

Assessment of the quality of honey of various botanical and geographical origins based on the pollen spectrum and physico-chemical properties

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Summary

Authenticity of honey presents significant challenges to food quality control, necessitating continuous modernization and enhancement of analytical methodologies. This study aimed to characterize 24 honey samples collected from eight regions in northeastern Algeria by analysing their physico-chemical parameters and pollen profiles. The results revealed significant pollen diversity across all samples, predominantly monofloral honeys, particularly eucalyptus honey, along with *Hedysarum coronarium* L., *Arbutus unedo* L., *Lavandula stoechas* L., *Ziziphus lotus* (L.) Lam. and *Citrus* sp. Multifloral honeys contained pollen from diverse taxa including *Eucalyptus camaldulensis* Dehnh., *Hedysarum coronarium* L., *Echium plantagineum* L., *Lavandula stoechas* L., *Raphanus raphanistrum* L. and *Malva sylvestris* L., common to northeastern Algeria's ecosystems. Most honey samples met international physico-chemical standards, indicating high quality. However, honey quality is predominantly influenced by its botanical origin, as demonstrated by principal component analysis, cluster analysis and co-inertia analysis, which grouped the samples into seven distinct physico-chemical units. Precise characterization is essential for enhancing local honey production by elucidating the complex relationships between pollen composition, botanical origin and physico-chemical properties.

Keywords

honey; melissopalynology; physico-chemical property; quality; geographical provenance; northeastern Algeria

Honey is a natural sweet substance produced from the nectar and secretions of plants and the excretions of plant-sucking insects that honeybees (*Apis mellifera* L.) collect and mix with their excretions [1]. It is the oldest sweetener substance consumed by humans and it has proven to have beneficial effects on health [2].

The quality of honey is determined by its composition, which is also related to the botanical origin of the nectar and honeydew, as well as to climate, environmental conditions, and beekeeping practices [3]. Therefore, it is strictly related to the geographical origin of the honey and, because

of that, information on the physico-chemical parameters and the botanical origin of honey from various regions are usually demanded by consumers [4]. In order to increase the commercial value of honey, beekeepers generally give it a particular name, referring to the specificities linked to its botanical and/or geographical origin. This is the case of monofloral honeys, which mainly come from a single botanical origin and are perceived by consumers as high-quality honeys with distinct individual characteristics [5, 6].

Algeria boasts a rich diversity of vegetation, encompassing over 3 152 species of spermatophyte

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plants [7]. This abundance of flora lends itself to the production of a wide array of exquisite honeys, both polyfloral and monofloral. In the north of the country, where the typical Mediterranean climate occurs, various types of monofloral honey such as the honeys of *Eucalyptus* sp., *Hedysarum coronarium* L., *Echium plantagineum* L., *Erica* sp., *Myrtus communis* L., *Rubus* sp., *Capparis* sp. or *Erica arborea* L. [7, 8] are found. On the other hand in the west of the country, *Thymus munbyanus* Boiss. & Reut., *Citrus* sp., *Eucalyptus* sp., *Lavandula angustifolia* Mill., *Lavandula stoechas* L. and *Hedysarum coronarium* L. can be found. In the south of the country, honey from *Ziziphus lotus* (L.) Lam., *Peganum harmala* L. and *Euphorbia* sp. [9, 10] can be found. In addition, the Algerian market boasts a variety of honeys beyond the commonly known types. These include those from *Thymus* sp., *Citrus* sp., *Lavandula stoechas*, *Eucalyptus* sp., *Acacia* sp. and others.

In recent years, several fragmental studies about the composition of Algerian honeys were carried out [7–11]. However, the pollen spectrum of some Algerian honeys remains unknown. The contribution aims to improve information on the diversity and quality of honeys, which will increase interest in local products and allow for the establishment of denomination of origin according to their type.

Thus, the aim of this study was a) characterize

the honeys of certain regions of the north-east of Algeria according to their botanical origin, their geographical origin and their physico-chemical profile, and b) highlight the link between pollen composition and the physico-chemical quality of honey.

MATERIALS AND METHODS

Characteristics of the geographic origin of honey

Twenty-four honey samples were collected from 8 wilayas (departments; Fig. 1). The samples were obtained by beekeepers from various regions of northeastern Algeria during the period 2016–2020. An initial appellation was assigned to each sample of honey based on the knowledge of beekeepers (Tab. 1). Half of the samples came from 4 neighbouring wilayas of the small Kabylia-Numidia subsector (K2 and K3 according to the subdivision proposed by QUÉZEL and SANTA [12], while the second half came from the wilayas of the Constantine Tell “C1” (Guelma, Souk Ahras, Constantine and Mila). The samples were kept in airtight glass containers in refrigerator at 4–5 °C for one week. For each sampled locality, the following variables were considered (Tab. 1): geographic coordinates, altitude, average annual maximum and minimum temperatures, average annual total rainfall and type of vegetation. According to Tab. 1,

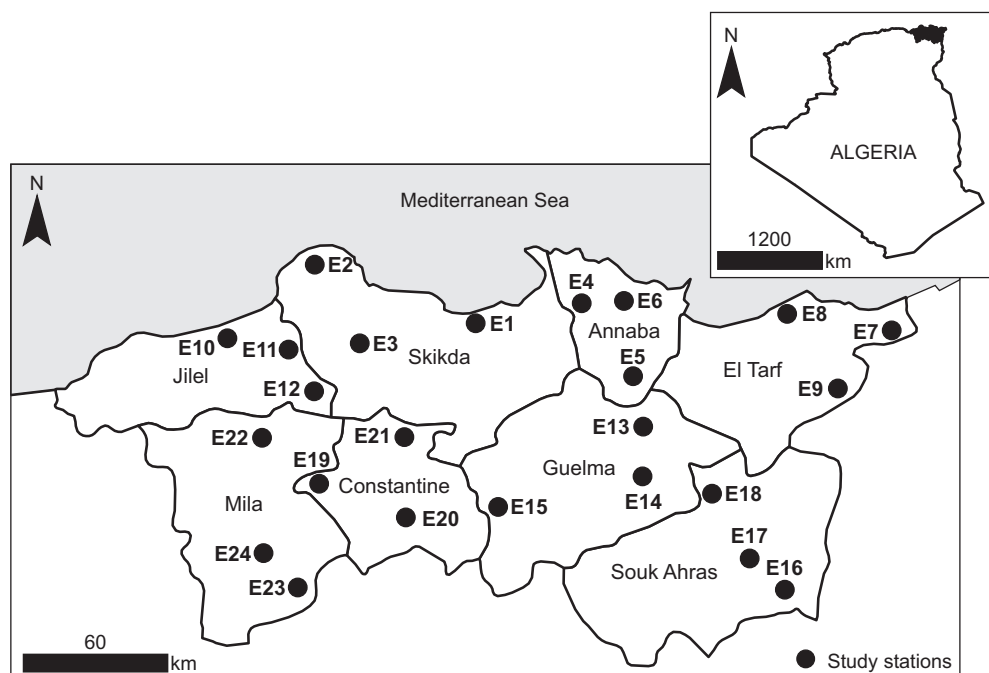


Fig. 1. Geographical location of the honey samples collected.

