

## Ethnomelissological, melissopalynological and physicochemical characterization of honeys from the El Tarf region, Northeastern Algeria



Caracterización etnomelissológica, melisopalinológica y fisicoquímica de las mieles de la región de El Tarf, noreste de Argelia

Caracterização etnomelissológica, melisopalinológica e físico-química de méis da região de El Tarf, nordeste da Argélia.

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### Food technology

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### Abstract

Honey is a natural product produced by bees from floral nectar or plant secretions. This study evaluated apicultural practices and honey quality from the El Tarf region (northeastern Algeria) using an integrated approach combining ethnomelissological surveys, melissopalynological analysis, and physicochemical characterization. A total of 36 samples representing six honey types (white heather, eucalyptus, lavender, mountain, multifloral, and orange blossom) were collected from four localities (Aïn Khiyar, Zitouna, Bougous, and Aïn Karma). Pollen analysis revealed a predominance of polyfloral honeys with variable monofloral representation. Physicochemical parameters showed significant variability among honey types ( $p < 0.05$ ), with pH ranging from 3.73 to 4.37, moisture content from 13.90 % to 18.19 %, and electrical conductivity from 330.33 to 719.33  $\mu\text{S}\cdot\text{cm}^{-1}$ . Hydroxymethylfurfural (HMF) content varied between 41.35 and 49.80  $\text{mg}\cdot\text{kg}^{-1}$ , while total acidity ranged from 41.50 to 63.64  $\text{meq}\cdot\text{kg}^{-1}$ . Diastase activity (9.50 – 14.00 DN) and proline content (310 – 420  $\text{mg}\cdot\text{kg}^{-1}$ ) also showed significant differences according to botanical origin ( $p < 0.05$ ). All samples complied with international honey quality standards. Principal Component Analysis (PCA) showed that honey samples were clearly structured according to botanical origin, with electrical conductivity, proline, and acidity contributing most to variability (PC1 = 46.38 %, PC2 = 28.04 %). Monofloral honeys exhibited more homogeneous profiles, whereas multifloral samples were more dispersed, confirming significant compositional variability ( $p < 0.05$ ). The results demonstrate that honey quality in the El Tarf region is primarily influenced by floral origin and local beekeeping practices, highlighting the relevance of integrating traditional knowledge with physicochemical and palynological approaches for honey authentication and quality assessment.

## Resumen

La miel es un producto natural producido por las abejas a partir del néctar floral o las secreciones vegetales. Este estudio evaluó las prácticas apícolas y la calidad de la miel en la región de El Tarf (noreste de Argelia) mediante un enfoque integrado que combinó estudios etnomelissológicos, análisis melissopalínológicos y caracterización fisicoquímica. Se recolectaron 36 muestras de seis tipos de miel (brezo blanco, eucalipto, lavanda, de montaña, multifloral y azahar) en cuatro localidades (Aïn Khiyar, Zitouna, Bougous y Aïn Karma). El análisis de polen reveló un predominio de mieles multiflorales con una representación variable de mieles monoflorales. Los parámetros fisicoquímicos mostraron una variabilidad significativa entre los tipos de miel ( $p < 0,05$ ), con un pH que osciló entre 3,73 y 4,37, un contenido de humedad entre 13,90 % y 18,19 %, y una conductividad eléctrica entre 330,33 y 719,33  $\mu\text{S}\cdot\text{cm}^{-1}$ . El contenido de hidroximetilfurfural (HMF) varió entre 41,35 y 49,80  $\text{mg}\cdot\text{kg}^{-1}$ , mientras que la acidez total osciló entre 41,50 y 63,64  $\text{meq}\cdot\text{kg}^{-1}$ . La actividad de la diastasa (9,50–14,00 DN) y el contenido de prolina (310–420  $\text{mg}\cdot\text{kg}^{-1}$ ) también mostraron diferencias significativas según el origen botánico ( $p < 0,05$ ). Todas las muestras cumplieron con los estándares internacionales de calidad de la miel. El análisis de componentes principales (ACP) mostró que las muestras de miel presentaban una clara estructura según su origen botánico, siendo la conductividad eléctrica, la prolina y la acidez los parámetros que más contribuyeron a la variabilidad (CP1 = 46,38 %, CP2 = 28,04 %). Las mieles monoflorales exhibieron perfiles más homogéneos, mientras que las multiflorales mostraron una mayor dispersión, lo que confirma una variabilidad composicional significativa ( $p < 0,05$ ). Los resultados demuestran que la calidad de la miel en la región de El Tarf está influenciada principalmente por el origen floral y las prácticas apícolas locales, lo que subraya la importancia de integrar el conocimiento tradicional con enfoques fisicoquímicos y palinológicos para la autenticación y evaluación de la calidad de la miel.

**Palabras clave:** apicultura, espectro polínico, parámetros de calidad de la miel, conocimiento tradicional, El Tarf-Argelia.

