the Lane–Eynon titration, 61 for the Winkler method and 76 for HPLC-UV. Most of the gain comes from three changes: the copper, tartrate and methylene blue reagents are replaced by 1.6 mg of $K_3[Fe(CN)_6]$ and 53 mg of Na_2CO_3 per test; Carrez clarification and *p*-toluidine are no longer used; and the waste per sample falls to about 13 mL of dilute aqueous solution. The AGREE score of 0.73 was also the highest of the four methods (0.49–0.54 for the others). The lowest AGREE segments for the proposed method were principle 7 (the procedure is off-line and generates some waste) and principle 10 (reagents are not bio-based), which leaves room for later improvement, for example by automating the reaction in a flow system.

Table 7. Greenness assessment of the proposed and conventional methods.

Criterion	Proposed method	Lane–Eynon	Winkler	HPLC-UV (IHC)
Main reagents	K ₃ [Fe(CN) ₆], Na ₂ CO ₃ , NaHSO ₃	CuSO ₄ , NaOH, K-Na tartrate, methylene blue	p-toluidine, barbituric acid, isopropanol, acetic acid, Carrez I/II	Methanol, Carrez I/II
Reagent mass per test	< 0.1 g	≈ 4 g	≈ 1.5 g	≈ 0.5 g + mobile phase
Waste per sample	≈ 13 mL (aqueous)	≈ 60 mL (Cu)	≈ 25 mL (organic)	≈ 30 mL (organic)
Energy per sample	< 0.1 kWh	< 0.1 kWh (boiling)	< 0.1 kWh	≈ 0.5 kWh
Operator hazard	Low	Corrosive alkali	Toxic, carcinogenic	Flammable, toxic
Eco-Scale penalty points	11	26	39	24
Eco-Scale score	89 (excellent)	74 (acceptable)	61 (acceptable)	76 (excellent)
AGREE score	0.73	0.54	0.49	0.53

**Figure 10.** AGREE pictograms of the proposed procedure and three conventional methods. Numbers 1–12 are the principles of green analytical chemistry; the central value is the overall score.

Quality of the analysed honeys. Table 8 summarises compliance with the legal limits. Fifteen samples (83%) met every Codex and EU criterion, and all of them were local honeys. Twelve of these reached Grade A. H8 and H12 were placed in Grade B because of HMF values of 16–20 mg kg⁻¹, and H14 because its moisture of 18.3 g/100 g is slightly high for long storage. All three imported blends were Grade C. H16 failed only the HMF limit. H17 failed HMF, sucrose and moisture. H18 failed five of the six criteria, which is not compatible with authentic, properly stored honey.

Table 8. Compliance of the honey samples with Codex/EU limits and quality grading.

Criterion / grade	Compliant samples or count	Non-compliant or listed samples
Reducing sugars ≥ 60 g/100 g	17/18	H18
Sucrose ≤ 5 g/100 g	16/18	H17, H18
HMF ≤ 40 mg kg ⁻¹	15/18	H16, H17, H18
Moisture ≤ 20 g/100 g	16/18	H17, H18
Free acidity ≤ 50 meq kg ⁻¹	17/18	H18
EC ≤ 0.8 mS cm ⁻¹	18/18	None
Grade A (fresh, well ripened)	12	H1–H7, H9–H11, H13, H15
Grade B (compliant)	3	H8, H12, H14
Grade C (non-compliant)	3	H16, H17, H18

These results agree with surveys from other countries, where imported and supermarket blends more often exceed HMF and moisture limits than honey bought directly from beekeepers [21,31,32].

4. Conclusion

A simple, low-cost, and environmentally friendly spectrophotometric procedure was successfully developed and validated for routine honey quality control. By quantifying reducing sugars via the decrease in hexacyanoferrate(III) absorbance at 420 nm and determining HMF at 284 nm against a bisulfite reference following membrane filtration, the method completely eliminates hazardous reagents such as Fehling's solution, Carrez clarifiers, and carcinogenic p-toluidine. The protocol reduces chemical waste to approximately 13 mL per sample and total reagent mass below 0.1 g, achieving excellent greenness metrics with an Eco-Scale score of 89 and an AGREE index of 0.73. Full validation according to ICH Q2(R2) guidelines confirmed excellent linearity, sensitivity, precision, and recovery, while comparative analysis against official Lane–Eynon and HPLC-UV methods demonstrated equivalent accuracy without statistical divergence ($p > 0.05$). Application to 18 market samples revealed full compliance among local Iraqi honeys, whereas all imported blends failed established HMF thresholds, with multivariate analysis (PCA and HCA) confirming clear separation between fresh local products and heat-damaged or adulterated blends.

