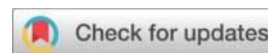




## **Study Of Variations In Honey Types According To Temporal Factors (Years) And Geographical Factors (Regions)**



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### **Abstract**

The present study aims to evaluate the quality of honeys produced in three wilayas of eastern Algeria, namely Souk Ahras, Tébessa and El Tarf, through the analysis of their physicochemical and palynological properties. Over five distinct years, namely 2007, 2008, 2012, 2013 and 2016, samples of honey were collected from local beekeepers, in order to apprehend the spatio-temporal variations likely to influence the composition of the honey. The classification of Botanical families and their associated species according to their frequency of occurrence showed that the Asteraceae family (17.1%) is the most represented, testifying to its diversity and floristic richness. The Fabaceae family (14.3%) is followed by the Lamiaceae, Poaceae, Brassicaceae and Apiaceae which contribute to the floristic composition. The results show that the pollen bouquet of Algerian honey bees is dominated by the Asteraceae-Fabaceae

association, with secondary families that participate in the floristic and apicultural value of the product. The results of the study of the pollen composition reveal a great variability according to the regions and years. The samples are classified mainly in class II and III, with a marked presence of class V in some cases. The contents oscillate between 1 117 and 23 196 grains/g, with 66.7 % in class II, 23.3 % in class III and only 10 % in class I. The pollen content (class I) is low, less than 10 % in some cases. The physicochemical characteristics of the honeys are very variable which depend on the local flora and apicultural factors. The spatio-temporal study of the physicochemical parameters reveals a variability depending on the year of harvest and the geographical area. The results showed that the floristic richness and dominance were positively correlated with pH, density and acidity and negatively with humidity and protein content.

**Keyword:** Honey quality, Physicochemical properties, Palynology, Floristic composition, Spatio-temporal variability

## INTRODUCTION

Algeria possesses abundant melliferous resources, favoring a variety of honeys. According to Oudjet (2012), beekeeping is particularly widespread there in coastal, mountainous areas, high plateaus, maquis and forests. In 2015, national production culminated at 6427 tons of honey (FAO, 2019). It is estimated that Algeria has an area of 471 184 ha dedicated to honey production, with a predominance of forests and maquis which constitute 71% of this potential. The highest yields are observed in citrus cultivation and on natural meadows, each representing 9% of the area suitable for beekeeping, or approximately 44 205 hectares. This area has the capacity to accommodate a vast number of hives, estimated at 221 025, which represents 22% of the national total, thus ensuring remarkable honey production (Chenane, 2000).

Recent research concerning honeys from Algeria has highlighted their exceptional characteristics, including their antimicrobial virtues (Abid, 2017). Significant progress has been made in recent studies concerning honey in Algeria, including melissopalynological and physicochemical analyses (Chefrour, 2007 ; Ouchemoukh et al., 2007 ; Makhoulfi et al., 2010, 2015 ; Zerrouk et al., 2011, 2013, 2014 ; Nair et al., 2013 ; Draiaia et al., 2015 ; Haouam et al., 2016 ; Laouar et Tahar, 2017 ; Bouzebda et al., 2018 ; Necib et al., 2022 ; Ketfi et al., 2023).

The objective of this study is to examine the quality of honeys produced in three eastern wilayas of Algeria (Souk Ahras, Tébessa and El Tarf) by analyzing their physicochemical and palynological properties. Over five specific years (2007, 2008, 2012, 2013 and 2016), honey samples were collected from local beekeepers, which allowed evaluating the spatio-temporal

fluctuations likely to affect the composition of the honey. The aim is to design a comparative analysis profile of honeys based on their geographical location and year of harvest, to better analyze the environmental and apicultural elements that affect their quality. This will also contribute to the valorization of local beekeeping productions.

## MATERIALS AND METHODS

### I. Presentation of study areas

In the framework of this study, a total of 15 honey samples was collected from local beekeepers distributed in three wilayas of eastern Algeria: Souk Ahras, Tébessa and El Tarf (Fig. 1). These samples were collected over a period of five distinct years, namely 2007, 2008, 2012, 2013 and 2016, in order to ensure temporal and geographical representativeness of the apicultural productions of the region. This approach allowed taking into account the interannual and regional variations likely to influence the physicochemical quality and biological characteristics of the studied honeys.

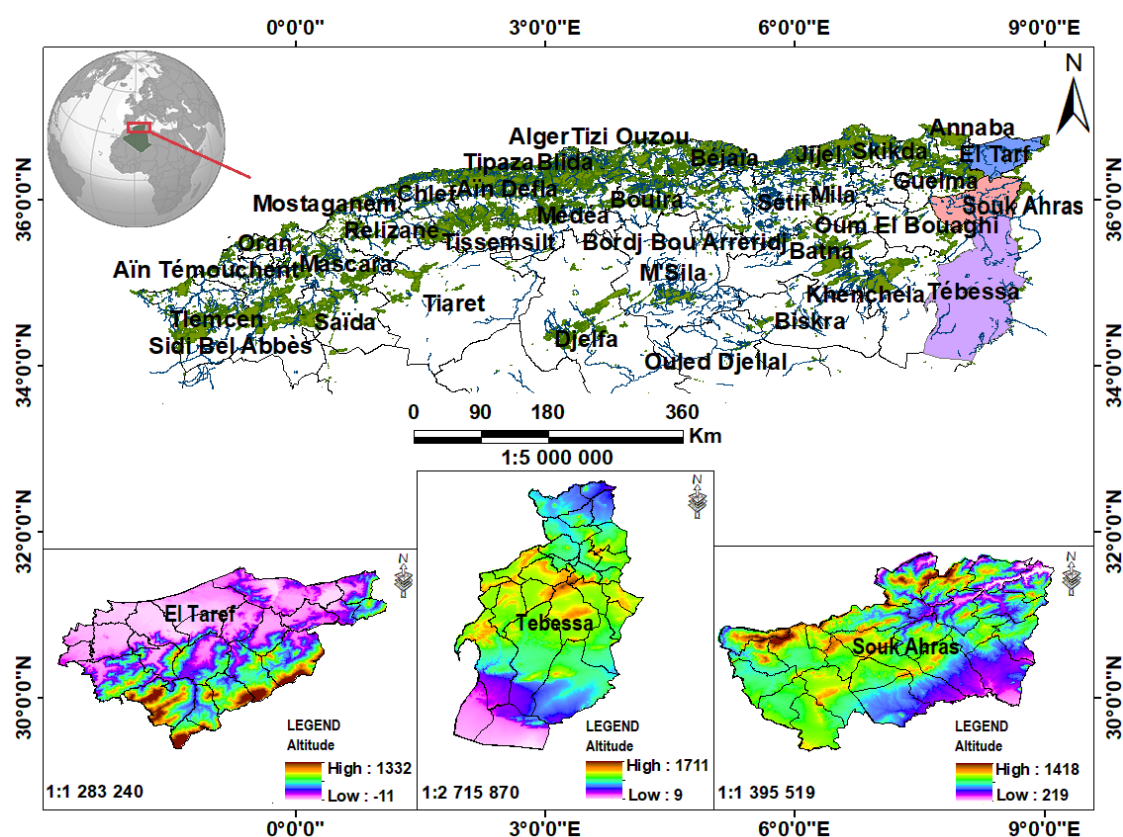


Figure 1. Geographic location of study sites.

