RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2026; 40 (2): 167 - 176
http://www.fusabil.org

Physicochemical Characterisation and Microbiological Quality of Chestnut, Pine, and Flower Honeys from Kastamonu Province, Türkiye: Botanical Differentiation and Quality Assessment *

Yasin AKKEMİK [1, a]
Sedat ÖZCAN [2, b]
Alper GÜNGÖREN [1, c]
Ahmet GÜNER [3, d]

[1] Kastamonu University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kastamonu, TÜRKİYE

[2] İzmir Bakırçay University, Menemen Vocational School, Veterinary Department, İzmir, TÜRKİYE

[3] Selçuk University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Konya, TÜRKİYE

[a] ORCID: 0000-0002-9086-0324
[b] ORCID: 0000-0001-9235-4049
[c] ORCID: 0000-0001-7818-1372
[d] ORCID: 0000-0001-9661-555X

This study presents the first integrated physicochemical and microbiological characterisation of chestnut (*Castanea sativa*; n=14), pine (*Pinus brutia*; n=14), and flower (*polyfloral*; n=12) honeys from Kastamonu Province, Türkiye. Fifteen physicochemical parameters were assessed; fourteen differed significantly among honey types (p<0.001), water-insoluble matter being the sole exception. For all parameters analysed, samples complied with the Turkish Food Codex (TGK 2020/7), Codex Alimentarius, and EU Directive 2001/110/EC. Pine honey was distinguished by markedly elevated maltose (7.87±1.27 g/100 g), a recognised biochemical signature of honeydew origin, and by the lowest moisture and HMF values. Chestnut honey exhibited the highest electrical conductivity (1.25±0.16 mS/cm), proline (514.84±6.26 mg/kg), and F/G ratio (1.62±0.10). Flower honey showed the highest HMF (21.32±1.37 mg/kg) and glucose content. HMF yielded the largest effect size (η^2p=0.931), and principal component analysis achieved clear three-way botanical separation. Microbiologically, total mesophilic aerobic bacteria counts were low across all honey types (1.56–1.81 log CFU/g), with no significant difference among groups (p=0.387). *Coliforms*, sulphite-reducing anaerobic bacteria, yeasts, moulds, and *Salmonella* spp. were not detected in any of the 40 samples examined. These findings establish a physicochemical and microbiological baseline for Kastamonu honey and support its potential for geographical origin designation.

Key Words: *Honey, food quality, food microbiology, hydroxymethylfurfural, principal component analysis*

Kastamonu İlinde Üretilen Kestane, Çam ve Çiçek Ballarının Fizikokimyasal ve Mikrobiyolojik Özellikleri: Botanik Ayrım ve Kalite Değerlendirmesi

Bu çalışma, Türkiye'nin Kastamonu ilinden temin edilen kestane (*Castanea sativa*; n=14), çam (*Pinus brutia*; n=14) ve çiçek (*polifloral*; n=12) ballarının fizikokimyasal ve mikrobiyolojik özelliklerini kapsamlı biçimde belirlemek amacıyla planlandı. Değerlendirilen on beş fizikokimyasal parametreden on dördü bal türleri arasında istatistiksel olarak anlamlı farklılık göstermiştir (p<0.001). Yalnızca suda çözünmeyen madde miktarı bakımından gruplar arasında fark saptanmamıştır. İncelenen parametreler bakımından tüm örneklerin Türk Gıda Kodeksi (TGK 2020/7), Codex Alimentarius ve AB Direktifi 2001/110/EC gerekliliklerini karşıladığı görülmüştür. Çam balı, yüksek maltoz (7.87±1.27 g/100g) ve en düşük nem ve HMF değerleriyle farklılık arz etmektedir. Kestane balı en yüksek elektriksel iletkenliğe (1.25±0.16 mS/cm), proline (514.84±6.26 mg/kg) ve F/G oranına (1.62±0.10) sahipti. Çiçek balı ise en yüksek HMF (21.32±1.37 mg/kg) ve glikoz değerlerini gösterdi. HMF en büyük etki büyüklüğünü (η^2p=0.931) verdi ve temel bileşen analizi net üç yönlü botanik ayrım sağladı. Mikrobiyolojik olarak, toplam mezofilik aerobik bakteri sayıları düşüktü ve gruplar arasında homojendi (1.56–1.81 log CFU/g; p=0.387); koliformlar, sülfit indirgeyici anaerobik bakteriler, mayalar, küfler ve *Salmonella* türleri hiçbir örnekte tespit edilmedi. Bu bulgular, Kastamonu balı için fizikokimyasal ve mikrobiyolojik bir temel oluşturmakta ve coğrafi menşe belirleme potansiyelini desteklemektedir.

Anahtar Kelimeler: *Bal, gıda kalitesi, gıda mikrobiyolojisi, hidroksimetilfurfural, temel bileşen analizi*

Introduction

Honey is a natural food of high nutritional and economic value, produced by *Apis mellifera* from floral nectar or honeydew secretions. Its complex composition (primarily sugars, together with organic acids, enzymes, amino acids, minerals, and phenolic compounds) varies considerably with botanical origin, geographical conditions, and beekeeping practices and is therefore of great importance for determining quality parameters (1, 2).