Based on these findings, food control laboratories in Iraq are encouraged to adopt this green spectrophotometric method for primary screening of honey quality parameters, reserving complex chromatographic techniques primarily for confirmatory testing of flagged samples. Regulatory authorities should enforce strict inspection protocols at border ports to monitor HMF, moisture, and sucrose levels in imported honeys, alongside mandating clear harvest and packaging dates on commercial labels. Furthermore, thermal processing above 40 °C should be strictly avoided during handling and storage kept below 25 °C, as kinetic modeling indicates HMF content exceeds legal safety limits after just two weeks at elevated temperatures (50 °C). Future research should aim to adapt this hexacyanoferrate(III) assay into automated microplate or flow-injection formats to further diminish reagent consumption, while expanding testing to additional quality markers such as diastase activity and proline content across broader geographical samples.

REFERENCES

- [1] Codex Alimentarius Commission, *Standard for Honey*, CXS 12-1981, Adopted 1981, Revised 1987 and 2001, Amended 2019, Rome: FAO/WHO, 2019.
- [2] J. M. Alvarez-Suarez, S. Tulipani, S. Romandini, E. Bertoli, and M. Battino, "Contribution of honey in nutrition and human health: A review," *Med. J. Nutrition Metab.*, vol. 3, no. 1, pp. 15–23, 2010.
- [3] O. Escuredo, M. Míguez, M. Fernández-González, and M. C. Seijo, "Nutritional value and antioxidant activity of honeys produced in a European Atlantic area," *Food Chem.*, vol. 138, no. 2–3, pp. 851–856, 2013.
- [4] P. M. da Silva, C. Gauche, L. V. Gonzaga, A. C. O. Costa, and R. Fett, "Honey: Chemical composition, stability and authenticity," *Food Chem.*, vol. 196, pp. 309–323, 2016.
- [5] J. W. White and L. W. Doner, "Honey composition and properties," in *Beekeeping in the United States*, Agriculture Handbook No. 335, Washington, DC: USDA, 1980, pp. 82–91.
- [6] D. W. Ball, "The chemical composition of honey," *J. Chem. Educ.*, vol. 84, no. 10, pp. 1643–1646, 2007.
- [7] C. Pita-Calvo and M. Vázquez, "Differences between honeydew and blossom honeys: A review," *Trends Food Sci. Technol.*, vol. 59, pp. 79–87, 2017.
- [8] S. Bogdanov, K. Ruoff, and L. Persano Oddo, "Physico-chemical methods for the characterisation of unifloral honeys: A review," *Apidologie*, vol. 35, no. Suppl. 1, pp. S4–S17, 2004.
- [9] Council of the European Union, "Council Directive 2001/110/EC of 20 December 2001 relating to honey," *Off. J. Eur. Communities*, vol. L10, pp. 47–52, 2002.
- [10] E. Anklam, "A review of the analytical methods to determine the geographical and botanical origin of honey," *Food Chem.*, vol. 63, no. 4, pp. 549–562, 1998.
- [11] A. J. Siddiqui, S. G. Musharraf, M. I. Choudhary, and A. Rahman, "Application of analytical methods in authentication and adulteration of honey," *Food Chem.*, vol. 217, pp. 687–698, 2017.
- [12] J. H. Lane and L. Eynon, "Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator," *J. Soc. Chem. Ind.*, vol. 42, pp. 32T–37T, 1923.
- [13] S. Bogdanov, P. Martin, and C. Lüllmann, *Harmonised Methods of the International Honey Commission*, Bern: Swiss Bee Research Centre, FAM, Liebfeld, 2002 (revised 2009).
- [14] G. L. Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal. Chem.*, vol. 31, no. 3, pp. 426–428, 1959.
- [15] H. C. Hagedorn and B. N. Jensen, "Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid," *Biochem. Z.*, vol. 135, pp. 46–58, 1923.
- [16] J. T. Park and M. J. Johnson, "A submicrodetermination of glucose," *J. Biol. Chem.*, vol. 181, no. 1, pp. 149–151, 1949.
- [17] S. Kowalski, M. Łukasiewicz, A. Duda-Chodak, and G. Zięć, "5-Hydroxymethyl-2-furfural (HMF): Heat-induced formation, occurrence in food and biotransformation. A review," *Pol. J. Food Nutr. Sci.*, vol. 63, no. 4, pp. 207–225, 2013.
- [18] E. Capuano and V. Fogliano, "Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies," *LWT - Food Sci. Technol.*, vol. 44, no. 4, pp. 793–810, 2011.
- [19] B. Fallico, M. Zappalà, E. Arena, and A. Verzera, "Effects of conditioning on HMF content in unifloral honeys," *Food Chem.*, vol. 85, no. 2, pp. 305–313, 2004.
- [20] E. Tosi, M. Ciappini, E. Ré, and H. Lucero, "Honey thermal treatment effects on hydroxymethylfurfural content," *Food Chem.*, vol. 77, no. 1, pp. 71–74, 2002.
- [21] M. I. Khalil, S. A. Sulaiman, and S. H. Gan, "High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year," *Food Chem. Toxicol.*, vol. 48, no. 8–9, pp. 2388–2392, 2010.
- [22] U. M. Shapla, M. Solayman, N. Alam, M. I. Khalil, and S. H. Gan, "5-Hydroxymethylfurfural (HMF) levels in honey and other food products: Effects on bees and human health," *Chem. Cent. J.*, vol. 12, art. no. 35, 2018.