### II. Sampling technique

Each sample was carefully conditioned in a clean, dry and hermetically sealed glass vial, then stored at a constant temperature of 4 °C in order to prevent any chemical or biological alteration likely to affect the quality of the honey. These samples were used for all the planned analyses, notably the physicochemical analyses.

### **III. Melissopalynological analysis**

The pollen analysis of the honey was carried out following the method of Layka (1989), integrating both quantitative and qualitative approaches. In order to evaluate the pollen richness, a microscopic slide was made from previously liquefied honey, then examined under an optical microscope with a magnification of 400x. The identification of pollen grains was based on the classifications of Louveaux et al. (1970) and Maurizio (1968), allowing to classify the honeys into five groups according to pollen density (from <2000 to >100 000 grains/g). Finally, the relative frequencies of pollen types were established from the observation of 200 to 300 grains per sample, divided into four classes: very frequent (>45 %), frequent (16–45 %), isolated (3–15 %) and rare (<3 %).

### **IV. Physicochemical parameters**

The physicochemical analyses were carried out according to recognized methods.

- Determination of pH: The measurement of the standard hydrogen potential (pH) was carried out using the method of the Association of Official Analytical Chemists (AOAC, 1990).
- Determination of electrical conductivity: It is measured using an auto-correcting conductimeter (20 °C), according to the method of Vorwohl (1964).
- Determination of acidity: The lactone and total acidity were determined by the titrimetric method (White, 1958).
- Determination of water content: The measurements are carried out with a refractometer at a temperature of 20 °C, the method used is that of Chataway (1932).
- Ash content: It is determined by the method of Williams (1998), which consists of carbonizing a mass ( $M_0 = 2$  g) in a tared platinum capsule. After evaporation of the water, the sample is incinerated at a temperature of 630 °C for 4 hours until white ashes are obtained. After cooling, it is weighed ( $M_1$ ).
- Protein assay: it is carried out according to the method of Bradford (1976), which uses coomassie brilliant blue (BBC) and bovine serum albumin (BSA) at 1 mg/ml as standard, the assay is carried out using a calibration range. The reading of the absorbance ( $D_0$ ) is done at a wavelength of 595 nm against a blank using a spectrophotometer.
- Density: it consists of weighing 5 ml of honey ( $m$ ) and 5 ml of distilled water ( $m_1$ ) and calculating the ratio where  $d=m/m_1$ .

### **V. Statistical analysis**

In order to study the effect of pollen parameters on the physicochemical properties of honey, and to group the samples according to the year of harvest and the wilaya of origin, a multivariate statistical analysis was carried out. The Spearman correlation test was used to identify the

relationships between pollen and physicochemical parameters (pH, conductivity, water content, ash content, density and proteins). Through principal component analysis (PCA), we were able to optimize the visualization of the data and distinguish the differences between the samples. The ascending hierarchical clustering (AHC) was used to classify the honeys according to their similarities, highlighting distinct profiles associated with certain wilayas and years. All analyses were performed with the R software (version 4.4.3).

## **RESULTS AND DISCUSSION**

### **I. Melissopalynological analysis**

#### **I.1. Circular classification of Botanical families and their associated species**

Figure 2 represents a hierarchical classification of Botanical families and their species, based on their frequency of occurrence. Indeed, the Asteraceae family (Astar) is the most represented with 17.1 % of the whole, which reflects its great variety and floristic richness in the examined ecosystem. The Fabaceae family (Fabac), which also represents 14.3 %, follows closely. Families such as Lamiaceae (Lamia), Poaceae (Poac), Brassicaceae (Brass) and Apiaceae (Apiac) also play a significant role, which testifies to their considerable contribution to the floristic composition.

The external segments describe the specific species linked to each family. Each group presents a variety of species, but families like Asteraceae or Fabaceae stand out by their high number of species, testifying to their significant ecological importance (Fig. 2).

These results are in agreement with those reported in the literature. Ghorab et al. (2021) emphasized a preponderance of Fabaceae, Asteraceae, Apiaceae and Lamiaceae, which alone group 36 % of the recorded pollen types. Similarly, Manamani et al. (2021) showed that Asteraceae, Rosaceae and Fabaceae were the most represented families. Makhloufi et al. (2020) have, for their part, identified 124 species dominated by *Papaver rhoeas* (Papaveraceae), *Eucalyptus* spp. (Myrtaceae), *Olea europaea* (Oleaceae), *Carduus* sp. (Asteraceae), *Hedysarum coronarium* (Fabaceae) and *Pimpinella anisum* (Apiaceae). Zerroukh et al. (2020) also reported a strong representation of Fabaceae, Boraginaceae and Asteraceae, while Laouar (2017) listed 38 families and 60 species, with a dominance of Ericaceae, Myrtaceae, Fabaceae and Boraginaceae. Conversely, Ouchemoukh et al. (2016) found a predominance of Myrtaceae, Apiaceae and Ericaceae. Finally, Makhloufi et al. (2010) highlighted the dominance of six main taxa: *Eucalyptus* spp., *Olea europaea*, *Papaver rhoeas*, *Pimpinella anisum*, *Carduus* sp. and *Hedysarum coronarium*.

Overall, these data confirm that the pollen bouquet of Algerian honeys is dominated by the Asteraceae–Fabaceae association, complemented by various secondary families that confer to

the product its floristic typicality and its apicultural value.