E1–E24 – honey samples (specified in Tab. 1).

Tab. 1. Characteristics of the geographic origin of the examined honey samples.

Code	Production region	Coordinates	Altitude [m]	T _{max} [°C]	T _{min} [°C]	Pp [mm]	Type of vegetation	Time of collection
E1	Fifila	36°51'01"N 7°13'30"E	59	21.50	14.33	440.30	Oleo-lentisk maquis	Summer 2019
E2	Kanoua	37°3'42"N 6°23'18"E	578	21.58	15.42	411.40	Oleo-lentisk maquis	Summer 2017
E3	Tamalous	36°51'10"N 6°37'28"E	55	21.50	14.33	440.30	Oleo-lentisk maquis	Spring 2018
E4	Treat	36°50'45"N 7°31'13"E	94	22.33	13.67	452.90	Oleo-lentisk maquis	Summer 2020
E5	Ain Berda	36°54'56"N 7°45'54"E	13	22.33	13.67	452.90	<i>Eucalyptus</i> forest and public garden	Summer 2019
E6	Ez-Reiba	36°55'45"N 7°40'44"E	540	22.33	13.67	452.90	<i>Quercus suber</i> forest	Summer 2019
E7	El Aloun	36°50'51"N 8°38'10"E	378	21.42	10.00	312.50	<i>Quercus suber</i> forest	Spring 2020
E8	El Bellah	36°53'58"N 8°8'30"E	47	22.83	13.83	170.60	<i>Quercus coccifera</i> forest	Summer 2017
E9	Bougous	36°42'22"N 8°23'59"E	209	22.83	13.83	499.40	Mixed forest of <i>Quercus suber</i> and <i>Eucalyptus</i>	Summer 2020
E10	Sidi Abdelaziz	36°51'43"N 6°4'22"E	163	21.83	14.67	476.30	Oleo-lentisk maquis	Spring 2019
E11	Settara	36°42'41"N 6°21'17"E	359	21.83	14.67	476.30	<i>Eucalyptus</i> forest	Summer 2019
E12	Sidi Maarouf	36°38'23"N 6°13'25"E	482	21.83	14.67	476.30	<i>Quercus suber</i> forest	Summer 2020
E13	Oued Fragha	36°32'06"N 7°42'34"E	80	23.58	11.33	581.60	Oleo-lentisk maquis	Summer 2017
E14	Hammam N'Badis	36°21'45"N 7°40'43"E	616	23.58	11.33	581.60	Oleo-lentisk maquis	Spring 2021
E15	Oued Zenati	36°19'8"N 7°7'47"E	790	23.58	11.33	581.60	<i>Eucalyptus</i> forest	Summer 2016
E16	Taoura	36°2'55"N 8°9'36"E	618	21.42	9.25	539.30	Steppe with <i>Ziziphus lotus</i>	Summer 2019
E17	Oued Medjerda	36°22'27"N 8°21'58"E	733	21.42	9.25	539.30	<i>Pinus halepensis</i> forest	Summer 2020
E18	Mechroha	36°21'51"N 7°50'45"E	715	21.42	9.25	539.30	<i>Quercus suber</i> forest	Summer 2020
E19	Ibn Ziad	36°19'47"N 6°26'05"E	887	22.08	10.50	503.10	<i>Eucalyptus</i> forest	Autumn 2019
E20	El Khroub	36°16'23"N 6°44'34"E	618	22.25	10.25	483.90	<i>Pinus pinaster</i> forest and <i>Eucalyptus</i> forest	Summer 2020
E21	El Kantour	36°34'24"N 6°45'01"E	521	22.08	10.50	503.10	Mixed forest of <i>Quercus suber</i> and <i>Eucalyptus</i>	Summer 2018
E22	Béni Haroun	36°34'35"N 6°16'46"E	232	22.17	11.00	524.00	<i>Pinus halepensis</i> forest	Spring 2020
E23	Teleghma	36°7'54"N 6°21'59"E	755	21.50	9.42	448.50	<i>Eucalyptus</i> forest	Summer 2018
E24	El Mechira	36°0'48"N 6°11'26"E	863	22.17	11.00	524.00	<i>Pinus halepensis</i> forest	Spring 2019

the types of vegetation were classified into nine classes:

1. Oleo-lentisk maquis;
2. *Eucalyptus* forest and public garden;
3. *Quercus suber* forest;
4. *Quercus coccifera* forest;
5. *Eucalyptus* forest;
6. Steppe with *Ziziphus lotus*;
7. *Pinus halepensis* forest;
8. *Pinus pinaster* forest and *Eucalyptus* forest;
9. mixed forest of *Quercus suber* and *Eucalyptus*.

Melissopalynological analysis

Extraction and analysis of pollen spectra were accomplished by using the methodology proposed by the International Commission for Plant-Pollinator Relationships, described by LOUVEAUX et al. [13]. Pollen was identified with the aid of an optical microscope Leica DM750 (Leica Microsystems, Wetzlar, Germany) at 400× and 600× magnification with the use of local pollen atlases and specialized publications [14, 15].

Quantitative analysis of pollen

Based on total amount of pollen in 10 g of honey, the richness in pollen grains was classified into five classes of frequencies (I–V) [13, 14]:

- class I – < 20 000 grains in 10 g of honey (honey poor in pollen),
- class II – 20 000–100 000 grains in 10 g of honey (honey moderately rich in pollen),
- class III – 100 000–500 000 grains in 10 g of honey (honey rich in pollen),
- class IV – 500 000–1 000 000 grains in 10 g of honey (honey very rich in pollen),
- class V – > 1 000 000 grains in 10 g of honey (honey extremely rich in pollen).

Qualitative analysis of pollen

The percentage of pollen types in each honey sample was determined from the total number of different types of pollen grains counted in each sample. The pollen types present in the honey samples were identified, counted and classified, according to their frequency classes as follows: dominant pollen ($\geq 45\%$), secondary pollen (15–45 %), important minor pollen (3–15 %), minor pollen (1–3 %) and present pollen ($< 1\%$) [12].

Electrical conductivity

Electrical conductivity (EC) of honey was measured with portable conductivity meter HI 99300 (Hanna Instruments, Woonsocket, Rhode Island, USA) on a sample of 20 g of honey (dry matter) in 100 ml of distilled water at 20 °C

according to the AOAC method No. 981.121 [16]. Results were expressed in millisiemens per centimeter, according to the unit of measurement in line with the measurement standards recommended by the harmonized methods endorsed by the European Honey Commission [17].

pH and free acidity

The pH measurement was conducted on a solution comprising 10 g of honey dissolved in 75 ml of distilled water. Free acidity (FA) was determined by the AOAC method No. 962.19 [16] by plotting the neutralization curve with NaOH solution and by determining the acidity (pH) of the equivalence point (pHe). Free acidity was expressed in milliequivalent of acid per kilogram of honey.