Physicochemical parameters constitute the cornerstone of honey quality evaluation. Moisture content determines fermentation risk; hydroxymethylfurfural (HMF) and diastase activity reflect thermal history and storage conditions with HMF additionally serving as a key indicator of honey quality, freshness, and heat treatment exposure; and free acidity, pH, electrical conductivity, sugar composition, and proline content collectively characterise botanical origin, maturity, and authenticity (1, 3, 4). Despite its

Received : 02.04.2026
Accepted : 08.05.2026

Correspondence

Yasin AKKEMİK
Kastamonu University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology Kastamonu – TÜRKİYE

yakkemik@kastamonu.edu.tr

* This study was supported by the Kastamonu University Scientific Research Projects Unit with project number KÜ-HIZDES/2023-58.

inherent antimicrobial properties, honey is not a sterile product (5). Contamination may occur through primary sources such as pollen and soil or through secondary routes during harvesting and processing. In microbiological assessments of honey, total mesophilic aerobic bacteria (TMAB), yeasts and moulds, coliform bacteria, sulphite-reducing anaerobic bacteria, and pathogen indicators constitute the primary targets of evaluation (4–6).

Integrated evaluation, combining physicochemical and microbiological data within a single analytical framework, provides a more realistic picture of honey quality and safety than either dimension alone (4, 7). Nevertheless, many regional studies still address these aspects separately, limiting their interpretive value for food safety management (8).

Türkiye is among the world's leading honey producers, endowed with a rich diversity of honey varieties owing to its varied climate, vegetation, and geographical conditions. The Western Black Sea region, and Kastamonu province in particular, supports the production of botanically distinct honey types, including chestnut, pine, and flower varieties, owing to its diverse forested flora (9–11). Prior studies from this locality have addressed palynological characterisation, selected physicochemical parameters, and mineral or heavy metal profiles (10, 11), but no study has systematically combined physicochemical and microbiological quality assessment within a single framework. The present study addresses this gap by providing integrated, region-specific data on chestnut, pine, and flower honeys from Kastamonu province, with the aim of establishing a physicochemical and microbiological quality baseline and contributing to evidence-based monitoring of honey produced in this region.

Materials and Methods

Honey Samples and Sampling Strategy: Forty honey samples (n=40) were collected from registered local beekeepers in Kastamonu province, Türkiye, between 1 June and 30 September 2024. The set comprised chestnut (*Castanea sativa*; n=14), pine (*Pinus brutia*; n=14), and flower honeys (n=12), sourced from ecologically distinct sub-regions: Inebolu and Bozkurt (chestnut), Tosya and Araç (pine), and central Kastamonu and Taşköprü (flower). The botanical type was designated by producer declaration, consistent with common practice where formal melissopalynological verification is beyond scope (10, 11). Samples were collected directly from producers at the point of extraction in sterile amber glass jars (250 mL), transported to the laboratory within 24 h, and stored at 20 ± 2°C in the dark until analysis. All analyses were performed in duplicate; results are expressed as mean ± SD (3, 4).

Physicochemical Analyses: Analyses followed the Turkish Food Codex Honey Communiqué (TGK 2020/7) and IHC harmonised methods (3, 12). Moisture content was determined refractometrically (Atago PAL-22S; Wedmore table) and dry matter calculated as the complement. pH was measured at 20°C with a calibrated pH meter (two-point calibration, pH 4.0 and 7.0) on a 10 g/75 mL honey–water solution; free acidity was determined by potentiometric titration of the same solution with 0.1 N NaOH to pH 8.3 and expressed as meq/kg. Electrical conductivity was measured in a 20% (w/v) solution in ultrapure water at 20°C (3, 4, 9).

Glucose, fructose, sucrose, and maltose were quantified by HPLC-RID (Agilent 1260 Infinity II; Aminex HPX-87C column, 300×7.8 mm, 80°C; ultrapure water mobile phase, 0.6 mL/min; injection volume 20 µL) after filtration through a 0.45 µm nylon membrane. HMF was determined by HPLC-UV (Phenomenex Kinetex C18, 150 × 4.6 mm; 95:5 water–acetonitrile with 0.1% formic acid; 1.0 mL/min; 284 nm) following Carrez clarification. Both analytes were quantified against five-point external calibration curves (r^2>0.999; Sigma-Aldrich standards, purity ≥ 99%) (1, 3). Diastase activity was determined by the Phadebas method at 40°C/30 min and expressed as diastase number (DN). Proline content was quantified colorimetrically by the ninhydrin method at 505 nm. Water-insoluble matter was determined gravimetrically after filtration through a 0.45 µm glass-fibre filter (3, 4,9).