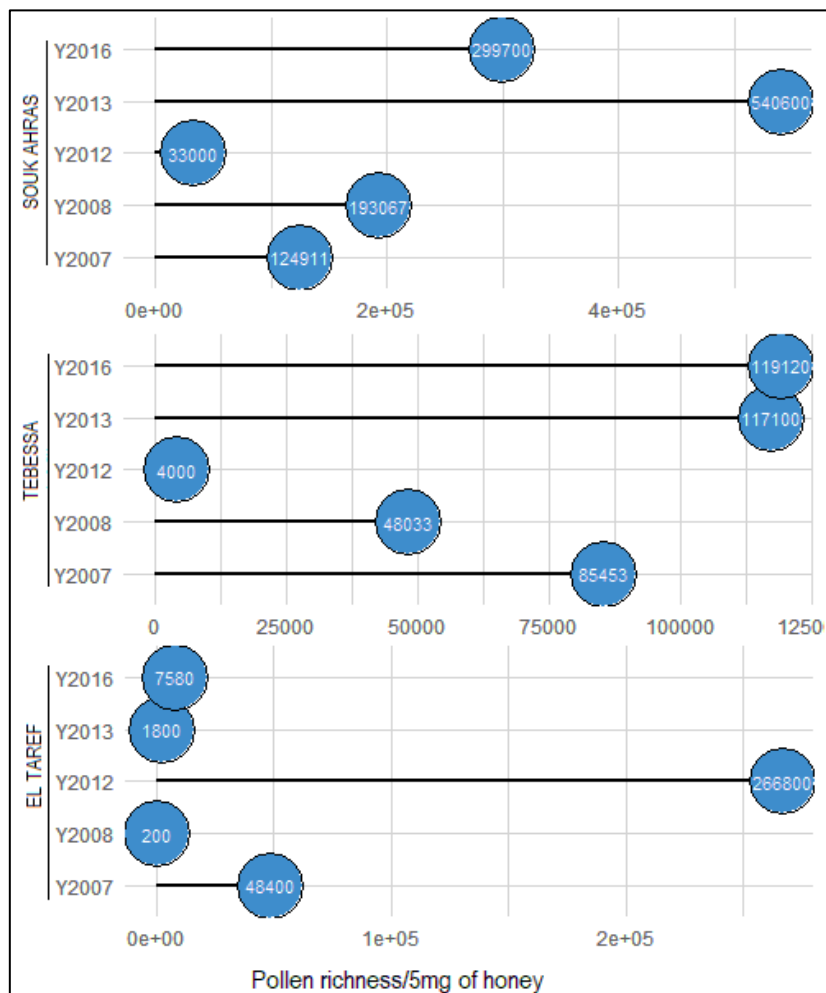


**Figure 2. Circular classification of Botanical families and their associated species according to their frequency of occurrence.**

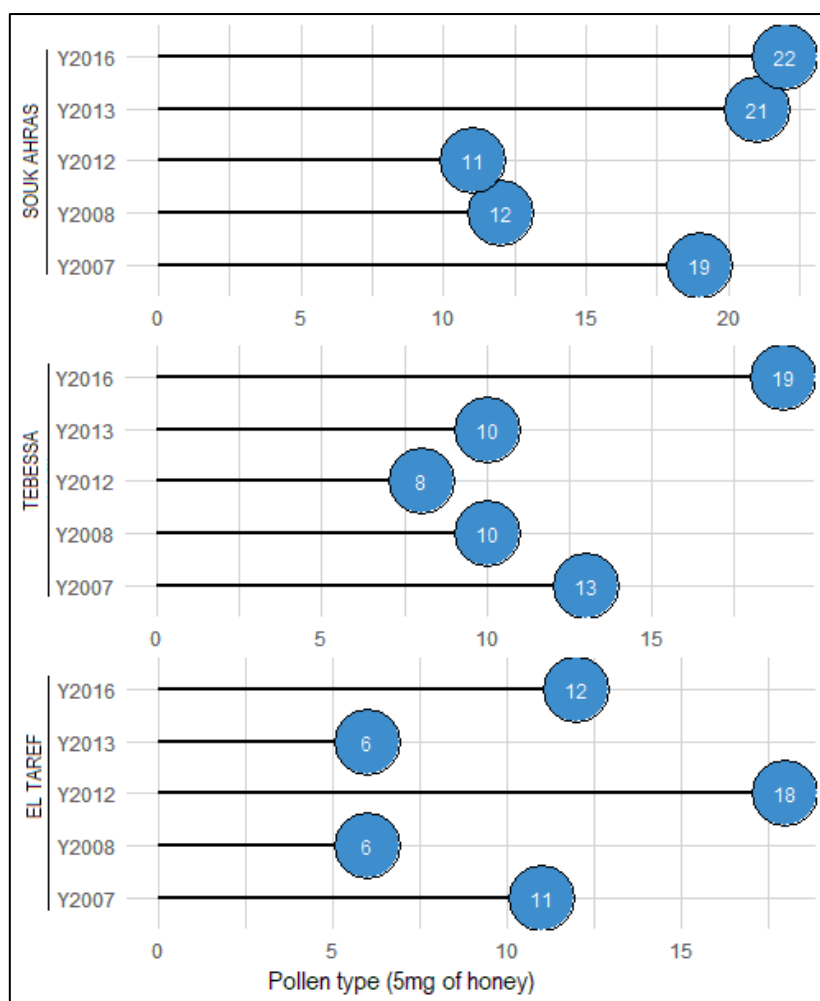
## **I.2. Spatio-annual evolution of pollen parameters of harvested honey**

The examination of the data (5mg of honey for each sample) shows that during the period from 2007 to 2016, the three studied cities present contrasting evolutions of pollen richness and pollen type, in link with the corresponding classes. In Souk-Ahras, a marked growth of pollen richness is observed between 2007 (124 911) and 2008 (193 067), although the pollen type decreases slightly from 19 to 12. In 2012 the drop to 33 000 and a pollen type reduced to 11 translate a passage to class CLIII, then a strong increase appears in 2013 with 540 600 for a pollen type of 21, maintaining the city in class CLV. In 2016, the pollen richness remains high (299 700) with a pollen type of 22, which confirms a stability in class CLV. Tebessa displays, in 2007, a R.P of 85 453 with a pollen type of 13 (CLIV). A decrease is noted in 2008 (48 033 ; pollen type = 10 ; CLIII) and continues until 2012 (4 000 ; pollen type = 8 ; CLII). From 2013, the situation improves markedly with a pollen richness of 117 100 and a pollen type of 10 (CLV), a trend that continues in 2016 (119 120 ; pollen type = 19 ; CLV). Finally, El Tarf presents in 2007 a pollen richness of 48 400 and a pollen type of 11 (CLIII), but experiences a

significant drop in 2008 (200 ; pollen type = 6 ; CLI). A recovery is recorded in 2012 (266 800 ; pollen type = 18 ; CLV), followed by a relapse in 2013 (1 800 ; pollen type = 6 ; CLI). In 2016, a slight rise occurs (7 580 ; pollen type = 12 ; CLII). In general, the highest values of pollen richness and pollen type are associated with classes CLV, while the decreases coincide with the lower classes (CLI to CLIII), illustrating a direct correlation between demographic performance and the assigned classification. (Fig. 3, 4).



**Figure 3. Spatio-temporal evolution of the pollen richness of harvested honey.**



**Figure 4. Spatio-temporal evolution of pollen type of harvested honey.**

All studies devoted to the pollen load of honey highlight a strong variability according to regions and years. Based on the classification of Maurizio (1939), several authors emphasize a predominance of classes II and III, with a strong representation of class V in some cases. Ghorab et al. (2021) observed concentrations between 1 117 and 23 196 grains/g (average: 6 710 grains/g), of which 66.7 % in class II, 23.3 % in class III and only 10 % in class I. Manamani et al. (2021) indicate that 33 % of the samples are in class V, 7 % in classes IV and III, 13 % in class II and 40 % in class I, while Homrani et al. (2020) report that nearly two thirds of the honeys belong to class II and less than a quarter to class III. The low pollen content (class I) remains marginal, with less than 10 % of cases. Similarly, Laouar (2017) notes that 66.66 % of the samples present an average floristic diversity (class II). In Nair et al. (2013), the samples display exclusively classes I (50 %) and II (50 %). Makhoulfi et al. (2010) emphasize for their part that 40.9 % of the analyzed honeys are in class II, 33.3 % in class I, 22.7 % in class III and only 3 % in class V.

On the qualitative level, the works converge towards an important floristic diversity. Ghorab et al. (2021) listed on average 26 pollen types (including 12 samples having more than 30 types).