## Resumo

O mel é um produto natural produzido pelas abelhas a partir do néctar floral ou de secreções vegetais. Este estudo avaliou as práticas apícolas e a qualidade do mel da região de El Tarf (nordeste da Argélia) utilizando uma abordagem integrada que combina levantamentos etnomelissológicos, análises melissopalínológicas e caracterização físico-química. Um total de 36 amostras, representando seis tipos de mel (urze branca, eucalipto, lavanda, montanha, multifloral e flor de laranjeira), foram recolhidas em quatro localidades (Aïn Khiyar, Zitouna, Bougous e Aïn Karma). A análise polínica revelou uma predominância de méis multiflorais com representação variável de méis monoflorais. Os parâmetros físico-químicos apresentaram uma variabilidade significativa entre os tipos de mel ( $p < 0,05$ ), com um pH a variar entre 3,73 a 4,37, um teor de humidade de 13,90 % a 18,19 % e uma condutividade elétrica de 330,33 a 719,33  $\mu\text{S}\cdot\text{cm}^{-1}$ . O teor de hidroximetilfurfural (HMF) variou entre 41,35 e 49,80  $\text{mg}\cdot\text{kg}^{-1}$ , enquanto a acidez total variou entre 41,50 a 63,64  $\text{meq}\cdot\text{kg}^{-1}$ . A atividade da diastase (9,50–14,00 DN) e o teor de prolina (310–420  $\text{mg}\cdot\text{kg}^{-1}$ ) também apresentaram diferenças significativas de acordo com a origem botânica ( $p < 0,05$ ). Todas as amostras cumpriram os padrões internacionais de qualidade do mel. A Análise

de Componentes Principais (ACP) mostrou que as amostras de mel apresentaram uma estrutura clara de acordo com a origem botânica, sendo que a condutividade elétrica, a prolina e a acidez contribuíram mais para a variabilidade (PC1 = 46,38 %, PC2 = 28,04 %). Os méis monoflorais exibiram perfis mais homogéneos, enquanto as amostras multiflorais foram mais dispersas, confirmando uma variabilidade composicional significativa ( $p < 0,05$ ). Os resultados demonstram que a qualidade do mel na região de El Tarf é influenciada principalmente pela origem floral e pelas práticas locais de apicultura, destacando a relevância da integração do conhecimento tradicional com abordagens físicoquímicas e palinológicas para a autenticação e avaliação da qualidade do mel.

**Palavras-chave:** apicultura, espectro polínico, parâmetros de qualidade do mel, conhecimento tradicional, El Tarf-Argélia.

## Introduction

Algeria is characterized by a wide range of bioclimatic zones, from humid coastal areas to semi-arid inland regions, supporting a rich floristic diversity that favors apiculture and the production of high-value honeys (Hamsas El Youbi *et al.*, 2016; Ayad *et al.*, 2021). Honey characteristics are largely influenced by botanical origin, seasonal flowering, and environmental conditions, as well as by beekeeping practices and post-harvest handling. Although honey is recognized for its nutritional and medicinal properties (Al-Habsi and Niranjan, 2012), it remains susceptible to quality degradation and adulteration, particularly in regions where quality control systems are limited (Bouddine *et al.*, 2024; Derrar *et al.*, 2024).

Despite the importance of honey production in Algeria, integrated studies combining ethnomelissological surveys, melissopalynological analysis, and physicochemical characterization remain scarce, especially in northeastern regions. The El Tarf area, characterized by forest, coastal, and agricultural ecosystems, represents a zone of high apicultural potential, however, the documentation of traditional knowledge and comprehensive honey quality assessment in this region is still limited (Boutabia *et al.*, 2016).

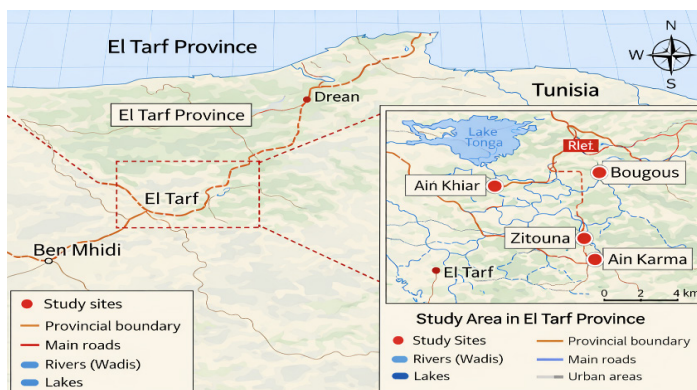
This study addresses this gap through a multidisciplinary approach integrating ethnomelissological investigation, pollen analysis, and physicochemical characterization. It aims to (i) document local beekeeping practices, (ii) identify high-potential apicultural zones, (iii) assess honey quality and botanical origin in relation to international standards, and (iv) propose strategies to improve honey quality and market value.

## Materials and Methods

### Study area

The wilaya of El Tarf is located in the extreme Northeast of Algeria, on the border with Tunisia (Boussaid *et al.*, 2018). It covers an area of 2,912.65  $\text{km}^2$  and belongs to the humid bioclimatic region of the Algerian coast. The capital, El Tarf, is located approximately 650 km east of Algiers, the country's capital. This wilaya is characterized by a varied topography including mountainous, forested, and coastal areas, favorable for the development of a rich and diverse melliferous flora (Boutabia *et al.*, 2016). The samples were collected from four study sites located in the El Tarf region (Figure 1).

These areas were chosen due to their floristic diversity, notable beekeeping activity, and their representativeness of the melliferous ecosystems of Northeastern Algeria.



**Figure 1.** Geographic location of the study area in El Tarf Province (northeastern Algeria), showing the sampling sites (Ain Khiair, Zitouna, El Tarf, Bougous, and Ain Karma). The map includes main geographical features such as roads, rivers, lakes, and urban areas.