Microbiological Analyses: For enumeration assays, 10 g of each sample was homogenised with 90 mL of 0.1% peptone water (BagMixer 400, Interscience, France) for 1 min to yield the initial 10^{-1} suspension; serial dilutions (10^{-2} to 10^{-4}) were prepared accordingly (13). The analytical detection limit for all enumeration methods was 10 CFU/g (1.0 log CFU/g). Total mesophilic aerobic bacteria (TMAB) were enumerated on Plate Count Agar (PCA; Merck) at 30±1 °C for 72 h (14). Total coliform bacteria were determined on Violet Red Bile Agar (VRBA; Merck) by double-layer pour plate at 30±1°C for 24 h (ISO 4832:2006) (15). Sulphite-reducing anaerobic bacteria were enumerated on SPS agar (Merck) under anaerobic conditions (AnaeroGen, Oxoid) at 35±2 °C for 18–48 h (16). Yeasts and moulds were counted on acidified PDA (pH 3.5±0.1; Merck) at 22±1 °C for 5–7 days (17). Results below the detection limit are reported as <1.0 log CFU/g or 'not detected in 1 g'.

Salmonella spp. detection followed ISO 6579-1:2017 using a 25 g test portion (18). Pre-enrichment was conducted in buffered peptone water (BPW; 37±1°C, 18 h), followed by selective enrichment in Rappaport–Vassiliadis soy broth (RVS; 41.5±1°C, 24 h) and Muller–Kauffmann tetrathionate/novobiocin broth (MKTTn; 37±1°C, 24 h). Isolates were plated onto XLD and *Salmonella* chromogenic agar (CAS; bioMérieux) and incubated at 37±1°C for 24–48 h. Suspected colonies were confirmed biochemically by API 20E (bioMérieux). Results are reported as '*Salmonella* spp. detected' or '*Salmonella* spp. not detected in 25 g'.

Statistical Analysis: Normality and variance homogeneity were assessed by Shapiro–Wilk and Levene's tests, respectively. Parametric data were analysed by one-way ANOVA with Tukey HSD post-hoc; non-parametric data were analysed by Kruskal–Wallis with Dunn–Bonferroni post-hoc. Effect sizes were

reported as partial η^2p (ANOVA) and ε^2 (Kruskal–Wallis), interpreted as small (<0.06), medium (0.06–0.13), or large (≥0.14). Spearman correlation and principal component analysis (PCA) on autoscaled data were used to explore multivariate structure. All analyses were performed in IBM SPSS Statistics v29.0 (IBM Corp., USA) at $p<0.05$.

Post-hoc statistical power analysis was conducted for all one-way ANOVA comparisons using Cohen's f as the effect size measure, calculated from partial eta-squared values (η^2p) as $f= \sqrt{[\eta^2p / (1 - \eta^2p)]}$, with a harmonic mean group size of $\tilde{n}= 13.26$ (derived from group sizes of n= 14, 14, and 12) and $\alpha= 0.05$. For twelve of the fourteen significant physicochemical parameters, observed power (1 − β) exceeded 0.90 and reached 1.000 for HMF ($\eta^2p=0.931$) and diastase activity ($\eta^2p=0.877$), confirming that the study was well-powered for the detection of large effects. Free acidity, while yielding a statistically significant result ($p<0.001$), returned a moderate observed power (1−β=0.531, f=0.735), reflecting a medium effect size ($\eta^2p=0.351$); the significant outcome nonetheless remains valid and is not subject to Type II error. For the two non-significant parameters, water-insoluble matter ($\eta^2p=0.014$, f=0.119, 1−β=0.061) and total mesophilic aerobic bacteria ($\eta^2p=0.050$, f=0.229, 1−β=0.092), the low observed power reflects near-zero effect sizes rather than inadequate sample size and is consistent with the biological interpretation that these parameters do not differ meaningfully among honey types.

Results

Physicochemical profiles of chestnut (*Castanea sativa*; n=14), pine (*Pinus brutia*; n=14), and flower (n=12) honeys are summarised in Table 1. Fourteen of fifteen parameters differed significantly among honey types ($p<0.001$); water-insoluble matter was the sole exception ($p=0.772$). For the parameters examined, all samples were within Turkish Food Codex and EU quality thresholds.

Moisture, pH, Free Acidity, and Electrical Conductivity: Pine honey had the lowest moisture content; chestnut and flower honeys were statistically comparable (Table 1, Figure 1). Chestnut and pine honeys did not differ in pH, but both were significantly higher than flower honey ($\eta^2p=0.676$), indicating a more acidic profile in the latter. Free acidity was lowest in chestnut honey, with pine and flower forming a common group. The electrical conductivity of chestnut and pine honeys significantly exceeded that of flower honey, consistent with the higher mineral load of honeydew-derived products.

Sugar Composition: Glucose was greatest in flower honey, inversely reflected by the F/G ratio, which was highest in chestnut honey. Pine honey was distinguished by markedly elevated maltose relative to both chestnut and flower honeys, a recognised biochemical signature of honeydew origin (Table 1, Figure 1). These patterns are reflected in the Spearman correlation matrix (Figure 2) and PCA loading directions (Figure 3).

HMF, Diastase Activity, and Proline: HMF yielded the largest effect size ($\eta^2p=0.931$), with all three honey types forming statistically distinct groups: flower > chestnut > pine (Table 1). Effect sizes across all physicochemical parameters are illustrated in Figure 4, ranging from negligible for water-insoluble matter ($\eta^2p=0.014$) to very large for HMF and diastase activity ($\eta^2p=0.877$). Diastase activity and proline content both decreased in the order chestnut > pine > flower, each group significantly different from the others. All samples remained above the legal diastase minimum (8 DN) and substantially exceeded the proline authenticity threshold (180 mg/kg), confirming the absence of sugar adulteration.