Hydroxymethylfurfural

The content of hydroxymethylfurfural (HMF) was determined by high-performance liquid chromatography (HPLC) [17]. Briefly, the honey samples (10 g each) were diluted to 50 ml with distilled water, filtered using a 0.45 μm pore size nylon membrane filter and injected into an HPLC system Waters 2695 (Waters, Milford, Massachusetts, USA) equipped with a photodiode array detector Waters 2996 (Waters) and treating the samples with Carrez solutions (Merck, Darmstadt, Germany). The HPLC column was Lichrospher, RP-18e (125 mm \times 4 mm, 5 μm particle size) fitted with a guard cartridge packed with the same stationary phase (Merck). The HPLC conditions were the following: isocratic mobile phase, 89 % water, 1 % acetic acid and 10 % methanol; flow rate of 0.7 $\mu\text{l}\cdot\text{min}^{-1}$; injection volume of 20 μl . All solvents were of HPLC grade (from Merck). The detection wavelength range was 220–660 nm with specific monitoring at 285 nm. The HMF content of each sample was calculated by comparing the corresponding peak areas of the sample and those of the standard solutions of HMF (Sigma, Aldrich, St. Louis, Missouri, USA) after correcting for the honey dilution. There was a linear relationship ($r^2 = 0.9997$) between the content and the area of the HMF peak. Results were expressed in milligrams per kilogram.

Diastase activity

The diastase activity (DA) of honey samples was determined following the Phadebas method according to the procedure of BOGDANOV et al. [17]. Absorbance was measured at 400 nm with a UV-Vis spectrophotometer Jenway 6305 (Fisher Scientific, Waltham, Massachusetts, USA). Diastase activity was expressed as the diastase

number in Schade units defined as follows: one diastase unit corresponds to the enzyme activity of 1 g of honey, which can hydrolyse 0.01 g of starch in 1 h at 40 °C.

Invertase activity

Invertase activity (*IA*) was determined following the Siegenthaler method, as described by BOGDANOV et al. [17]. Absorbance readings were taken using a spectrophotometer Jenway 6305 set at 400 nm. The relevant results were expressed as the units of enzyme per kilogram of sample.

Water content

The water content (*WC*) was determined by an Abbe-type refractometer (Fisher Scientific) read at 20 °C, according to the relationship between honey water content and refractive index. The water content was determined using the Chataway table that relates the percent of water with refractive index [17].

Ash content

Ash content was determined after the sample was burnt in an electric muffle furnace CFS 11/B (Carbolite Gero, Hope Valley, United Kingdom). First the ash dish was cleaned and heated in the electrical furnace at 550 °C. Later, it was cooled back to room temperature (20–25 °C) in a desiccator and 5–10 g of the sample were weighed with 0.001 g precision into the ash dish. Two drops of olive oil were added, then water was removed and the ashing procedure started without loss at a low heat rising to 350–400 °C. After the preliminary ashing, the dish was placed in the pre-heated furnace and heated for at least 1 h. Subsequently, the ash dish was cooled in a desiccator and weighed. The ashing procedure was continued until a constant weight was reached [17].

Colour measurement

The colour of honey depended on its botanical origin, ranging from almost transparent water white to dark brown almost blackish. Colour was measured according to Pfund colour scale, using the Lovibond comparator. The reading was expressed in millimetres.

Statistical analysis

The statistical analyses were performed using Microsoft Excel (Microsoft, Redmond, Washington, USA) and PAST 4.12 software (University of Oslo, Oslo, Norway). In the first step, honey samples were clustered according to their standardized physico-chemical characteristics using the similarity index of BRAY and CURTIS [18]

and the unweighted pair group method with arithmetic mean algorithm (UPGMA) [19]. According to these results, the operational physico-chemical units (OPU) were created. Likewise, principal component analysis (PCA) was carried out. After that, for each OPU, the floristic diversity of the samples was measured according to three metrics: Shannon H, Distinctiveness index, and Rarefaction index. With these metrics, the integrated diversity index (*IDI*) was calculated [20]. In order to determine if there was any association between the pollen composition of honey and its physico-chemical as well as environmental parameters, a co-inertia analysis was performed. The correlation between the species detected and various physico-chemical as well as environmental factors such as altitude or vegetation types was measured by the vector correlation coefficient (*VC*), ranging between 0 (all species are independent of environmental variables) and 1 (both tables are homothetic) [21]. These statistical analyses were conducted using R software version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Quantitative pollen analysis

The quantitative pollen analysis showed (Fig. 2) that pollen richness of honey samples ranged from medium (class III, 8.3 % of the samples) to high (class I, 50 % of the samples), where the pollen density ranged from 1724 to 144282 in 10 g of honey, with an average of 44620 grains per 10 g.

Qualitative pollen analysis

Qualitative analysis of the pollen spectrum of the 24 honey samples from the study area revealed a wide diversity of pollen sources, underscoring the variety of plants foraged by bees in the region. According to Tab. 2, the honey samples were divided into two categories: multifloral honey and monofloral honey, each with a different pollen composition. Upon examining the nature of the honey samples, it was interesting to note that the majority of them were monofloral in nature, characterized by predominance of a single pollen taxon, with 17 samples corresponding to this category. Nine of these samples were *Eucalyptus* honeys, three were *sulla* (*Hedysarum coronarium* L.) honeys, two were *Citrus* and *Arbutus* honeys, while a single sample was attributed to lavender (*Lavandula*) and jujube (*Ziziphus lotus* (L.) Lam.).