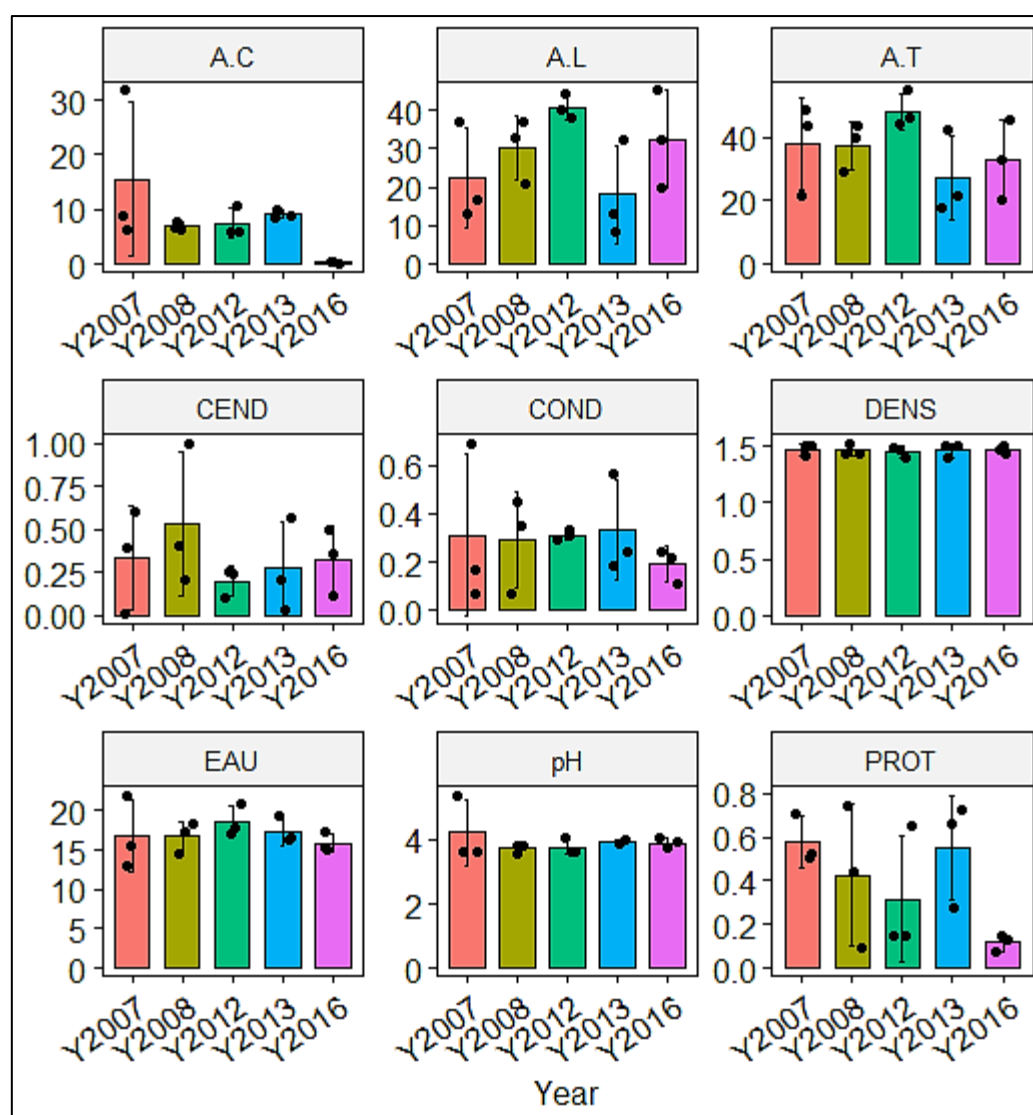
Manamani et al. (2021) identified grains from 36 Botanical families grouping 65 genera and 45 species. Homrani et al. (2020) highlighted 104 types belonging to 51 families, while Laouar (2017) reports a significant diversity in the humid areas of North-East Algeria. According to Nair et al. (2013), 36 taxa were identified ; Makhloufi et al. (2010) report 124 species (9 to 31 species per sample, average: 14). Finally, Ouchemoukh et al. (2016) note a regional variability, with more than 13 pollen types in Tizi-N'berber, Kherrata and Souk-El-Tenine, 11 to 13 types in Oued-Ghir, Amizour and H11, and 6 to 10 types in the five other sites. This diversity depends not only on the local flora, but also on apicultural factors such as the strength of the colony and the mode of extraction (Chefrour, 2007 ; Boutabia, 2016).

## **II. Physicochemical analysis**

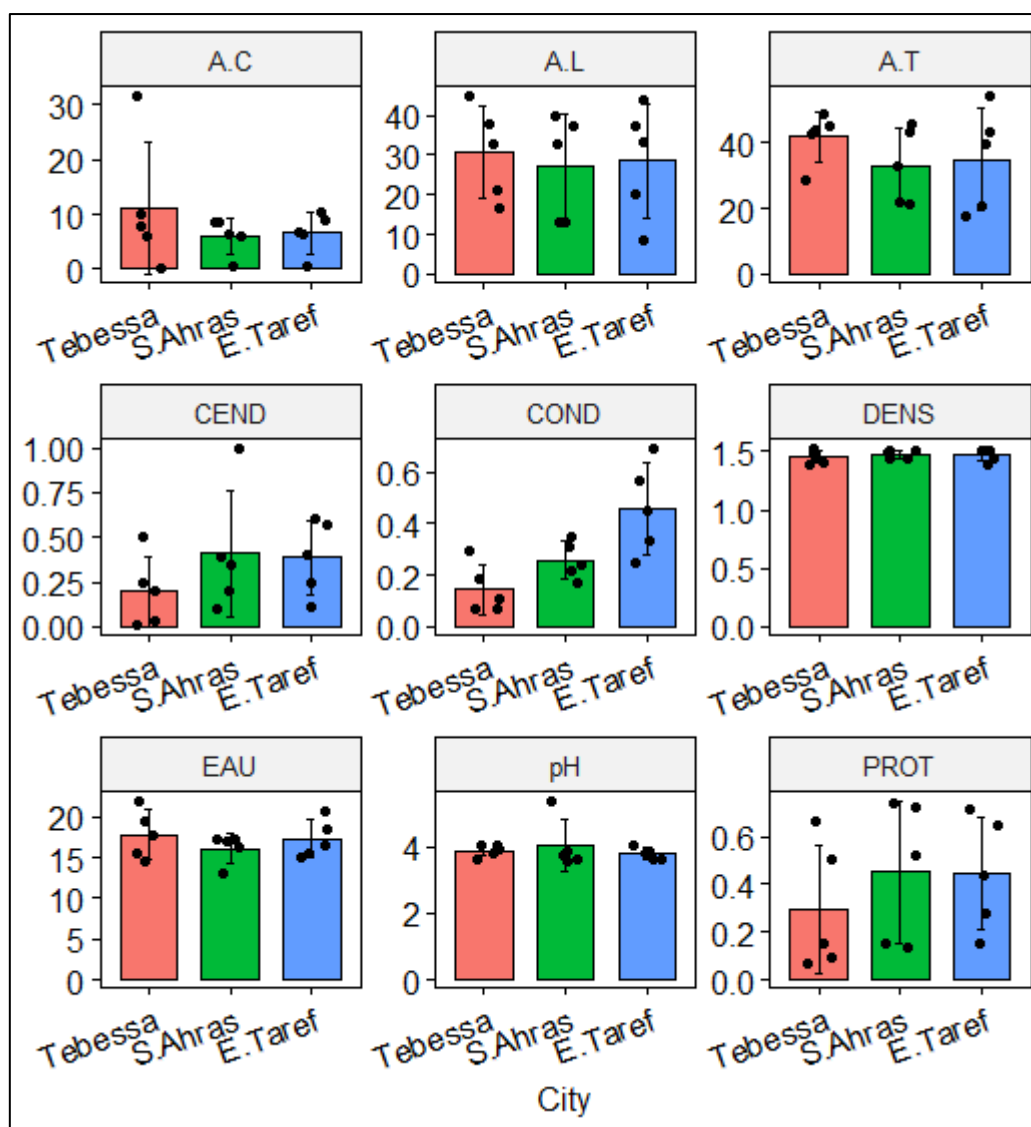
### **II.1. Spatio-annual evolution of physicochemical parameters of harvested honey**