Multivariate Structure: PCA achieved clear three-way botanical separation (Figure 3). PC1 was driven by HMF, diastase activity, and F/G ratio; PC2 discriminated along the maltose–EC axis. The group-level heatmap (Figure 5) consolidates these fingerprints: pine honey by elevated maltose, chestnut by high EC and F/G ratio, and flower honey by elevated HMF and glucose alongside suppressed diastase activity.

Table 1. Physicochemical parameters of chestnut, pine, and flower honeys from Kastamonu, Türkiye

Parameter	Chestnut (n=14)	Pine (n=14)	Flower (n=12)	*p*-value	η^2p / ε^2
Moisture (%)	16.997 ± 0.565^b	15.839 ± 0.656^a	17.060 ± 0.555^b	< 0.001***	0.495
Dry Matter (%)	83.003 ± 0.565^b	84.161 ± 0.656^a	82.940 ± 0.555^b	< 0.001***	0.495
pH	5.078 ± 0.296^a	4.844 ± 0.363^a	4.027 ± 0.275^b	< 0.001***	0.676
Free Acidity (meq/kg)	22.804 ± 2.110^a	27.760 ± 3.213^b	26.153 ± 3.532^b	< 0.001***	0.351
Electrical Conductivity (mS/cm)	1.249 ± 0.164^b	1.158 ± 0.241^b	0.733 ± 0.052^a	< 0.001***	0.658
Fructose (g/100g)	39.232 ± 1.462^b	34.939 ± 1.096^a	39.278 ± 1.211^b	< 0.001***	0.666
Glucose (g/100g)	24.269 ± 1.175^a	26.693 ± 1.387^b	29.872 ± 1.329^c	< 0.001***	0.765
Sucrose (g/100g)	0.664 ± 0.072b	0.638 ± 0.095^b	0.457 ± 0.073^a	< 0.001***	0.569
Maltose (g/100g)	2.156 ± 0.532^a	7.866 ± 1.273^c	3.313 ± 0.895^b	< 0.001***	0.791
Fructose + Glucose (g/100g)	63.501 ± 1.879^b	61.632 ± 1.383^a	69.151 ± 2.324^c	< 0.001***	0.749
F/G Ratio	1.620 ± 0.102^b	1.313 ± 0.089^a	1.316 ± 0.044^a	< 0.001***	0.767
HMF (mg/kg)	14.415 ± 0.671^b	11.546 ± 1.274^a	21.323 ± 1.374^c	< 0.001***	0.931
Diastase Activity (DN)	14.661 ± 0.252^c	13.168 ± 0.642^b	10.536 ± 0.353^a	< 0.001***	0.877
Proline (mg/kg)	514.839 ± 6.260^c	505.302 ± 3.545^b	494.432 ± 2.475^a	< 0.001***	0.787
Water-Insoluble Matter (g/100g)	0.068 ± 0.022	0.071 ± 0.013	0.073 ± 0.017	0.772 ns	0.014

Values are Mean ± SD. Different superscript letters within the same row denote significant differences ($p<0.05$). ***: $p<0.001$; ns: not significant. F/G: Fructose-to-Glucose ratio; HMF: Hydroxymethylfurfural; DN: Diastase Number.

Microbiological Quality: Microbiological results are summarised in Table 2. TMAB counts were uniformly low across all honey types, with no statistically significant difference among groups ($F(2.37) = 0.974$, $p=0.387$, $\eta^2p=0.050$). Yeasts and moulds were below the analytical detection limit (<1.0 log CFU/g) in all 40 samples. Coliform bacteria, sulphite-reducing anaerobic bacteria, and Salmonella spp. were not detected in any sample examined.

Table 2. Microbiological quality of chestnut, pine, and flower honeys from Kastamonu, Türkiye (log CFU/g)

Parameter	Flower honey (n=12)	Chestnut honey (n=14)	Pine honey (n=14)	*p* value	Partial η²
TMAB	1.80 ± 0.53[a]	1.56 ± 0.56[a]	1.81 ± 0.53[a]	0.387	0.050
Yeast–Mould	< 1.0	< 1.0	< 1.0	–	–
Total coliform	ND in 1 g	ND in 1 g	ND in 1 g	–	–
Sulphite-reducing anaerobes	ND in 1 g	ND in 1 g	ND in 1 g	–	–
Salmonella spp.	ND in 25 g	ND in 25 g	ND in 25 g	–	–

Values are expressed as mean ± SD. Different superscript letters within the same row indicate significant differences ($p<0.05$). ND denotes values below the limit of detection. TMAB counts were compared using one-way ANOVA, and effect size was expressed as partial η²