- [23] J. W. White, "Spectrophotometric method for hydroxymethylfurfural in honey," *J. Assoc. Off. Anal. Chem.*, vol. 62, no. 3, pp. 509–514, 1979.
- [24] O. Winkler, "Beitrag zum Nachweis und zur Bestimmung von Oxymethylfurfurol in Honig und Kunsthonig," *Z. Lebensm. Unters. Forsch.*, vol. 102, no. 3, pp. 161–167, 1955.
- [25] M. Zappalà, B. Fallico, E. Arena, and A. Verzera, "Methods for the determination of HMF in honey: A comparison," *Food Control*, vol. 16, no. 3, pp. 273–277, 2005.
- [26] I. N. Pasias, I. K. Kiriakou, and C. Proestos, "HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration," *Food Chem.*, vol. 229, pp. 425–431, 2017.
- [27] H. D. Chataway, "The determination of moisture in honey," *Can. J. Res.*, vol. 6, no. 5, pp. 532–547, 1932.
- [28] M. G. Piazza, M. Accorti, and L. Persano Oddo, "Electrical conductivity, ash, colour and specific rotatory power in Italian unifloral honeys," *Apicoltura*, vol. 7, pp. 51–63, 1991.
- [29] V. Kaškonienė, P. R. Venskutonis, and V. Čeksterytė, "Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania," *LWT - Food Sci. Technol.*, vol. 43, no. 5, pp. 801–807, 2010.
- [30] I. C. F. R. Ferreira, E. Aires, J. C. M. Barreira, and L. M. Estevinho, "Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract," *Food Chem.*, vol. 114, no. 4, pp. 1438–1443, 2009.
- [31] S. Gomes, L. G. Dias, L. L. Moreira, P. Rodrigues, and L. Estevinho, "Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal," *Food Chem. Toxicol.*, vol. 48, no. 2, pp. 544–548, 2010.
- [32] M. B. Sakač, P. T. Jovanov, A. Z. Marić, L. L. Pezo, Ž. S. Kevrešan, A. R. Novaković, and N. M. Nedeljković, "Physicochemical properties and mineral content of honey samples from Vojvodina (Republic of Serbia)," *Food Chem.*, vol. 276, pp. 15–21, 2019.
- [33] P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, New York: Oxford University Press, 1998.
- [34] S. Armenta, S. Garrigues, and M. de la Guardia, "Green analytical chemistry," *TrAC Trends Anal. Chem.*, vol. 27, no. 6, pp. 497–511, 2008.
- [35] M. Tobiszewski, A. Mechlińska, and J. Namieśnik, "Green analytical chemistry: Theory and practice," *Chem. Soc. Rev.*, vol. 39, no. 8, pp. 2869–2878, 2010.
- [36] A. Gałuszka, Z. Migaszewski, and J. Namieśnik, "The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices," *TrAC Trends Anal. Chem.*, vol. 50, pp. 78–84, 2013.
- [37] A. Gałuszka, Z. M. Migaszewski, P. Konieczka, and J. Namieśnik, "Analytical Eco-Scale for assessing the greenness of analytical procedures," *TrAC Trends Anal. Chem.*, vol. 37, pp. 61–72, 2012.
- [38] J. Płotka-Wasyłka, "A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index," *Talanta*, vol. 181, pp. 204–209, 2018.
- [39] F. Pena-Pereira, W. Wojnowski, and M. Tobiszewski, "AGREE: Analytical GREENness metric approach and software," *Anal. Chem.*, vol. 92, no. 14, pp. 10076–10082, 2020.
- [40] W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, and E. Psillakis, "AGREEprep: Analytical greenness metric for sample preparation," *TrAC Trends Anal. Chem.*, vol. 149, art. no. 116553, 2022.
- [41] International Council for Harmonisation, *ICH Q2(R2): Validation of Analytical Procedures, Step 4 Guideline*, Geneva: ICH, 2023.
- [42] R. Bro and A. K. Smilde, "Principal component analysis," *Anal. Methods*, vol. 6, no. 9, pp. 2812–2831, 2014.
- [43] J. H. Ward, "Hierarchical grouping to optimize an objective function," *J. Am. Stat. Assoc.*, vol. 58, no. 301, pp. 236–244, 1963.
- [44] L. Persano Oddo and R. Piro, "Main European unifloral honeys: Descriptive sheets," *Apidologie*, vol. 35, no. Suppl. 1, pp. S38–S81, 2004.

- [45] S. Ajlouni and P. Sujirapinyokul, "Hydroxymethylfurfuraldehyde and amylase contents in Australian honey," *Food Chem.*, vol. 119, no. 3, pp. 1000–1005, 2010.