Eucalyptus honey stands out particularly with

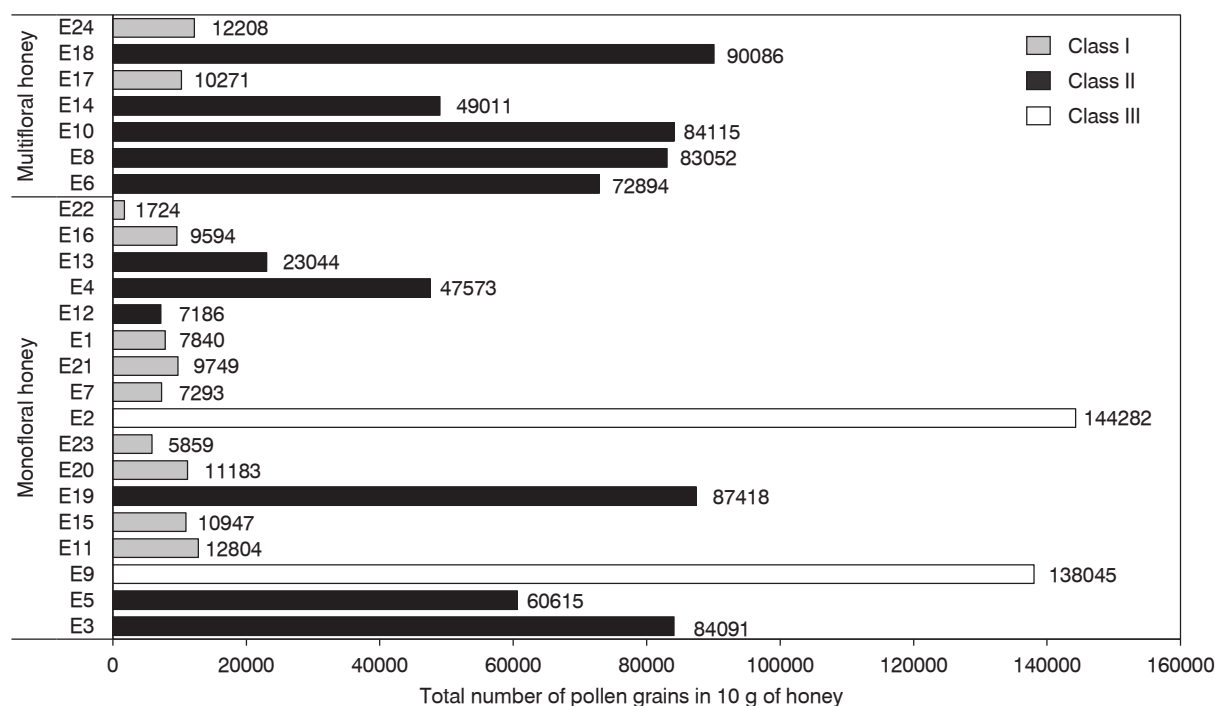


Fig. 2. Pollen richness of the studied honey samples and their classification according to the content of pollen.

E1–E24 – honey samples (specified in Tab. 1).

Class I – < 20 000 grains in 10 g of honey (honey poor in pollen), Class II – 20 000–100 000 grains in 10 g of honey (honey moderately rich in pollen), Class III – 100 000–500 000 grains in 10 g of honey (honey rich in pollen).

its high number of samples, illustrating the importance of this plant as the main nectar source for honey-producing bees. Similarly, honey from *Hedysarum coronarium* L., *Citrus* sp., *Ziziphus lotus* and *Lavandula stoechas* L. showed high relative frequencies, underscoring their predominance in monofloral samples. These results highlighted the specificity of monofloral honeys, where a single type of plant significantly contributed to the honey composition.

However, 7 samples turned out to be multifloral, meaning they contained pollen from several plant species.

By analysis of the 24 honey samples collected in northeastern Algeria, 87 pollen species were identified, which reflected the high biodiversity of this region despite the moderate pollen content of honey. In fact, northeastern Algeria belongs to the eleventh regional biodiversity hotspot in the Mediterranean called “Kabylas-Numidia-Kroumiria” [22]. These results were similar to those obtained by MAKHLOUFI et al. [8] on honey samples from Jijel and Mila located in eastern Algeria.

In our study area, pollen from *Eucalyptus* was the most representative pollen type, since it was dominant in 9 of the 24 honey samples analysed.

The presence of pollen grains of *Eucalyptus* species in the majority of honeys analysed revealed its omnipresence in the “four corners of Algeria”. In sample E11 from the Settara locality in Jijel, the relative abundance of this pollen type was 76.0 %. As previously reported, this species is an important source of nectar honey in Algeria [9, 10]. *Hedysarum coronarium* pollen dominated in 3 honey samples. LOUVEAUX et al. [13] pointed out that this taxon was dominant in North Africa, which is consistent with our results and those of BOUTABIA et al. [23]. *Lavandula stoechas* and *Citrus* sp. pollen dominated in two honey samples. These taxa do not produce high amounts of pollen but are highly nectariferous [8]. *Arbutus* honey was characteristic for the two coastal regions of Filfila and Sidi Maarouf. This honey is a poor-pollen honey according to the categories of LOUVEAUX et al. [13]. The honey samples without any dominant pollen type frequently contained pollen from *Echium plantagineum* L., *Lavandula stoechas* L., *Raphanus raphanistrum* L., *Daucus carota* L., *Calicotome villosa* (Poir.) Link, *Erodium moschatum* (L.) L’Hér., *Malva sylvestris* L., *Oxalis pes-caprae* and *Cistus* sp. [8, 9, 10, 23].

Tab. 2. Main pollen types of honey samples.

Pollen type	Monofloral honey																							Multifloral honey								RF [%]
	Eucalyptus											Hedysarum coronarium			Arbutus		Citrus	Eucalyptus-Citrus	Ziziphus lotus	Lavandula												
	E3	E5	E9	E11	E15	E19	E20	E23	E2	E7	E21	E1	E12	E4	E13	E16	E22	E6	E8	E10	E14	E17	E18	E24								
	Number of pollen grains counted for the taxon																															
Eucalyptus camaldulensis	65	51	51	74	14	47	70	63		5			4	7	38	5			14	27	14	3	7	17	79.2							
Hedysarum coronarium	8	4	7			3		47	48	51				10	3		28	11	10	15	12	10	13	9	70.8							
Echium plantagineum.						3	8	17			3			3	3		5	8	3		10		13	12	50.0							
Lavandula stoechas							3			10	3						49	13	3	15			10	15	37.5							
Raphanus raphanistrum			3		5								4	4				12	12	3					29.2							
Malva sylvestris										8								11	7		12	10	3		25.0							
Eucalyptus globulus.	17				43							10					7	5							20.8							
Daucus carota														4					4	4	8		3		20.8							
Thymus cf. algeriensis						3															7	12	6		16.7							
Trifolium cf. repens												3			4			4				3			16.7							
Ziziphus lotus					5	3										47								8	16.7							
Erodium moschatum					6														6	3		3			16.7							
Genista cf. ferox									4	11								3							12.5							
Oxalis pes-caprae																	3			6		6			12.5							
Cichorium intybus	3																		4		3				12.5							
Acacia mearnsii			3																5						8.3							
Citrus spp.														49	29										8.3							
Hedysarum spinosissimum					7																5				8.3							
Quercus suber			3																				4		8.3							
Rhamnus alaternus																		3					7		8.3							
Rosmarinus cf. eriocalyx																12						20			8.3							

Principal pollen types and their relative frequency classes in the honey samples calculated respectively, for pollen grains of Taxa exceeding 2 % and for pollen grains present in more than two samples.

E1–E24 – honey samples (specified in Tab. 1). RF – relative frequency.