The study of the spatio-temporal variation of the physicochemical characteristics of honey shows that the years 2007, 2008 and 2013 stand out by exceptional values that testify to a notable fluctuation of the quality of the product. In 2007, the honey displays a rather important combined acidity (A.C) (mean: 15.5) and a maximum protein content (mean: 0.58). The year 2008 stands out by a maximum electrical conductivity (mean: 0.38 mS/cm) and a composition in ashes of about (mean: 0.50), signaling an increase in mineral richness. In 2012, a peak of total acidity (A.T) approaching 49 on average is noticed (Fig. 5).

The comparative analysis of the parameters according to the city factor reveals notable differences between Tebessa, Souk Ahras and El Tarf. The combined acidity (A.C) is more marked in Tebessa (11) compared to Souk Ahras and El Tarf (mean: 6–7), while the free acidity (A.L) and total (A.T) remain relatively homogeneous between the three sites (mean: 27–30 and 35–40 respectively). The ash content (CEND) is lower in Tébessa (mean: 0.2) than in the two other localities (mean: 0.3–0.4), while the conductivity (COND) reaches its maximum in El Tarf (mean: 0.45). The density (DENS) remains stable in all regions (mean: 1.3–1.5). The humidity (EAU) varies little, oscillating between 15 and 17 on average, as well as the pH (mean: 3.5–4.2). Finally, the protein content (PROT) is the lowest in Tébessa (mean: 0.29), while Souk Ahras and El Tarf present higher values (mean: 0.44–0.45) (Fig. 5, 6).



**Figure 5. Annual evolution of the physicochemical parameters of harvested honey.**



**Figure 6. Spatial evolution of the physicochemical parameters of harvested honey.**

### III. Statistical study

#### III.1. Correlations of honey parameters by wilayas

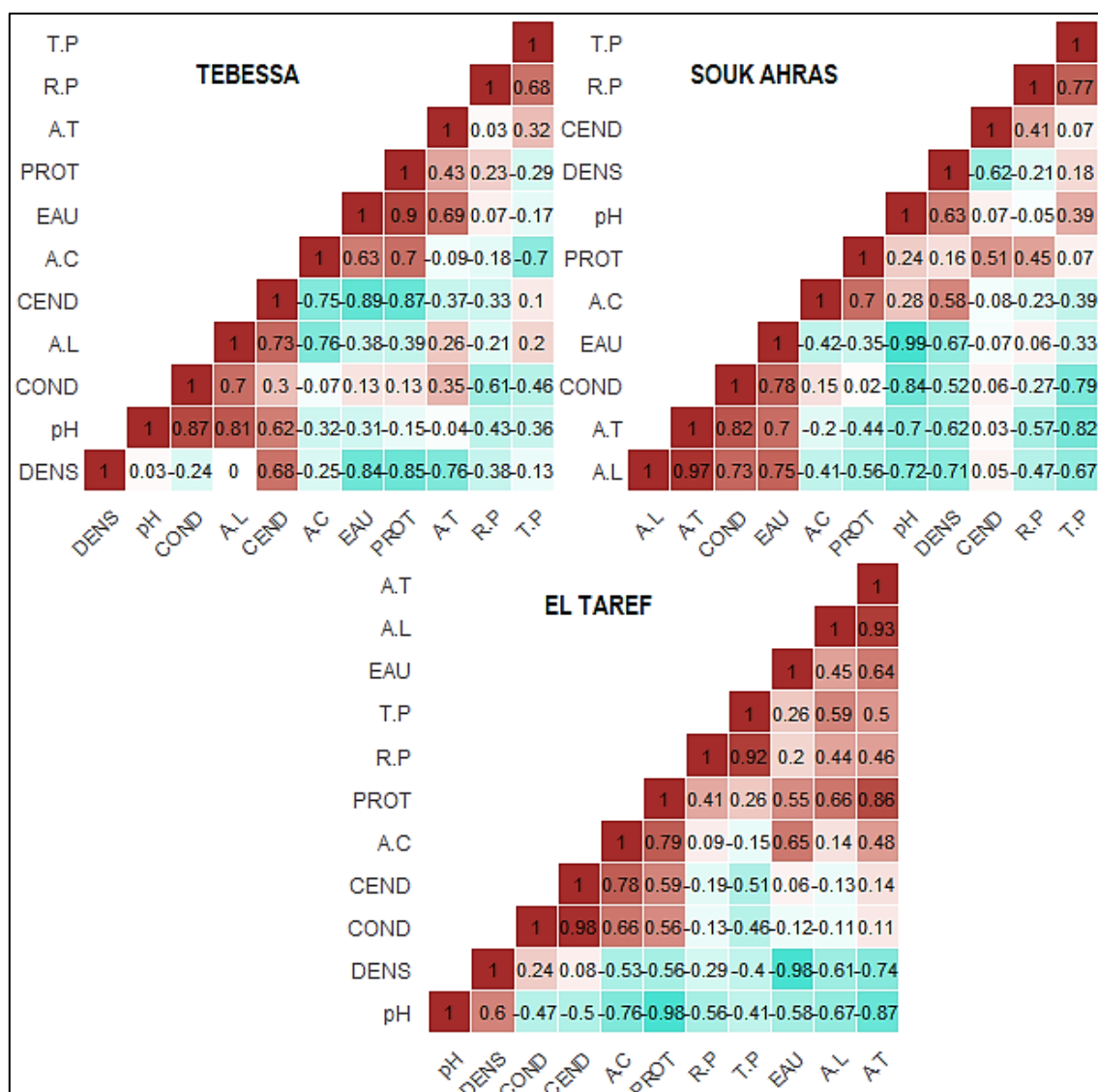
The analysis of the correlogram reveals several significant relationships between pollen and physicochemical variables, which vary according to the wilayas (Fig. 7).