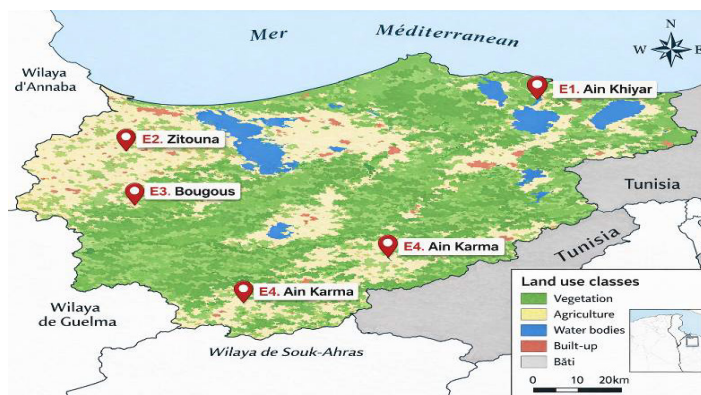
### Ethnomelissological survey (ethnographic)

An ethnomelissological survey was conducted among local beekeepers using a semi-structured questionnaire administered through direct interviews in four study areas (Ain Khiair, Zitouna, Bougous, and Ain Karma). Field visits and hive inspections were performed to document beekeeping practices, surrounding flora, and apiairy distribution.

### The survey focused on the following areas

The survey addressed floristic resources and honey typology (main melliferous species and their influence on honey characteristics), harvesting practices (seasonality and limited transhumance), local quality criteria, extraction and preservation techniques, commercialization, and traditional therapeutic uses.

Site-specific ethnomelissological features were identified across the four study areas. Ain Khiair was characterized by an agroforestry system producing mainly polyfloral honeys used for digestive and general health purposes. Zitouna represented a forest–agriculture transition zone, where honeys were commonly used for respiratory ailments. Bougous, a mountainous forest area, produced dark honeys dominated by *Arbutus unedo* and *Quercus* spp., highly valued for medicinal applications. Ain Karma, with a forested semi-rural landscape, was associated with honeys used for preventive and fortifying purposes (Figure 2).



**Figure 2.** Spatial distribution of beekeepers and sampling sites in El Tarf Province (northeastern Algeria): E1 (Ain Khiair), E2 (Zitouna), E3 (Bougous), and E4 (Ain Karma), overlaid on land use/land cover.

### Data collection

A semi-structured interview was conducted with approximately 30 beekeepers selected using a snowball sampling approach across four sites: E1 (Ain Khiair), E2 (Zitouna), E3 (Bougous), and E4 (Ain Karma) (Figures. 2, 3).



**Figure 3:** Photograph of the study stations (BERGAL, 2024)

The dataset included independent variables related to production conditions (site, harvest season, botanical origin, extraction method, and hive type), and dependent variables describing honey characteristics, including sensory attributes, traditional uses, and physicochemical parameters.

### Pollen analysis (melissopalynology)

The pollen analysis of honeys was carried out in accordance with the reference method described by Louveaux *et al.* (1978).

### Physicochemical analyses

Physicochemical analyses were performed according to harmonized methods of the International Honey Commission (IHC) and Codex Alimentarius standards (CODEX STAN 12-1981). Honey samples were stored in airtight glass containers, protected from light, at room temperature (20–25 °C) and analyzed within two weeks.

All measurements were conducted in triplicate and expressed as mean  $\pm$  standard deviation. The analyzed parameters included pH, free and total acidity, hydroxymethylfurfural (HMF), proline, protein, moisture content, diastatic activity, electrical conductivity, salinity, and sugar composition (Ghorab *et al.*, 2021).

pH, acidity, moisture, conductivity, and diastase activity were determined using standard analytical methods (Da Silva *et al.*, 2016). HMF was measured spectrophotometrically (Khalil *et al.*, 2012), while proline and protein contents were determined using ninhydrin and Coomassie methods, respectively (Da Silva *et al.*, 2016).

Sugar composition (glucose, fructose, and sucrose) was determined by HPLC-RID after dilution, filtration (0.45  $\mu$ m), and separation on a carbohydrate column using an acetonitrile:water (80:20, % v/v) mobile phase. Quantification was performed using external standards (Derrar *et al.*, 2024; Khalil *et al.*, 2012).

### Experimental design and statistical analysis

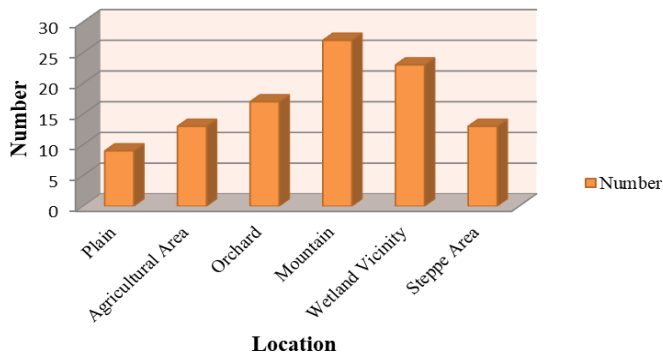
A factorial ANOVA (general linear model) was applied to evaluate the effects of site, season, and honey type, as well as their interactions, on physicochemical parameters. When significant differences were observed, Tukey's HSD test was used for multiple comparisons ( $p < 0.05$ ). Principal Component Analysis (PCA) was performed to explore relationships among honey samples and physicochemical variables and to identify the main factors explaining variability between honey types.