Physico-chemical characteristics

The *WC* analysis of the honey samples revealed a range spanning from 14.3 % to 16.9 %. Notably, honey sourced from coastal regions (E1, E4, E10 and E12) exhibited the highest *WC*, fluctuating between 16.0 % and 16.8 %, while honey originating from inland areas displayed a narrower range, from 14.5 % to 16.9 % (Tab. 3). Concurrently, *EC* of the honey samples varied between 0.2 mS·cm⁻¹ and 0.5 mS·cm⁻¹. *FA* of the samples ranged from 9.4 meq·kg⁻¹ to 14.44 meq·kg⁻¹, with lower *WC* correlating with reduced *FA*, notably observed in honey sourced from the Tell Constantinois wilayas. pH values of the samples fell within a range pH 2.74–4.84, indicative of varying degrees of acidity. Additionally, analysis of *AC* suggested mineralization across the samples, with values ranging from 0.2 % to 0.7 %. Further examination revealed a range of 3.8 mg·kg⁻¹ to 7.1 mg·kg⁻¹ for HMF levels and *DA* spanning from 5.3 to 19.3 Schade units. The dominant honey types, i.e. *Hedysarum coronarium*, *Citrus* honey and *Eucalyptus-Citrus* honeys, showed low colour variations (44–59 mm in Pfund scale). Colour of the honey samples dominated by *Arbutus unedo* ranged from 103 mm to 127 mm (Tab. 3).

The physico-chemical characteristics of the honey samples generally matched international standards [17]. According to BOGDANOV et al. [25], *WC* is crucial for the preservation of honey during its storage, given that a high *WC* can lead to deterioration of honey quality. These results were similar to those found by MAKHLOUFI et al. [8] for all flower honeys from the region of Bejaia, with values ranging from 14 % to 19 %, reflecting the high quality of Algerian honey.

The *EC* values did not exceed 0.5 mS·cm⁻¹ in any of the honey samples, thus allowing them to be classified as honey from flower nectar [26]. ZERROUK et al. [27] suggested that *EC* of honey is closely related to the concentration of mineral salts, organic acids and proteins, which means that it is highly variable depending on the floral origin. Therefore, it is one of the best parameters for differentiating between flower honey and honeydews. The acidity levels obtained were similar to those reported by NAMAN et al. [28] on 10 samples of Moroccan honey.

The pH values of the studied honey samples ranged from 2.4 to 4.8 and were close to the values obtained by LOUVEAUX et al. [13] in the steppes of Djelfa (northern Algeria) and those by MAKHLOUFI et al. [8] in the coastal regions of Algeria. In Morocco, the pH values of the analysed honey samples usually ranged from 3.2 to 4.5 [28]. In Egypt, BADAWY et al. [29] indicated that

the pH values of the studied honey varied between 4.1 and 5.2.

The results of *AC* were similar to those reported by MAKHLOUFI et al. [8] who found *AC* ranging from 0.1 % to 0.5 %. The values obtained for HMF ranged from 3.82 mg·kg⁻¹ to 7.19 mg·kg⁻¹, which were under the maximum limit (40 mg·kg⁻¹).

The results on *DA* showed that 66.6 % of the studied samples could be considered high-quality, fresh honey according to the standards. Based on the European Honey Commission [25], *DA* of honey determined after processing and/or blending should be, in general, not less than 8 Schade units and in the case of honeys with a low natural enzyme content, not less than 3 Schade units. Such *DA* values were not reached in samples E4, E8, E10, E13 and E15. These low values can be explained by the age of these samples or by the natural lack of enzymes [30, 31]. According to PERSANO ODDO et al. [32], the *DA* level in honey varies depending on several factors, including its sugar content, floral and geographic origins, collection duration, the age of the bees and the bee colony. The lower *DA* observed in some honey samples in this study may be related to the internal characteristics of the honey and their botanical origin. Additionally, BOGDANOV et al. [25] noted that *DA* is often used as a quality parameter of honey despite the fact that some honeys may have a lower level of enzymes intrinsically.

The *LA* levels ranged from 13.44 U·kg⁻¹ to 19.92 U·kg⁻¹. The European legislation does not give any reference value for *LA*. However, according to PERSANO ODDO et al. [32], the majority of high-quality honeys typically exhibit *LA* within the range of 5–20 U·kg⁻¹. This interval includes largely the results obtained for the 24 honey samples in this study. The variability in enzyme activity found in the different honey types was probably due to a series of factors, such as nectar collection period, abundance of nectar flow and its sugar content, age of the bees or pollen consumption.

The colour of honey samples dominated by *Arbutus unedo* ranged from 103 mm to 127 mm on the Pfund scale, corresponding to a spectrum from light to dark brown. This colour variation is linked to HMF and mineral content [3, 10, 33].

Principal component analysis

PCA showed (Fig. 3) a cophenetic correlation of 0.8. By drawing a phenon line at 38.0 % similarity, 7 OPUs were formed. The percentage of variance explained by the first three components was 66.6 %. On the other hand, data in Tab. 4 revealed that water content and electrical conduc-

Tab. 3. Physico-chemical characterization of the 24 honey samples from northeastern Algeria.