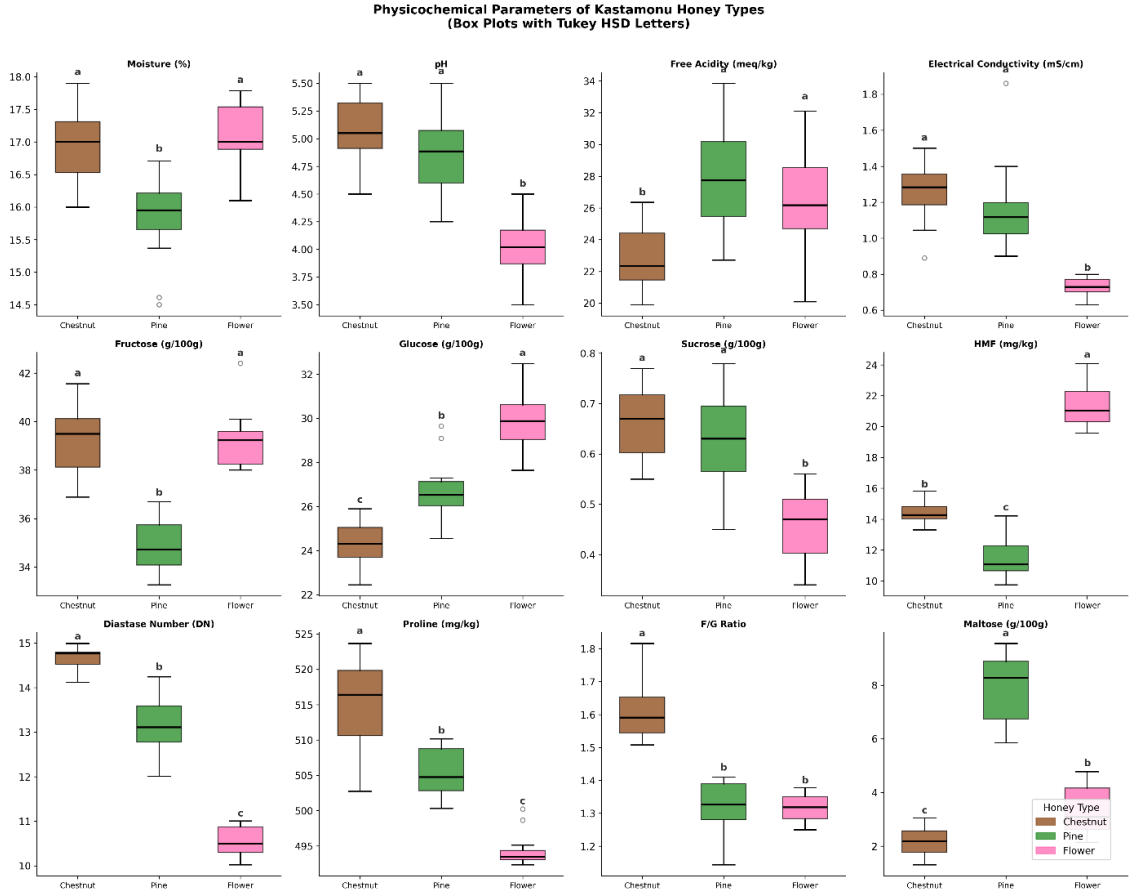

Figure 1. Distribution of physicochemical parameters (moisture, pH, free acidity, electrical conductivity, F/G ratio, and HMF) in Kastamonu honey types. Boxes show IQR with median; whiskers extend to 1.5 × IQR; dots represent individual observations. Different letters indicate significant differences (Tukey HSD or Dunn–Bonferroni, $p<0.05$).

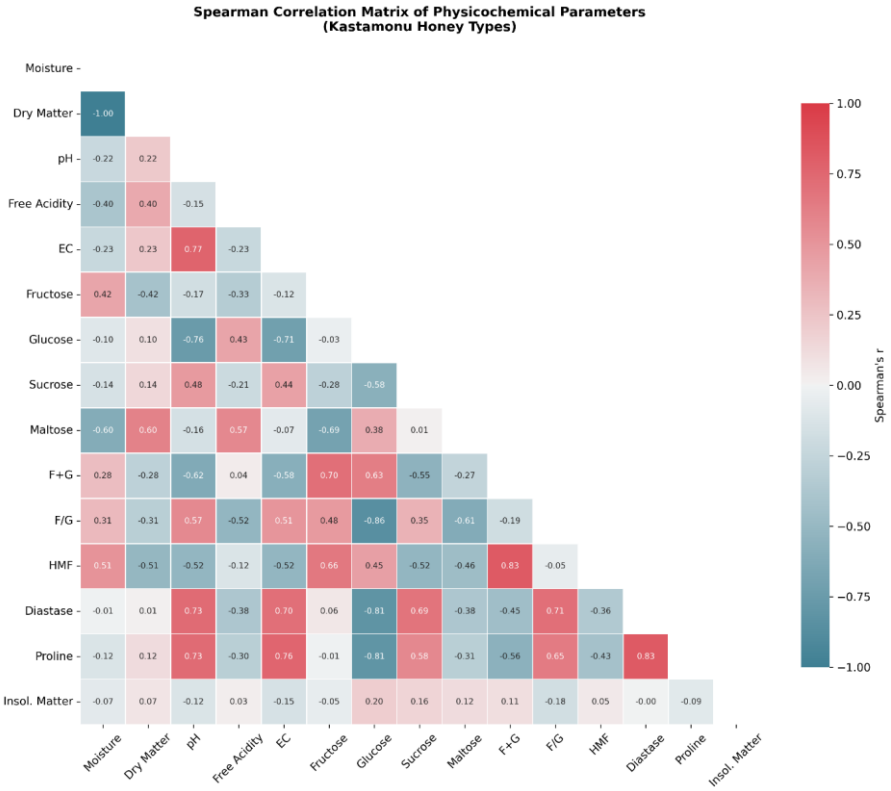

Figure 2. Spearman rank correlation matrix of physicochemical parameters of Kastamonu honey types. Colour intensity reflects the magnitude of rs; blue = positive, red = negative. Significance levels: * p<0.05, ** p<0.01, *** p<0.001.