Statistical analyses were performed using IBM SPSS Statistics (v.24). Results were expressed as mean  $\pm$  standard deviation based on triplicate measurements.

## Results and discussion

### Survey

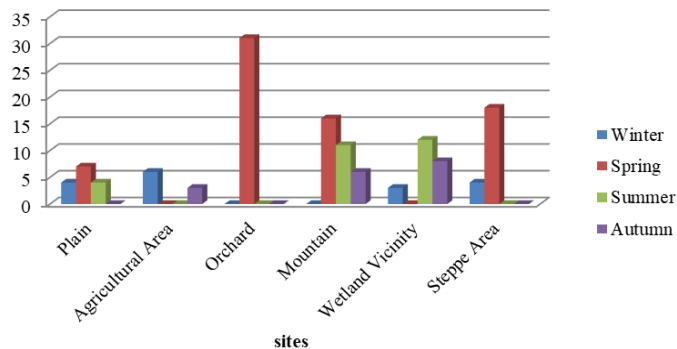
During the spring flowering period (Figure 4), hive placement was mainly concentrated in mountainous areas characterized by high floristic richness and abundant melliferous species.



**Figure 4 : The locations of the hives during the flowering period**

Orchards represented the second most frequent location due to fruit tree flowering, followed by wetland-associated areas offering continuous floral resources and favorable microclimatic conditions. In contrast, agricultural plains and steppe zones were less used, likely due to intensive farming practices and reduced spontaneous flora diversity (Derrar *et al.*, 2024). These differences in hive distribution among environments were statistically significant ( $p < 0.05$ ). These results are consistent with previous studies highlighting the importance of natural and semi-natural ecosystems in supporting beekeeping activity and the production of high-quality polyfloral honeys in North Africa and Mediterranean regions (Bouddine *et al.*, 2024; Al-Habsi and Niranjana, 2012; Anjos *et al.*, 2023; Haderbache and Mohammadi, 2015).

The different areas for hive placement in relation to the season are shown in figure 5.



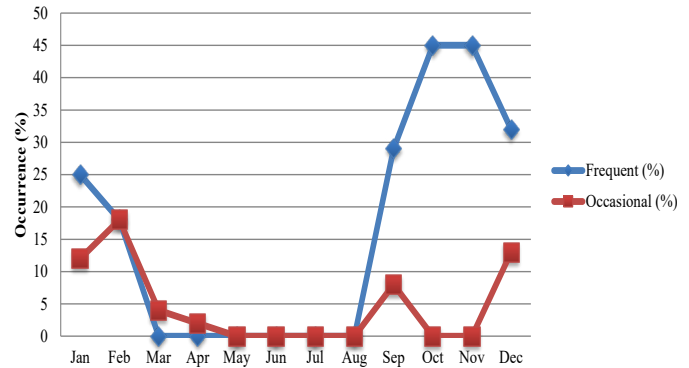
**Figure 5: The different areas for hive placement in relation to the season**

Beekeeping activity shows clear seasonal dynamics across habitats. During winter, hive placement is limited and mainly concentrated in agricultural and mountainous areas due to reduced floral availability and climatic constraints. In spring, hive distribution increases in all environments, particularly in orchards, corresponding to peak flowering of melliferous species (Boutabia *et al.*, 2016). In summer, hives are mainly relocated to mountainous and wetland areas offering favorable microclimatic conditions and sustained floral resources, while activity decreases in plains and orchards (Ghorab *et al.*, 2021). In autumn, overall beekeeping activity declines, with hives mostly concentrated in mountainous and wetland zones (Derrar *et al.*, 2024).

These seasonal patterns reflect a transhumance strategy driven by floral phenology and climatic conditions, highlighting the key role of natural ecosystems in shaping honey diversity and quality in

the El Tarf region (Ketfi *et al.*, 2023). Seasonal differences in hive distribution were statistically significant ( $p < 0.05$ ).

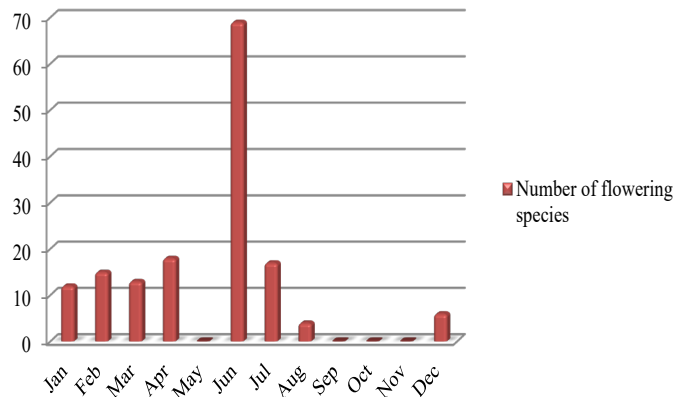
The feeding periods during the year 2024 are presented in figure 6.