Code	WC [%]	EC [mS·cm ⁻¹]	FA [meq·kg ⁻¹]	pH	AC [%]	HMF [mg·kg ⁻¹]	DA	IA [U·kg ⁻¹]	Col [mm]
E1	16.0 ± 0.2	0.53 ± 0.17	13.22 ± 0.22	4.57 ± 0.15	0.4 ± 0.1	6.12 ± 0.18	10.28 ± 0.71	17.27 ± 0.37	103
E2	14.7 ± 0.4	0.24 ± 0.35	13.78 ± 0.15	4.18 ± 0.22	0.3 ± 0.0	5.48 ± 0.27	18.33 ± 0.48	19.92 ± 0.61	44
E3	15.2 ± 0.5	0.41 ± 0.12	13.92 ± 0.13	4.41 ± 0.21	0.4 ± 0.2	5.23 ± 0.25	17.38 ± 0.41	15.38 ± 0.14	75
E4	16.8 ± 0.1	0.22 ± 0.33	14.12 ± 0.23	4.53 ± 0.18	0.3 ± 0.1	4.42 ± 0.14	9.72 ± 0.27	17.92 ± 0.23	51
E5	15.1 ± 0.1	0.47 ± 0.43	14.44 ± 0.25	4.52 ± 0.06	0.2 ± 0.3	4.81 ± 0.31	17.28 ± 0.12	19.58 ± 0.31	71
E6	14.8 ± 0.3	0.34 ± 0.22	13.81 ± 0.27	4.18 ± 0.18	0.3 ± 0.0	7.19 ± 0.26	15.22 ± 0.33	14.38 ± 0.09	66
E7	15.9 ± 0.4	0.27 ± 0.32	13.55 ± 0.29	3.81 ± 0.23	0.4 ± 0.0	6.55 ± 0.12	19.38 ± 0.51	19.08 ± 0.17	49
E8	15.2 ± 0.1	0.36 ± 0.08	13.88 ± 0.26	3.84 ± 0.19	0.5 ± 0.0	4.85 ± 0.34	5.84 ± 0.22	15.43 ± 0.23	69
E9	14.3 ± 0.5	0.41 ± 0.11	11.25 ± 0.43	3.91 ± 0.21	0.7 ± 0.1	4.96 ± 0.17	18.57 ± 0.14	19.17 ± 0.70	73
E10	16.0 ± 0.6	0.37 ± 0.07	13.88 ± 0.33	4.17 ± 0.26	0.4 ± 0.1	6.22 ± 0.43	7.33 ± 0.52	13.41 ± 0.42	78
E11	14.6 ± 0.4	0.48 ± 0.19	14.02 ± 0.41	4.43 ± 0.07	0.3 ± 0.2	6.08 ± 0.11	17.84 ± 0.23	18.65 ± 0.29	69
E12	16.0 ± 0.2	0.57 ± 0.21	14.28 ± 0.23	4.84 ± 0.25	0.7 ± 0.0	6.04 ± 0.35	12.55 ± 0.17	19.86 ± 0.64	127
E13	16.8 ± 0.4	0.33 ± 0.07	13.08 ± 0.12	4.28 ± 0.27	0.6 ± 0.0	5.04 ± 0.27	5.35 ± 0.47	14.72 ± 0.33	59
E14	15.0 ± 0.3	0.39 ± 0.18	12.87 ± 0.09	4.15 ± 0.31	0.5 ± 0.0	4.66 ± 0.41	19.08 ± 0.62	19.28 ± 0.18	65
E15	16.9 ± 0.5	0.45 ± 0.04	13.59 ± 0.15	4.42 ± 0.14	0.5 ± 0.0	5.71 ± 0.21	7.72 ± 0.28	13.44 ± 0.41	78
E16	15.4 ± 0.2	0.29 ± 0.07	14.01 ± 0.37	2.47 ± 0.31	0.4 ± 0.2	3.82 ± 0.39	13.28 ± 0.52	15.22 ± 0.52	62
E17	14.9 ± 0.1	0.45 ± 0.20	12.95 ± 0.23	4.41 ± 0.45	0.6 ± 0.0	4.79 ± 0.14	16.39 ± 0.19	18.39 ± 0.61	61
E18	15.1 ± 0.1	0.47 ± 0.42	12.33 ± 0.47	4.38 ± 0.33	0.4 ± 0.0	4.31 ± 0.23	17.71 ± 0.28	14.77 ± 0.18	69
E19	15.2 ± 0.2	0.48 ± 0.32	13.47 ± 0.03	4.39 ± 0.37	0.4 ± 0.0	6.03 ± 0.16	18.33 ± 0.53	18.07 ± 0.42	71
E20	15.3 ± 0.3	0.45 ± 0.06	14.15 ± 0.18	4.77 ± 0.24	0.2 ± 0.1	5.44 ± 0.19	15.07 ± 0.66	15.41 ± 0.39	76
E21	14.5 ± 0.1	0.23 ± 0.12	12.64 ± 0.21	4.33 ± 0.23	0.4 ± 0.0	5.18 ± 0.24	11.47 ± 0.71	16.33 ± 0.19	52
E22	14.6 ± 0.1	0.24 ± 0.17	9.85 ± 0.41	4.51 ± 0.37	0.5 ± 0.1	6.49 ± 0.17	18.64 ± 0.72	19.41 ± 0.47	71
E23	15.3 ± 0.2	0.48 ± 0.19	12.57 ± 0.15	4.43 ± 0.22	0.4 ± 0.0	5.52 ± 0.19	13.76 ± 0.49	13.94 ± 0.19	78
E24	14.6 ± 0.2	0.44 ± 0.08	12.29 ± 0.17	4.27 ± 0.19	0.4 ± 0.0	4.95 ± 0.31	14.89 ± 0.33	16.84 ± 0.53	67

E1–E24 – honey samples (specified in Tab. 1).

WC – water content, EC – electrical conductivity, FA – free acidity, AC – ash content, HMF – hydroxymethylfurfural, DA – diastase activity (expressed as diastase number in Schade units), IA – invertase activity, Col – colour (expressed in millimetres of Pfund scale).

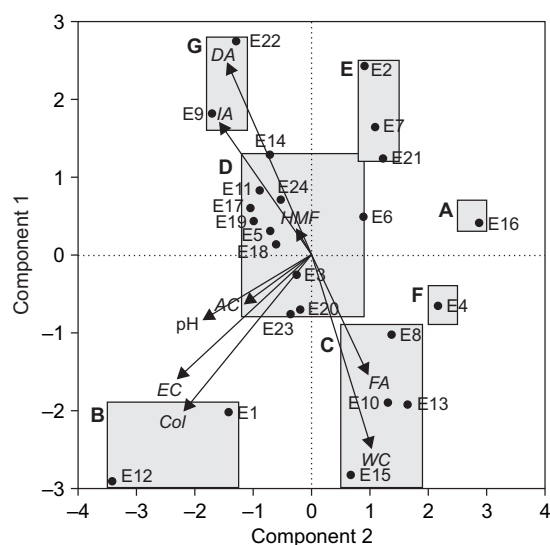


Fig. 3. Principal components analysis of the honey samples based on their physico-chemical characteristics.

Rectangles connect samples with more than 38 % of similarity. A–G – operational physico-chemical units, E1–E24 – honey samples (specified in Tab. 1).

WC – water content, EC – electrical conductivity, FA – free acidity, AC – ash content, HMF – hydroxymethylfurfural, DA – diastase activity, IA – invertase activity, Col – colour.

Tab. 4. Weight of individual variables in the principal components 1, 2 and 3.

Parameters	PC 1	PC 2	PC 3
WC [%]	0.7 *	–0.3	0
EC [mS·cm ^{–1}]	0.493	0.709 *	0.103
FA [meq·kg ^{–1}]	0.477	–0.299	0.370
pH	0.259	0.574	0.453
AC [%]	0.1	0.3	–0.8 *
HMF [mg·kg ^{–1}]	–0.104	0.082	0.699
DA	–0.760 *	0.444	0.159
IA [U·kg ^{–1}]	–0.524	0.486	–0.072
Col [mm]	0.620	0.674	–0.037

PC – principal component (the most relevant variables for each PC are marked with an asterisk).

WC – water content, EC – electrical conductivity, FA – free acidity, AC – ash content, HMF – hydroxymethylfurfural, DA – diastase activity (expressed as diastase number in Schade units), IA – invertase activity, Col – colour (expressed in millimetres of Pfund scale).

tivity were crucial for the sample aggregations, while DA and AC were inversely related. The most relevant variables for the principal components were WC and EC. OPUs obtained in by PCA were confirmed by cluster analysis. The double-crossed dendrogram (Fig. 4) obtained by means of a biplot model compared, on the one hand, the groupings of the sampled localities (a cophenetic correlation

of 0.76) against the chemical characteristics. By drawing a phenon line at 88.0% similarity, 7 OPUs were formed, whose differentiating characteristics could be deduced from the colour quadrant. Thus, OPU A (E16) was distinguished by its low pH value, OPU B (E12, E1) was characterized by higher coloration and EC values. OPU C (E8, E10, E13, E15) was distinguished by its low values of IA and DA at high values of WC, OPU D stood out for presenting high mean values of EC and DA at the same time but had a high variability for most physico-chemical properties, OPU E (E2, E7, E21) had in common low average values of EC and colour, OPU F separated sample E4 from the rest, which had high WC and low HMF, EC, AC as well as DA, and OPU G (E9, E22) that had low WC but high FA, IA, AC and DA. The proposed OPUs generally clustered honey with similar botanical origins. OPU A separated jujube honey from the rest, OPU B corresponded to *Arbutus* honey and OPU E to *sulla* honey. The remaining OPUs included mixtures of multifloral and *Eucalyptus* honeys.