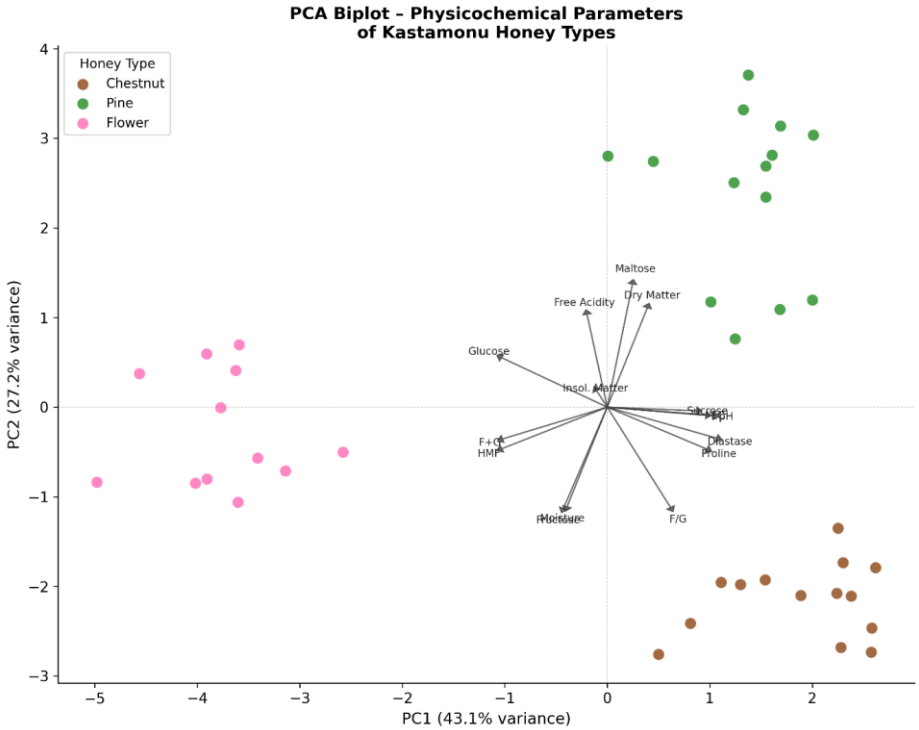

Figure 3. PCA biplot of physicochemical parameters of Kastamonu honey types. Scores are coloured by honey type; loading vectors indicate variable contributions to PC1 and PC2. Data were autoscaled prior to analysis

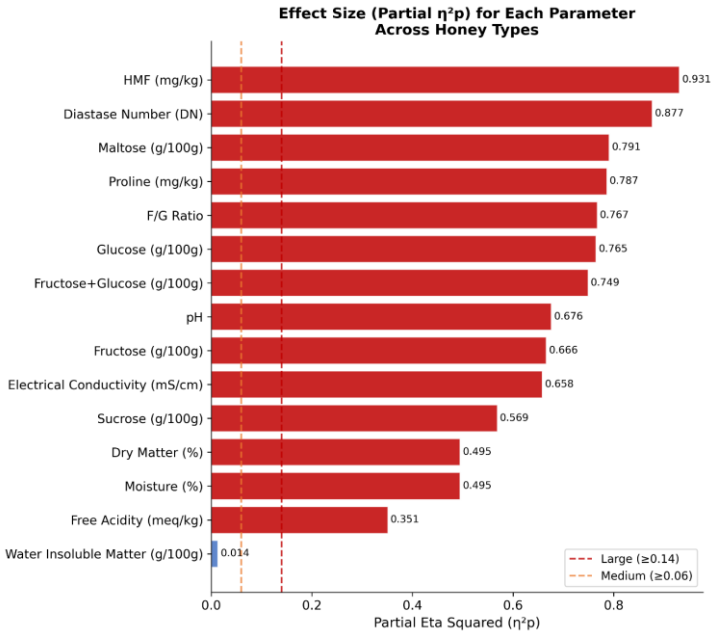

Figure 4. Effect sizes (partial eta-squared, η^2p) for physicochemical parameters of Kastamonu honey types (chestnut, pine, and flower). Parameters are ranked in descending order. Dashed lines indicate conventional thresholds for medium ($\varepsilon^2 \geq 0.06$) and large ($\varepsilon^2 \geq 0.14$) effects

Figure 5. Heatmap of z-score normalised group means for physicochemical parameters of Kastamonu honey types. Colour gradient: blue=below group mean; red=above group mean.