· In Souk Ahras: In this area, the analyses reveal a weak correlation between the pollen and physicochemical properties of honey. There is a positive correlation between pollen richness (R.P) and proteins ( $r = +0.48$ ), conductivity ( $r = +0.44$ ), pH ( $r = +0.42$ ) ; and to a lesser extent with density ( $r = +0.30$ ). These correlations suggest that the honeys of this wilaya that contain the most pollen are also those that contain the most nitrogen compounds, minerals and soluble substances, which enhances their quality. Conversely, pollen richness is negatively correlated with humidity ( $r = -0.38$ ), which means that pollen-rich honeys are more concentrated and less watery. As for the pollen type (T.P), it is very highly correlated negatively with pollen richness ( $r = -0.91$ ). T.P is also negatively correlated with proteins ( $r = -0.31$ ) and conductivity ( $r = -$

0.29), indicating that unifloral honeys are poorer in nutritional elements (Fig. 7).

- In Tébessa: The correlations are weaker but still revealing. Pollen richness shows a positive non-significant correlation with proteins ( $r = +0.35$ ), conductivity ( $r = +0.30$ ), and pH ( $r = +0.28$ ). These results suggest that in this wilaya, even if the flora is more homogeneous, the most diversified honeys remain slightly better in terms of physicochemical quality. Humidity is slightly negatively linked to R.P ( $r = -0.27$ ), translating somewhat more concentrated honeys. As for the pollen type, it is negatively correlated with R.P ( $r = -0.88$ ), but presents very few correlations with the other parameters ( $r$  between  $-0.1$  and  $-0.2$ ), which means that unifloral honeys do not differ markedly from multifloral ones on the chemical level in this region (Fig. 7).

- In El Taref: The honeys of El Taref reveal intermediate correlations between pollen diversity and chemical composition. Pollen richness is positively correlated there with proteins ( $r = +0.40$ ), conductivity ( $r = +0.33$ ) and pH ( $r = +0.36$ ). This shows that multifloral honeys are richer in nutrients and minerals. Humidity is weakly negatively linked to R.P ( $r = -0.29$ ), which reinforces the idea that more pollen = less water. The pollen type is for its part slightly positively correlated with humidity ( $r = +0.25$ ), and negatively with conductivity ( $r = -0.30$ ), indicating that unifloral honeys are overall more watery and less mineralized in this wilaya (Fig. 7).



**Figure 7. Comparative analysis of Spearman correlations between the physicochemical and biochemical parameters of honey in the wilayas of Tébéssa, Souk Ahras and El Taref.**

### III.2. Principal component analysis (PCA)

The principal component analysis (PCA) was carried out to explore the relationship between the pollen parameters T.P (pollen type) and R.P (pollen richness) and the physicochemical parameters of honey, as well as to characterize the year and wilaya factors. The first two dimensions of the PCA explain nearly 100 % of the total variance (Dim 1 = 89.5 %, Dim 2 = 10.5 %), which shows an excellent quality of representation of the data in the factorial plane (Fig. 8). Indeed,

The PCA highlights a strong influence of pollen parameters on the distribution of samples according to physicochemical characteristics. On the graph, these two pollen parameters are projected positively and very closely on axis 1 (Dim 1), which explains 89.5 % of the total variance. This strong contribution means that floristic diversity and dominance greatly influence the structuring of honeys.

In contrast, the vectors of physicochemical parameters (in blue) show varied correlations with these two axes:

- The pH, density, as well as free and total acidity are moderately positively correlated to pollen richness.
- Conversely, humidity (EAU) and proteins (PROT) are projected in opposition to the R.P vector, indicating a negative correlation. In other words, a greater abundance of pollens seems linked to a reduced content in water and proteins.
- It seems that conductivity (COND) and ash content (CEND) are little connected to pollen parameters, which indicates that they are undoubtedly influenced by other environmental elements, such as soil quality or conservation conditions.

To synthesize, diversity and pollen character have a notable impact on certain physicochemical characteristics of honey, such as pH, density and humidity. This testifies to a close relationship between floral origin and technological quality of honey (Fig. 8, A).

The in-depth analysis of the PCA shows a significant variation of honey samples both according to the year of harvest (interannual variation) and according to the geographical region of origin (intersite variation), from physicochemical parameters (pH, humidity, conductivity, proteins, etc.) and pollen (richness and pollen type) (Fig. 8, B-C).

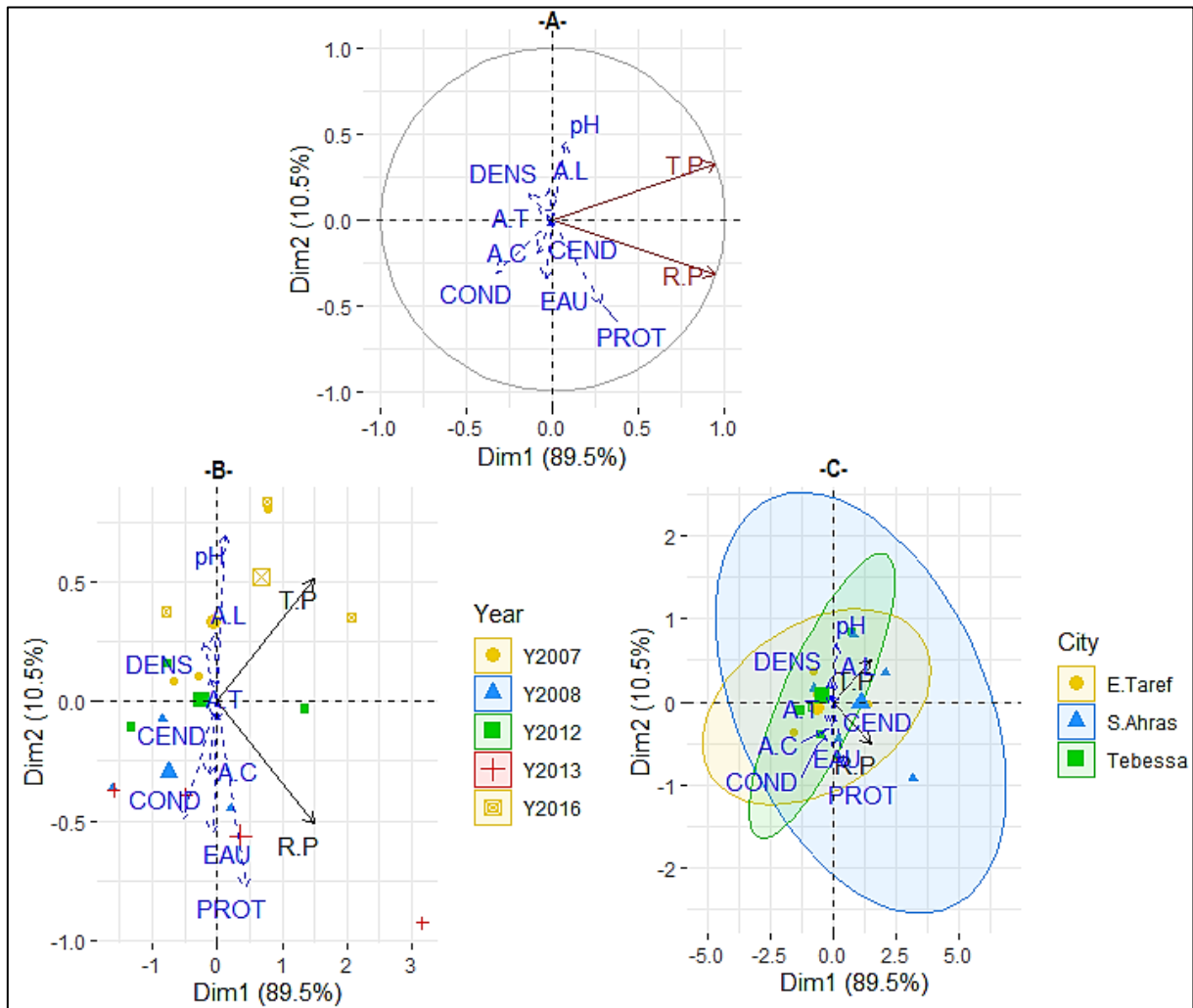
On the interannual level, the results indicate that the year 2016 stands out by a great diversity of pollens, reflecting climatic and floristic conditions conducive to an extended flowering. However, the honeys of the years 2007, 2008 and 2012 stand out by constant physicochemical properties (moderate pH, adequate density, balanced acidity), although they have a less important pollen content. The year 2013 stands out markedly by a specific profile: its sample is isolated in the factorial space, due to extreme values for certain parameters, which could reflect a punctual environmental change or a particular dominant flora that year (Fig. 8, B).