**Figure 6: The feeding periods during the year 2024**

The data show a clear seasonal dynamic in feeding activity, with high values in winter (January–February), a sharp decline from March to August, and a marked increase in autumn (September–November), reaching a peak in October–November. Overall differences between months were statistically significant ( $p < 0.05$ ). This pattern reflects the influence of climatic and floristic seasonality on beekeeping practices in the El Tarf region (Boutabia *et al.*, 2016; Al-Habsi and Niranjana, 2012). The autumn peak coincides with the flowering of late melliferous species such as *Arbutus unedo*, *Erica* spp., and *Quercus* spp., which contribute significantly to honey production (Machado De-Melo *et al.*, 2018). The low activity observed in spring and summer is likely related to reduced floral availability, high temperatures, and transhumance or reduced colony management during this period (Bouddine *et al.*, 2024; Ayad *et al.*, 2021). These results are consistent with previous studies in Mediterranean regions reporting a bimodal beekeeping activity pattern, with peaks in early spring and autumn (Derrar *et al.*, 2024; Machado De-Melo *et al.*, 2018).

The Figure 7 shows a marked seasonal pattern in flowering in El Tarf, with two main periods.

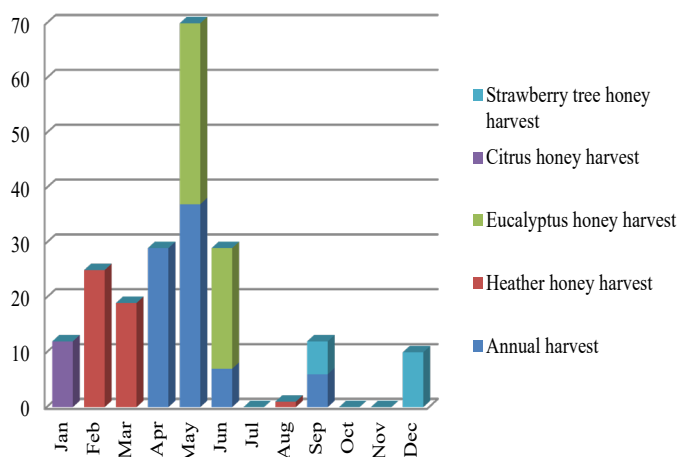


**Figure 7: Chronology of flowering during the year 2024**

A moderate flowering phase occurs in winter (January–February), dominated by early-flowering species providing initial nectar resources for bee colonies. From March to August, flowering is very limited due to high temperatures and water stress. The main flowering

peak occurs in autumn (September–November), particularly in October–November, driven by favorable climatic conditions such as rainfall and cooler temperatures, which support colony resource accumulation. Sporadic flowering species contribute marginally throughout the year (Cohen *et al.*, 2022). Overall, flowering intensity showed significant seasonal variation ( $p < 0.05$ ). This phenological pattern highlights the importance of forest and shrub ecosystems in sustaining beekeeping activity and supports adaptive practices such as transhumance and supplemental feeding. It also emphasizes the relevance of flowering seasonality in apicultural management, with a stronger autumn bloom compared to other Mediterranean regions (Ketfi *et al.*, 2023; Machado De-Melo *et al.*, 2018).

The annual honey harvest shows a marked seasonal pattern, with a main peak in summer (June–July), reflecting high overall beekeeping activity (Figure 8).



**Figure 8: Harvest periods**

Heather honey is mainly produced in spring (March–May), citrus honey in early spring (March), eucalyptus honey in summer (July–August), and *Arbutus unedo* honey in late autumn to early winter (November–December), extending the harvesting period beyond the main season. These patterns reflect the strong dependence of honey production on floral phenology (Al-Habsi and Niranjana, 2012; Ayad, 2021). The summer peak highlights the importance of eucalyptus as a key nectar source (Bouddine *et al.*, 2024), while *Arbutus unedo* contributes to late-season production, supporting colony sustainability during nectar scarcity periods (Ketfi *et al.*, 2023). Overall, honey production differed significantly across harvest periods ( $p < 0.05$ ), confirming the strong seasonal influence of floral availability on beekeeping yields.

Table 1 shows the characteristics of honeys from the El Tarf region.

A factorial ANOVA revealed significant variability in honey characteristics according to site, season, and botanical origin. A significant site effect ( $p < 0.05$ ) indicated that geographical origin strongly influences honey composition and sensory properties. Seasonal variation was also significant ( $p < 0.05$ ), with spring honeys generally showing lighter floral profiles, while autumn honeys exhibited darker and more woody characteristics. Botanical origin had a highly significant effect ( $p < 0.01$ ), confirming clear differences between monofloral and polyfloral honeys, with monofloral types showing more homogeneous profiles (Khalil *et al.*, 2012; Machado De-Melo *et al.*, 2018).

Significant interactions were observed between site  $\times$  season and season  $\times$  honey type ( $p < 0.05$ ), indicating that environmental and botanical factors jointly influence honey variability.

#### Pollen taxa identified in the samples

Pollen analysis of the 36 honey samples (Table 1) revealed taxa mainly associated with forest, Mediterranean, and agricultural flora characteristic of the El Tarf region (Al-Kafaween *et al.*, 2023; Anjos *et al.*, 2015).

Ethnographic data showed that spring was the dominant harvesting season (60 %), followed by autumn (40 %). Centrifugation was the most common extraction method (60 %), compared to pressing (40 %), and Langstroth hives were predominant (60 %) over traditional hives (40 %).

Polyfloral honeys were more frequent (60 %) than monofloral types (40 %). *Thymus* species represented the most commonly cited floral source (60 %), followed by *Arbutus unedo* and *Quercus* spp. (40 %). Honey use was mainly associated with medicinal and nutritional purposes. Overall differences in frequencies among categories were statistically significant ( $p < 0.05$ ), indicating a non-random distribution of beekeeping practices and honey types across the studied samples.