Discussion

Regulatory Compliance and General Quality Assessment: For all fifteen physicochemical parameters analysed, samples were within the limits of the Turkish Food Codex (12), Codex Alimentarius (19), and EU Directive 2001/110/EC (20). Moisture remained below 20% across all types (15.84–17.06%), consistent with the 13.9–18.0% range reported for Eastern Anatolian honeys by Demir et al. (21) and the 15.91±1.05% mean reported by Gürbüz et al. (22) for Southeastern Anatolian honeys. Free acidity values (22.80–27.76 meq/kg) were well below the 50 meq/kg regulatory limit and comparable to those reported for Turkish multifloral and chestnut honeys in the literature (22, 23). HMF values (11.55–21.32 mg/kg) were substantially below the 40 mg/kg maximum, confirming minimal heat exposure and appropriate storage conditions; similar HMF ranges have been reported for fresh Turkish honeys of comparable botanical origin (23). Diastase activity consistently exceeded the 8 DN regulatory minimum across all types (10.54–14.66 DN), in agreement with Aslan and Arık Kibar (24), who similarly reported diastase values above the legal threshold in Turkish pine and flower honeys. Collectively, these findings confirm appropriate post-harvest handling and product integrity across all three honey types.

Moisture, Dry Matter, and Electrical Conductivity: Pine honey's lower moisture content is consistent with the prolonged evaporation associated with honeydew-based production (25). Elevated EC in chestnut and pine honeys relative to flower honey reflects the higher mineral load of honeydew-derived and chestnut-origin products, corroborating findings from comparable Turkish localities. Demir Kanbur et al. (26) reported the highest electrical conductivity values in chestnut honey from Senoz Valley, Rize (a river valley located in the Eastern Black Sea region of Türkiye) compared with highland honeys from the same area, and Saral (27) documented EC values of 0.56–1.12 mS/cm in Artvin chestnut honeys, which is consistent with the 1.249±0.164 mS/cm observed here. That pine honey's EC was statistically indistinguishable from chestnut honey in this dataset may reflect the specific mineralogical profile of the Kastamonu locality (25).

pH, Free Acidity, and Their Mechanistic Relationship: Flower honey had significantly lower pH (4.027±0.275) than chestnut (5.078±0.296) and pine honeys (4.844±0.363), despite comparable free acidity values–an apparent inconsistency that can be explained by differences in buffering capacity among honey types. The higher electrical conductivity of chestnut honey, indicative of a higher mineral load, may confer greater buffering capacity, potentially maintaining a higher pH despite moderate titratable acidity; flower honey's lower mineral-associated buffering reserve may yield a lower pH at similar acidity levels (28). Comparable pH differentials between chestnut and polyfloral honeys have been reported by Akgün et al. (29), who documented higher pH in chestnut honey relative to multifloral and acacia types from Ordu province, and by Gürbüz et al. (22), who reported a mean pH of 4.10±0.73 across mixed-botanical Turkish honeys, which is consistent with the flower honey values observed here. This distinction is functionally relevant: the more acidic environment of flower honey may enhance hydrogen peroxide generation via glucose oxidase activity, a principal antimicrobial mechanism that operates in conjunction with osmotic pressure and phenolic compounds (30); however, as water activity, osmolarity, and phenolic content were not directly measured in the present study, this interpretation should be regarded as mechanistically plausible rather than conclusively demonstrated.

Sugar Composition: Botanical Differentiation and Crystallisation Behaviour: Sugar profiles reflected well-established botanical fingerprints. The highest F/G ratio in chestnut honey (1.620±0.102) indicates the lowest crystallisation tendency among the three types, consistent with Ucurum et al. (31), who reported F/G ratios exceeding 1.3 in both chestnut and pine honeydew honeys relative to multifloral flower types and identified sugar profile and electrical conductivity as key discriminant parameters between honeydew and flower honeys. The markedly elevated maltose in pine honey (7.866±1.273 g/100 g versus 2.156±0.532 in chestnut and 3.313±0.895 in flower) is a recognised biochemical signature of honeydew origin, consistent with the enzymatic processing of oligosaccharide-rich honeydew secretions on Pinus brutia (25, 32). This pattern aligns with findings from Ucurum et al. (25), who documented elevated maltose and higher proline and diastase activity as characteristic features of Turkish pine honeydew honeys across 373 geographically distributed samples, and with Aslan and Arık Kibar (24), who similarly confirmed higher diastase and acidity in pine honeys relative to Turkish flower types. Cobanoglu et al. (33) further demonstrated that sugar and phenolic profiles together constitute reliable botanical differentiation markers between Turkish pine and flower honeys. The specific honeydew-producing insect(s) active in the Kastamonu locality were not determined in the present study. The strong negative maltose–F/G correlation ($r_s \approx -0.80$; Figure 2) is consistent with these botanical patterns, as increasing maltose reduces the relative fructose fraction, a relationship previously documented in comparative studies of honeydew and flower honeys (25, 31, 32).

HMF and Diastase Activity and Proline: HMF formation proceeds via acid-catalysed fructose dehydration, predisposing lower-pH honeys, such as flower honey, to higher accumulation irrespective of thermal history (34, 35). Conversely, the mineral-rich matrix of pine honey confers a buffering effect that suppresses HMF formation, explaining the lowest values in that type. The inverse diastase gradient (chestnut > pine > flower) reflects not only processing history but also botanical differences in enzyme input: chestnut and honeydew honeys receive higher salivary enzyme contributions than most flower types (25–27). Despite the lowest diastase activity in flower honey, all values exceeded the 8 DN legal minimum.