The Principal Component Analysis (PCA) allows differentiating the three analysis zones, based on the intersite variation. The region of Souk Ahras shows an extended distribution of samples, testifying to an important floristic and ecological diversity. This variety has a direct impact on the composition of honey, both from a pollen and chemical point of view.

In contrast, Tébéssa stands out by a pronounced homogeneity, reflecting environmental stability and a more uniform flora over the years. Finally, El Taref occupies an intermediate but specific position in the factorial space, suggesting a typical local flora (potentially of coastal or humid origin), which influences the pollen and chemical composition of the produced honey (Fig. 8, C).

Thus, the PCA clearly demonstrates that the quality, floristic richness and physicochemical

properties of honey vary according to the year and geographical location, confirming that these two factors play a determining role in the typicality and value of the final product (Fig. 8).



**Figure 8. Principal component analysis; A: Correlation circle of pollen and physicochemical parameters. B: Factorial plane (Dim 1 vs Dim2) according to the "Year" factor. C: Factorial plane (Dim 1 vs Dim2) according to the "Wilaya" factor.**

### III.3. Hierarchical classification (AHC)

The factorial map below, resulting from a principal component analysis (PCA) coupled with an ascending hierarchical classification (AHC), allows visualizing the structuring of honey samples according to their physicochemical and pollen characteristics over several years and locations (Fig. 9).

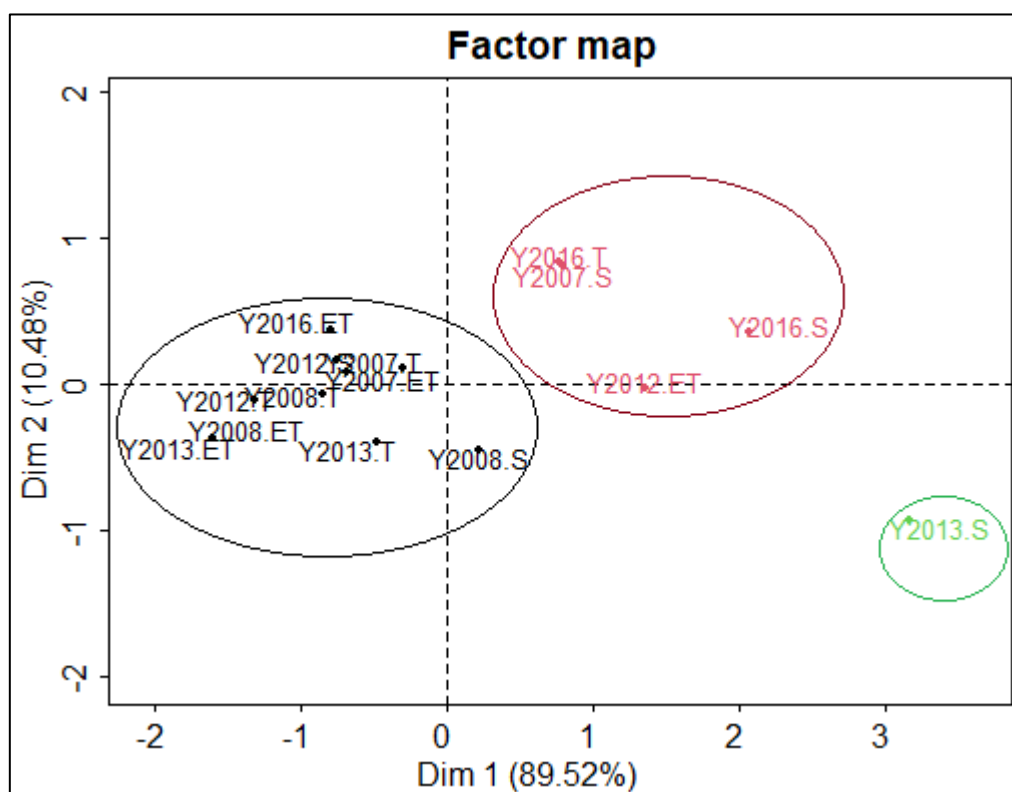
Two main axes (Dim 1 = 89.52 % and Dim 2 = 10.48 %) explain nearly 100 % of the total variance, which attests to a very good quality of projection of the data on this plane. Indeed, three distinct groups of samples emerge:

Group 1: It groups the majority of samples, notably those of the years 2007, 2008, 2012, 2013 and 2016, coming from the three wilayas. These honeys present a homogeneity in their

physicochemical characteristics (pH, conductivity, humidity, etc.) and pollen (type and richness), suggesting a stability of environmental and floristic conditions during these years for these sites (Fig. 9).

Group 2: Mainly constituted of samples Y2007.S, Y2012.ET, Y2016.S and Y2016.T, this group is characterized by a pollen or physicochemical particularity, probably linked to a specific flowering or particular meteorological conditions that influenced the composition of honey during these years (Fig. 9).

Group 3: This distinct group is only represented by the sample Y2013.S. It highlights the remarkable specificity of this honey, which stands out markedly from the others thanks to its analytical properties. This could be due to an exceptional floral dominance or particular harvest or storage conditions having influenced its properties (Fig. 9).