Botanical composition

According to the *IDI* values and its components, OPU A was clearly distinguished from all the others by its low rarefaction (0.054) and very low *IDI* (0.5; Tab. 5). In contrast, OPU G had a high distinction (4.46) but a low species diversity (2.5). Among the other OPUs, B, C, and D had the highest specific diversity (3.12, 3.16 and 3.36, respectively), followed by E and F. On the other hand, the rarefaction indices of OPU E and OPU F had the highest values (3.84 and 3.68, respectively) compared to this index in OPU B, C and D. In comparison to the other OPUs, *IDI* had the highest values (33.61 and 29.41) in OPUs E and D.

The Shannon *H* index reflected a high diversity of pollen types in OPUs B, C and D, with predominant *Arbutus* and multifloral honey [34]. Comparatively, the monofloral *Eucalyptus* and *Lavandula* honeys of OPU G had lower diversity. Generally, lower diversity values were observed in a study carried out by SONG et al. [1] with 19 honey samples from Shanxi, China, with Shannon *H* index values ranging between 1.79 and 2.21 for the multifloral samples, qualifying Algeria's honey as being of better quality with regard to its rich sources of nectar and pollen [23, 35].

Co-inertia analysis

A co-inertia analysis was carried out considering the botanical origin of the honey samples and their physico-chemical properties (Fig. 5).

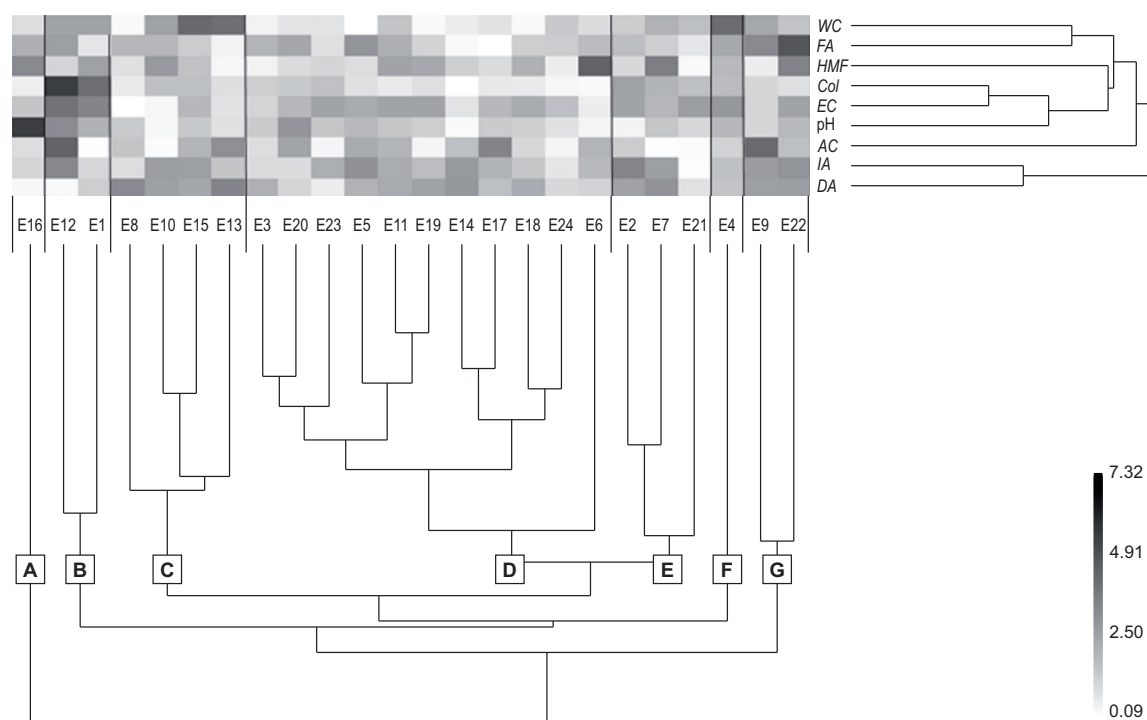


Fig. 4. Cluster aggregation of the honey samples according to their physico-chemical characteristics.

Dashed line – the phenon line established the groups of sampling locations with more than 38% similarity.

A–G – operational physico-chemical units, E1–E24 – honey samples (specified in Tab. 1).

WC – water content, EC – electrical conductivity, FA – free acidity, AC – ash content, HMF – hydroxymethylfurfural, DA – diastase activity, IA – invertase activity, Col – colour.

There was a co-structure defined by the first two axes of co-inertia, showing 55.3 % and 20.1 % inertia, respectively. These axes revealed a good correspondence between the structures reflected by the botanical and physico-chemical analyses and those provided by co-inertia. The vector correlation coefficient (VC) of the co-inertia analysis highlighted the relationship between the species and the descriptors studied. This coefficient was high ($VC = 0.47$), thus indicating the presence of a moderately significant co-structure between the parameters analysed and the pollen flora. The first axis differentiated the samples of *Arbutus* honey from coastal regions from the rest, which matched OPU B obtained in the cluster analysis. These samples were associated with dark colouration, important DA, very high AC and FA. Additionally, the first axis was dominated by several physico-chemical descriptors of honey (WC, HMF, IA, DA) and it showed strong co-linearity between these factors. In contrast, the samples of *sulla* honey (E2, E7, and E21) appeared relatively isolated and were characterized by low HMF content and high IA. On the contrary, the second axis isolated the samples of multifloral or all-flower honey from the Constantine Tell stations and separated the Algerian

steppe samples from jujube honey in relation to vegetation near hives, altitude, EC and pH from other variables. This analysis reflected that the co-structure between the studied variables and the pollen spectrum in honey was associated with the characteristics of the vegetation that surrounds the hives.

The OPUs obtained in PCA generally grouped honey with the same botanical origin and the same aggregations were obtained when perform-

Tab. 5. Characterization of the operational physico-chemical units according to the diversity of the botanical origin of honey.