Proline decreased significantly in the order chestnut > pine > flower, yet all samples substantially exceeded the 180 mg/kg authenticity threshold; chestnut honey additionally surpassed the 500 mg/kg type-specific limit (12), consistent with the proline-rich nectars of *Castanea sativa* reported across Turkish Black Sea localities (22, 27).

Multivariate Structure: PCA achieved clear three-way botanical separation, with PC1 driven by HMF, diastase, and F/G ratio, and PC2 discriminating along the maltose–EC axis – a structure paralleling multivariate differentiation reported for Turkish (25) and Greek (36) unifloral honeys, reinforcing cross-regional validity of these parameters as botanical discriminants. The Spearman matrix (Figure 2) captured mechanistically interpretable associations: maltose–F/G ($r_s \approx -0.80$), EC–free acidity (joint mineral–organic acid influence), and the inverse HMF–diastase relationship. The group heatmap (Figure 5) consolidates these as coherent typological signatures.

Microbiological Quality and Its Relationship to Physicochemical Properties: Yeasts and moulds were below the analytical detection limit (<1.0 log CFU/g) in all 40 samples, while coliform bacteria, sulphite-reducing anaerobic bacteria, and *Salmonella* spp. were not detected in any sample examined. These findings are consistent with a growing body of evidence documenting the microbiological safety of high-quality honeys across different geographical and botanical origins. Gomes et al. (4) reported the complete absence of faecal coliforms, sulphite-reducing clostridia, and *Salmonella* in commercial Portuguese honeys, attributing this to the synergistic inhibitory effects of low water activity, acidic pH, and hydrogen peroxide generation. Tornuk et al. (7) similarly reported undetectable yeast and mould counts in artisanal Turkish flower honeys, and Luca et al. (8) comprehensively documented the antimicrobial mechanisms (including osmotic stress, low pH, and hydrogen peroxide generation) that collectively suppress microbial proliferation in honey. Kędzierska-Matysek et al. (37) found that 52% of Polish artisanal varietal honeys contained fewer than 10 CFU/g of total bacteria, with fungal counts below 10 CFU/g in 81% of samples, while Pauliuc et al. (38) reported satisfactory microbiological profiles with no detectable *Salmonella* or pathogenic *Enterobacteriaceae* across five botanical honey types from Bucovina, Romania. The absence of a microbiological gradient mirroring the substantial physicochemical differences observed among honey types in the present study is consistent with a threshold effect: although pH, EC, and sugar composition differed significantly, all types maintained inhibitory conditions sufficient to prevent microbial proliferation. Moisture content data (15.8–17.1%) correspond to expected a_w values of approximately 0.55–0.62, based on published reference values for comparable honey types (5). These values provide indirect evidence that water activity suppression was maintained throughout. Once moisture and pH fall within inhibitory ranges, botanical-origin differences in antimicrobial intensity become of limited relevance to routine microbiological safety (8, 30).

Chestnut, pine, and flower honeys from Kastamonu province were comprehensively characterised across fifteen physicochemical and five microbiological parameters. Fourteen physicochemical parameters differed significantly among types ($p<0.001$); for all parameters analysed, samples were within TGK 2020/7, Codex Alimentarius, and EU Directive 2001/110/EC thresholds, confirming compliance with compositional quality criteria.

The most discriminant parameters (HMF, diastase activity, maltose, glucose, and F/G ratio) closely mirror the botanical and biochemical logic of each honey type. The markedly elevated maltose content of pine honey constitutes a robust biochemical fingerprint of honeydew origin; the identity of the honeydew-producing insect(s) active in the Kastamonu locality was not determined in the present study and warrants further investigation. The pH–free acidity paradox in flower honey is explained by differences in buffering capacity. Divergent HMF–diastase profiles reflect both post-harvest conditions and intrinsic botanical differences in enzyme input and acid-catalysed fructose dehydration, underscoring the need for type-specific rather than uniform quality interpretation.

Microbiologically, all types were free from coliforms, pathogens, and detectable yeast–mould growth, with uniformly low and statistically indistinguishable TMAB counts. This supports the view that honey's microbiological safety is determined primarily by process hygiene and moisture-mediated water activity suppression, rather than botanical origin (5, 8, 30).

Collectively, the physicochemical and microbiological data generated in this study provide a comprehensive quality baseline for chestnut, pine, and flower honeys from Kastamonu province, Türkiye. The parameters examined (including moisture, HMF, diastase activity, sugar composition, electrical conductivity, free acidity, and proline) are established indicators of honey quality, maturity, and post-harvest handling integrity, and the dataset may serve as a regional reference for routine quality monitoring. It should be acknowledged, however, that the establishment of geographical indication status requires additional analytical dimensions, particularly melissopalynological profiling, phenolic fingerprinting, and stable isotope analysis. These were beyond the scope of the present study. Future research incorporating these approaches would more directly address botanical authentication and geographical indication documentation for Kastamonu honeys.

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