The main pollens identified are presented in Table 2.

Monofloral strawberry tree honeys were mainly associated with forested areas (Bougous, Aïn Karma), confirming the importance of sylvatic formations. Citrus honeys were linked to agricultural areas (Zitouna and Aïn Khiyar) (Table 2, 3). The high frequency of *Quercus* and *Cistus* pollen reflects the strong influence of Mediterranean forest ecosystems (Boutabia *et al.*, 2016; Ketfi *et al.*, 2023). Overall, pollen frequencies differed significantly among taxa and ecological groups ( $p < 0.05$ ), indicating a non-random distribution of floral resources in the studied honeys.

Melissopalynological analysis revealed a predominance of forest and Mediterranean taxa, particularly *Arbutus unedo*, *Quercus* spp., *Thymus* spp., and *Cistus* spp. These results confirm the presence of monofloral and polyfloral honeys and are consistent with ethnomelissological data obtained from local beekeepers (Table 3 and 4) (Hamsas El Youbi *et al.*, 2016; Anjos *et al.*, 2023).

Overall, the distribution of honey types based on pollen criteria showed significant differences ( $p < 0.05$ ), indicating a non-random pattern of botanical origin across the samples.

The pollen spectrum revealed a strong dominance of forest and Mediterranean taxa, particularly *Arbutus unedo*, *Citrus* spp., and *Rubus* spp. Most samples exhibited a polyfloral character, although several monofloral honeys were identified, mainly dominated by *Arbutus* and *Citrus*. These results highlight the importance of local floristic diversity and confirm the information obtained from ethnomelissological surveys conducted among beekeepers in the region (Boutabia *et al.*, 2016; Ayad *et al.*, 2021). Overall, pollen distribution among taxa showed significant heterogeneity ( $p < 0.05$ ), indicating a non-random botanical structure in the studied honeys. This melissopalynological profile reflects the forest–agricultural mosaic of El Tarf and supports the valorization and certification of local honeys.

#### Physico-chemical analyzes

The results of physicochemical analyzes of different types of honey are shown in Table 5.

**Table 1. Ethnomellissological table of honeys from the El Tarf region (Algeria)**

Site	Harvest date	Season	Extraction method	Type of beehive	Floral sources cited	Traditional uses	Sensory notes	Type of honey
Ain Khiyar	April 2024	Spring	Centrifuge	Langstroth	Strawberry tree, Oak	Cough, cold	Dark, woody	Monofloral Strawberry Tree
	October 2024	Autumn	Pressing	Traditional	Thymus, Rockrose	Digestive	Golden, herbaceous	Polyfloral
Zitouna	April 2024	Spring	Centrifuge	Langstroth	Citrus fruits, Thymus	Food use	Light yellow, floral	Monofloral Citrus
Bougous	April 2024	Spring	Centrifuge	Langstroth	Thymus, Bramble	Traditional infusion	Golden, fruity	Polyfloral
Ain Karma	October 2024	Autumn	Pressing	Traditional	Strawberry tree, Quercus	Local medicinal use	Dark, woody	Polyfloral

**Table 2. Main pollens identified**

Pollen taxon	Family	Frequency of occurrence	Role in honey
<i>Arbutus unedo</i>	Ericaceae	Very common	Monofloral / dominant honey
<i>Oak</i> spp.	Fagaceae	Frequent	Forest indicator
<i>Thymus</i> spp.	Lamiaceae	Frequent	Major nectar source
<i>Cistus</i> spp.	Cistaceae	Frequent	Mediterranean flora
<i>Citrus</i> spp.	Rutaceae	Moderate to high	Citrus honey
<i>Rubus</i> spp.	Rosaceae	Moderate	Blackberry honey
<i>Eucalyptus</i> spp.	Myrtaceae	Casual	Secondary contributions
<i>Various Asteraceae</i>	Asteraceae	Low to moderate	Pollen accessories
<i>various Fabaceae</i>	Fabaceae	Weak	Secondary pollens

**Table 4. Main pollen taxa identified in the studied honeys**

Pollen taxon	Frequency (%)	Pollen class	Botanical interpretation
<i>Arbutus unedo</i>	35–55	Dominant	Monofloral strawberry tree honey
<i>Cistus</i> spp.	25–55	Dominant to secondary	Citrus honey
<i>Thymus</i> spp.	20–45	Secondary	Marked aromatic influence
<i>Cistus</i> spp.	10–30	Secondary	Mediterranean vegetation
<i>Oak</i> spp.	5–20	Secondary	Forest origin
<i>Rubus</i> spp.	15–50	Dominant	Blackberry honey
<i>Eucalyptus</i> spp.	5–15	Minor	Additional contribution
Other taxa	<10	Minor	Polyfloral character