OPU	Shannon H index	Distinctiveness	Rarefaction	ID/I
A	2.318	4.747	0.054	0.59
B	3.129	2.154	3.063	20.65
C	3.168	2.675	2.734	23.17
D	3.367	3.036	2.877	29.41
E	2.750	3.181	3.842	33.61
F	2.583	2.938	3.689	28.00
G	1.538	4.464	3.599	24.70

OPU – operational physico-chemical unit, ID/I – integrated diversity index.

ing the cluster analysis. This was also supported by the co-inertia analysis results, which reflected the association between the physico-chemical characteristics and the botanical origin of honey [35]. These findings highlighted the importance of maintaining the local vegetation near the hives to preserve the properties and quality of honey, which are of great interest to beekeepers when selecting places to install the hives. Additionally, these results should encourage public admi-

nistrations to develop conservation strategies and reforestation plans in vulnerable ecosystems. Disturbed sites should maintain their melliferous native plant species to preserve their ecosystem services and the natural heritage of the area.

According to pollen analysis, more than two-thirds of the honey samples analysed were monofloral, with the remaining third being multifloral. Among the monofloral varieties, *Eucalyptus* honey dominated, comprising half of the samples, while

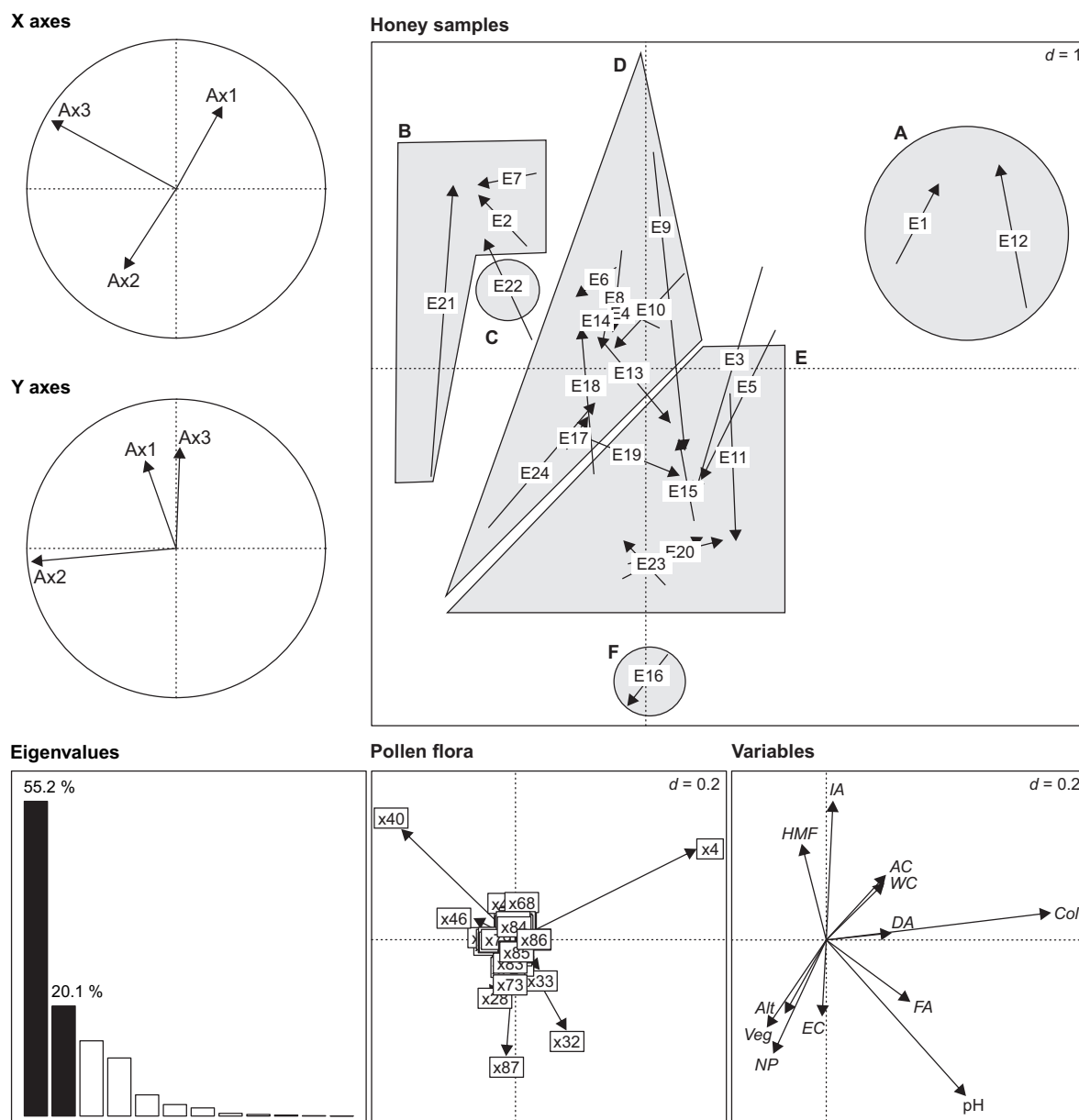


Fig. 5. Co-inertia analysis carried out between the pollen flora and the physico-chemical variables.

A–F – operational physico-chemical units (A – *Arbutus* honey, B – *sulla* honey, C – *Lavandula* honey, D – multifloral honey, E – *Eucalyptus* honey, F – *jujube* honey).

E1–E24 – honey samples (specified in Tab. 1).

WC – water content, EC – electrical conductivity, FA – free acidity, AC – ash content, HMF – hydroxymethylfurfural, DA – diastase activity, IA – invertase activity, Col – colour, NP – number of pollen, Veg – vegetation, Alt – altitude.

the remainder included *sulla*, *Arbutus*, *Citrus*, jujube and *Lavandula* varieties. The multifloral honey samples exhibited remarkable diversity in their pollen composition, resulting in a wide range of physico-chemical properties [35]. Most of these honeys met the established standards, with their physico-chemical parameters primarily influenced by botanical origin.

CONCLUSIONS

The study provided an in-depth assessment of the quality of 24 honey samples collected from eight different wilayas in northeastern Algeria, representing a diversity of vegetation, including oak and pine forests and maquis. By analysing their pollen composition, botanical origins and physico-chemical properties, it offers a detailed overview of the diversity and richness of honeys produced in the region. The results of the pollen analysis highlighted the predominance of monofloral honeys, mainly those containing *Eucalyptus* pollen, while revealing the significant presence of other species such as *Hedysarum coronarium*, *Arbutus unedo*, *Lavandula stoechas*, *Ziziphus lotus* and *Citrus* sp. Multifloral samples also showed a variety of taxa, confirming the diversity of floral sources in the region. Regarding properties, the majority of honey samples conformed to international standards, indicating their quality and adherence to production requirements [17]. Nevertheless, a few honey samples did not meet some of the international quality requirements [17], underscoring the need for continuous monitoring and an improved beekeeping practices. The data collected in this study laid a strong groundwork for the creation of honey evaluation and valorization programs in the northeastern region of Algeria. They could also contribute to the attribution of quality signs, characterization of honey and its protection under a geographical certificate of origin.

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