**Figure 9. Factorial map resulting from the ascending hierarchical classification concerning the spatio-annual evolution of harvested honey by physicochemical and pollen parameters.**

The spatio-temporal examination of the physicochemical parameters of honey highlights a marked variability according to the years and studied localities. In 2007, the combined acidity reaches 15.5, accompanied by a relatively high protein content (0.58), while in 2008 the electrical conductivity (0.38) and the ash content (0.50) translate a more important mineral richness. The year 2012 stands out by a peak of total acidity (49), underlining notable fluctuations of the composition. The geographical analysis confirms these differences: in

Tébessa, the combined acidity is more marked (11), but the ash contents (0.2) and proteins (0.29) remain inferior to those observed in Souk Ahras and El Tarf (0.3–0.4 and 0.44–0.45 respectively). In El Tarf, the conductivity reaches its maximum value (0.45), while the free acidity (27–30), total acidity (35–40), density (1.3–1.5), humidity (15–17) and pH (3.5–4.2) appear globally stable between the sites. The results of Ghorab et al. (2021) confirm, similarly concerning humidity (16.8–19.6) and pH (3.6 - 4.4). However, the conductivity measured by Ghorab (0.57–1.35 mS/cm) is markedly higher, translating a richer mineral composition. The free acidity, oscillating between 15.5 and 39 meq/kg, approaches the values of 27–30 observed in El Tarf, confirming a global coherence despite different amplitudes. In comparison, Manamani et al. (2021) present ranges of values very close. The pH (3.54–4.09) corresponds closely to the values of 3.5–4.2, and the conductivity (0.186–0.639 mS/cm) joins the 0.38–0.45 reported in 2008 and in El Tarf. The protein content (0.27–0.76 %) is comparable to the 0.29 measured in Tébéssa and to the 0.44–0.45 noted in El Tarf and Souk Ahras. Finally, the total acidity (15.14–54.54 meq/kg) overlaps the peak of 49 identified in 2012, confirming a significant temporal variability. However, the works of Guerzou et al. (2021) also corroborate our data. The humidity (14.03–18.80 %) remains coherent with that observed in El Tarf, and the pH (3.50–4.50) corresponds to the range of 3.5–4.2. The conductivity (0.038–0.641 mS/cm) joins the values reported in 2008 and in El Tarf, while the free acidity (11–47 meq/kg) encompasses the results of 27–30 meq/kg reported locally. The ash contents (0.06–0.48 %) also align with the 0.20–0.50 measured in the different localities of our work. Dahmani et al. (2020), reveal a lower humidity in arid zones (15.8 %) than in humid regions (16.9 %), as well as a more acidic pH in the latter (3.06). The two studies agree on the role of climate, directly or indirectly, in the regulation of key parameters such as humidity, pH and free acidity, while showing that the mineral and protein composition can also vary according to the year and geographical location. In Homrani et al. (2020), the spatial variability is particularly marked, joining the spatio-temporal observations of our results. Humidity reaches 22.5 in El Tarf, translating a direct climatic influence. Similarly, conductivity varies from 0.133 to 1.460 mS/cm, a much wider amplitude than that described in our results, illustrating differences in mineral richness according to sites. The pH (3.5–4.7) remains however in agreement with the data reported in El Tarf. The results of Laouar and Tahar (2017) highlight a notable variability of the physicochemical parameters of honey with a humidity between 14.47 and 23 g/100 g ; the pH varies from 3.15 to 4.50 ; the ash content between 0.02 to 0.53 g/100 g ; the electrical conductivity between  $1 \times 10^{-4}$  and  $3 \times 10^{-4}$  S/cm ; the free, lactic and total acidity are between 10.16 and 28.03 meq/kg, 2.38 and 8.55 meq/kg and 15.46 and 34.27 meq/kg, respectively ; the

protein varies between 0.11 and 2.85 mg/g ; the density varies between 1.32 and 1.55. The observations of Makhoulfi et al. (2017) join our data by emphasizing a stable humidity (16.5–16.8 % against 15–17 % in El Tarf) and a moderate pH (3.4–4.2, similar to 3.5–4.2). In contrast, the higher conductivity in Eucalyptus (0.60 mS/cm) contrasts with the maximum value of 0.45 observed by us, indicating a more marked mineral composition. Similarly, Laredj et al. (2017) report a humidity of 16–17 % and a pH of 3.4–4.2, perfectly coherent with our observations. The higher conductivity of Eucalyptus described by Laredj et al. (2017) contrasts however with the maximum values of 0.45 reported by us, confirming a floristic and regional variability. Nair et al. (2017) highlight an even wider variability, whose humidity (14.21–16.59) is in the same range as that reported in El Tarf (15–17), but the conductivity (10–606  $\mu$ S/cm, i.e. 0.010–0.606 mS/cm) covers a more extended spectrum. The pH (3.7–4.78) slightly exceeds the range 3.5–4.2 observed by us, and the total acidity (12–30 meq/kg) remains inferior to the peak of 49 noted in 2012, confirming the importance of spatio-temporal factors. However, Ouchemoukh et al. (2007) report that the analyzed samples stand out by a low water content, which puts them safe from a risk of fermentation. Among them, the honey of Oued-Des stands out particularly, by presenting the most representative attributes of melliferous honeys, characterized by a relatively high pH and a strong concentration in ashes.

The analyzed samples revealed a strong heterogeneity both on the physicochemical and pollen level. In order to synthesize this variability and to draw the main trends, a principal component analysis (PCA) was carried out. The results indicate that pollen parameters constitute a major factor in the differentiation of honeys. Axis 1 explaining 89.5 % of the variance. Floristic richness and dominance are positively associated with pH, density and acidity, while they are negatively correlated with humidity and proteins. In contrast, conductivity and ashes seem little linked to pollen parameters, suggesting the action of other environmental factors. These results confirm the importance of floral origin in the physicochemical quality of honeys. These results join those of Makhoulfi et al. (2017), who emphasize that the pH of honeys from the south differs significantly from that of other regions (ANOVA:  $F = 12.410$  ;  $P ; 0.001$ ), while no notable difference was noted for the other parameters. This singularity could be attributed to steppe and pre-Saharan soils, often rocky and alkaline (Nedjraoui, 2001), although a secondary role of the flora is not excluded, some samples resulting from both floral nectar and honeydew. Similarly, Ouchemoukh et al. (2007) show that physicochemical characteristics allow distinguishing the Botanical origin, whether floral or melliferous.

Other studies support these relationships: Laouar and Tahar (2017) report a positive correlation between total acidity and free acidity, between lactonic acidity and water content, as well as

between proteins and ashes. Homrani et al. (2020), through PCA, highlight that pH, conductivity and humidity are closely correlated and depend on the Botanical origin. In the same logic, Dahmani et al. (2020) observe a positive relationship between water and free acidity, as well as between conductivity and pH, confirming the floral influence on humidity and pH. Finally, Ghorab et al. (2021) report a joint correlation between pH, conductivity, humidity and acidity, reinforcing the idea that these parameters are directly modulated by the Botanical origin.

## CONCLUSION

The results of this study provide a better understanding of the influence of floristic, geographical and climatic factors on the quality of Algerian honey. The combined approach between pollen analysis, physicochemical parameters and multivariate statistical methods allowed identifying the main determinants of honey variability according to the year of harvest and the wilaya of origin.

This work showed a strong variability of the pollen and physicochemical characteristics of honey according to the year of harvest and the wilaya of origin. Floristic richness is dominated by the Asteraceae and Fabaceae families, especially in the region of Souk Ahras. For pollen richness significantly influences certain physicochemical parameters like pH, density and humidity.

The ascending hierarchical classification (AHC) allowed grouping the honeys into homogeneous classes, confirming the joint influence of environmental and floristic factors.

The year 2013 and the wilaya of Souk Ahras stand out by specific physicochemical and pollen profiles.

These results underline the importance of an integrated geo-temporal approach for the traceability, valorization and standardization of Algerian honey.

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