Classification used • Dominant: > 45 % • Secondary: 16–45 % • Minor: < 15 %

**Table 3. Pollen spectrum of the samples**

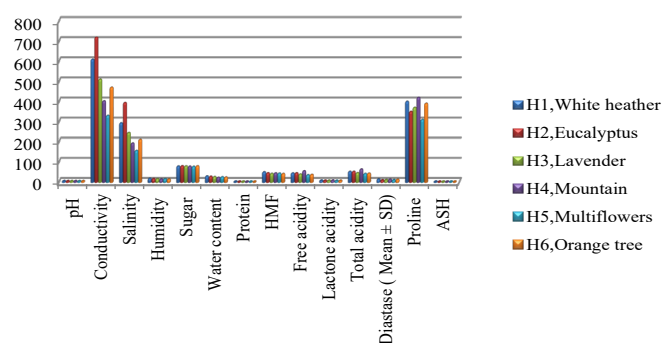
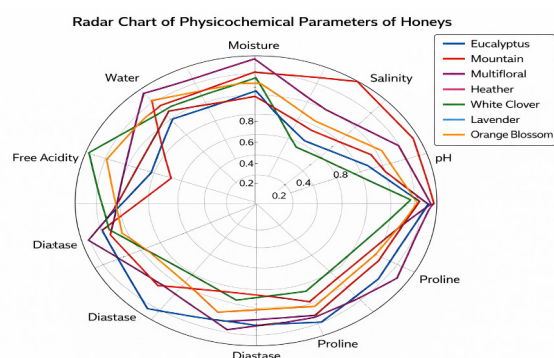
Type of honey	Pollen criterion	Number of samples
Monofloral Strawberry Tree	<i>Arbutus</i> > 45 %	11
Monofloral Citrus	<i>Citrus</i> > 45 %	8
Monofloral Bramble	<i>Rubus</i> > 45 %	6
Polyfloral	No pollen > 45 %	11
Total		36

Overall, physicochemical parameters such as electrical conductivity, diastase activity, proline, and protein content effectively discriminate between dark, mineral-rich honeys (eucalyptus, white heather, lavender) and lighter honeys (orange, multifloral, mountain) (Gheldof *et al.*, 2002; Ketfi *et al.*, 2023) (Table 5, Figure 9 and 10). Significant differences were observed among honey types for the measured parameters ( $p < 0.05$ ), confirming that floral origin strongly influences honey physicochemical profiles. Mountain and multifloral honeys showed lower mineralization due to diverse floral origins, while orange honey displayed typical citrus characteristics with low conductivity, moderate acidity, and high enzymatic activity, indicating good quality and freshness.

The Comparison of the different honey samples studied are presented in Figure 9. Each axis represents a physicochemical parameter (pH, electrical conductivity, salinity, etc.), and the curves show the normalized mean profiles of each honey type, allowing visual comparison of their overall physicochemical signatures (Amessis-Ouchemoukh *et al.*, 2021). The radar analysis highlights clear inter-floral variability, reflecting ecological and botanical diversity. White heather and lavender honeys exhibited richer biochemical profiles, with higher enzymatic and mineral-related parameters, consistent with honeys derived from woody and aromatic flora. Eucalyptus honey also displayed a profile typical of mineral-rich dark honeys (Figure 10).

**Table 5. Comparative table of physicochemical analyzes (Mean±SD) of different types of honey.**

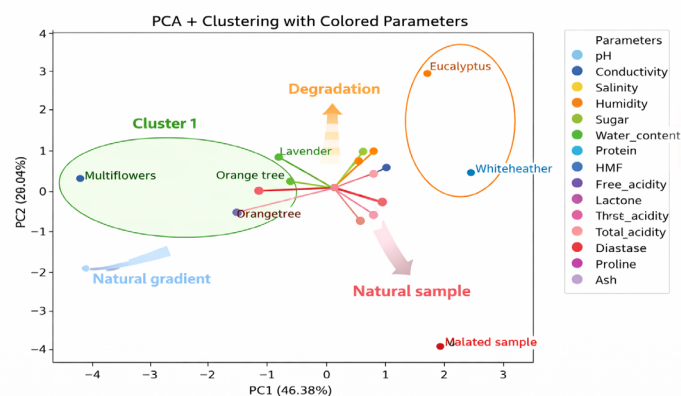
Physicochemical characteristics	White heather	Eucalyptus	Lavender	Mountain	Multiflowers	Orange tree
pH	4.310± 0.010	4.367 ± 0.015	4.110 ± 0.010	4.230 ± 0.010	3.730 ± 0.010	3.980 ± 0.010
Conductivity	609.333 ± 2.517	719,333 ± 2,517	510.333 ± 1.528	402.333 ± 1,528	330.333 ± 1.528	470.000 ± 2,000
Salinity	293.100 ± 0.656	394.100 ± 1.054	245.233 ± 0.351	192.733 ± 0.802	155.733 ± 0.862	210,600 ± 0.557
Humidity	18.187 ± 0.035	16.590 ± 0.036	15.447 ± 0.025	14.127 ± 0.025	15.807 ± 0.025	13.900 ± 0.020
Sugar	78.50 ± 0.50	80.20 ± 0.40	79.10 ± 0.45	77.80 ± 0.35	76.90 ± 0.50	79.50 ± 0.40
Water content	29.340 ± 0.040	27.777 ± 0.025	26.210 ± 0.036	22.700 ± 0.010	25.020 ± 0.020	23.883 ± 0.031
Protein	0.063 ± 0.006	0.043 ± 0.006	0.073 ± 0.006	0.053 ± 0.006	0.083 ± 0.006	0.063 ± 0.006
HMF	49.800 ± 0.100	45.117 ± 0.176	42.100 ± 0.100	44.550 ± 0.050	43.700 ± 0.100	41.350 ± 0.050
Free acidity	44.307 ± 0.200	45.077 ± 0.166	38.750 ± 0.250	55.140 ± 0.212	35.100 ± 0.100	36.767 ± 0.252
Lactone acidity	7.800 ± 0,000	7.200 ± 0,000	7.000 ± 0,000	8.500 ± 0,000	6,400 ± 0,000	6.500 ± 0,000
Total acidity	52.107 ± 0.200	52.277 ± 0.166	45.750 ± 0.250	63.640 ± 0.212	41.500 ± 0.100	43.267 ± 0.252
Diastase	14.000 ± 0.000	9.500 ± 0.000	11.000 ± 0.000	13.000 ± 0.000	10.500 ± 0.000	12.500 ± 0.000
Proline	400.000 ± 0.000	350.000 ± 0.000	370.000 ± 0.000	420.000 ± 0.000	310.000 ± 0.000	390.000 ± 0.000
ASH	0.240 ± 0.000	0.180 ± 0.000	0.190 ± 0.000	0.220 ± 0.000	0.160 ± 0.000	0.200 ± 0.000

**Figure 9. Comparison of the different honey samples studied****Figure 10: The radar of the physico-chemical analyzes of the different honey samples.**

These differences among honey types were statistically significant ( $p < 0.05$ ), confirming the influence of floral origin on physicochemical composition.

#### Multivariate analysis and physicochemical variability

Principal Component Analysis (PCA) combined with hierarchical clustering (Ward's method) was used to explore relationships among physicochemical parameters and classify honey samples (Figure 11). The first two principal components explained a substantial proportion of the total variance (PC1 = 46.38 %, PC2 = 28.04 %), indicating a reliable representation of the dataset.



**Figure 11. Combined Principal Component Analysis (PCA) and hierarchical clustering of honey samples.** Physicochemical variables are represented as colored-coded vectors (legend provided for clarity). Ellipses indicate clusters obtained using Ward's hierarchical method. The first two principal components (PC1 and PC2) explain 46.38 % and 28.04 % of the total variance, respectively.

PC1 was mainly driven by mineral-related variables such as electrical conductivity, salinity, total acidity, and proline, which contributed to the differentiation of forest and mountain honeys (e.g., eucalyptus and white heather), in agreement with previous studies on Mediterranean honeys (Amessis-Ouchemoukh *et al.*, 2021; Da Silva *et al.*, 2016; Al-Kafaween *et al.*, 2023). PC2 was associated with quality and freshness indicators, particularly moisture content, HMF, and diastase activity, reflecting storage and environmental effects (Anjos *et al.*, 2015; Machado De-Melo *et al.*, 2018). The PCA score plot revealed a clear separation of honey types according to botanical origin. Monofloral honeys clustered into more homogeneous groups, while multifloral and mountain honeys exhibited greater dispersion, indicating higher variability in floral composition. The clustering analysis confirmed these patterns and supported the robustness of group separation (Boussaid *et al.*, 2018 ; Al-Habsi and Niranjana, 2012).

Strong positive correlations were observed between conductivity and proline, whereas an inverse relationship between HMF and diastase activity confirmed the effect of thermal exposure on honey quality (Amessis-Ouchemoukh *et al.*, 2021; Da Silva *et al.*, 2016).

#### Statistical analysis of physicochemical parameters

One-way ANOVA revealed significant differences among honey types for most physicochemical parameters ( $p < 0.05$ ), including electrical conductivity, total acidity, proline content, moisture, and HMF. These results confirm that botanical origin is the main factor influencing honey composition (Gheldof *et al.*, 2002; Amessis-Ouchemoukh *et al.*, 2021; Boussaid *et al.*, 2018). Electrical conductivity and proline showed the strongest discriminatory power, with significantly higher values in forest and mountain honeys compared to multifloral and citrus honeys. Moisture content and total acidity also varied significantly ( $p < 0.05$ ), while pH showed limited variability and weak discriminating ability (Da Silva *et al.*, 2016; Hamsas El Youbi *et al.*, 2016).

#### Integration and interpretation

The combined PCA and ANOVA results demonstrate that honey variability is primarily structured by botanical origin, followed by environmental and management factors. These findings are statistically supported by significant differences among groups ( $p < 0.05-0.01$ ), confirming a non-random distribution of honey physicochemical profiles and supporting previous reports on Mediterranean honeys (Al-Kafaween *et al.*, 2023; Anjos *et al.*, 2023; Bouddine *et al.*, 2024).

## Conclusion

This study provides an integrated assessment of the ethnomellissological, melissopalynological, ecological, and physicochemical characteristics of honeys from the El Tarf region (northeastern Algeria). The results demonstrate a strong relationship between traditional beekeeping practices, floristic diversity, and seasonal flowering dynamics, which together structure honey composition and quality.

Melissopalynological and physicochemical analyses confirmed the botanical origin and quality of the studied honeys, with significant differences observed among honey types according to their floral origin and environmental conditions. These findings were further supported by multivariate analyses, which clearly separated samples according to their physicochemical profiles. Overall, the honey samples complied with international quality standards, indicating good physicochemical stability and regional specificity. The El Tarf region therefore represents a significant apicultural potential supported by rich biodiversity and traditional knowledge.

To enhance honey quality and market value, improvements in harvesting and storage practices, reinforcement of quality control, prevention of adulteration, and promotion of certified monofloral honeys are recommended. The development of traceability systems and regional branding could further strengthen the competitiveness of Algerian honeys in national and international markets.

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