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Physicochemical and Melissopalynological Study of Some Honey Samples from the Algerian East Region

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A R T I C L E I N F O

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A B S T R A C T

The quantitative and qualitative analysis of honey components is the object of this study. These analyses are considered a physicochemical and melissopalynological tool that allows the study of some characteristics of honey. The analysis of ten (10) samples of Algerian honey is carried out to determine the pH, water content, electrical conductivity, ash content, acidity, hydroxymethylfurfural content, proteins, and sugar content. According to the obtained results, the physico-chemical parameters of the studied honeys comply with the European and international standards. The pollen spectrum indicated the presence of 23 plant families, mainly Fabaceae, encompassing a total of 65 honey species visited by foragers. Of the 10 honeys sampled and analyzed, six were monofloral, with the remainder showing no apparent dominance of any honey type. The most frequent plant species were eucalyptus (*Eucalyptus* sp.), Italian sainfoin (*Hedysarum coronarium*), and chickweed (*Lathyrus* sp.). Furthermore, the results obtained indicate that all honey (monofloral and polyfloral) showed a high degree of variability in the number of pollen grains.

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Introduction

Honey is the natural sweet product produced by *Apis mellifera* bees species from plant nectar or from the secretions of living parts of melliferous plants. This substance is supersaturated with carbohydrate (glucose, fructose, polysaccharides), water and, other molecules, such as proteins, enzymes, vitamins, minerals, phenolic components, and amino acids that have important nutritional value for human health (Tsavea et al., 2022). In addition, honey contains an important amount of total polyphenols contents, which are responsible for the significant antioxidant activity (Necib et al., 2022).

Honey chemical composition and physicochemical parameters are variable and related to the botanical origin, geographical area, and environmental conditions (Tarapatskyy, 2021).

Its characteristics are depending to the food source of bees; in fact, it can have all the virtues and active principles of the plant that was at the origin of its production (Mekious et al., 2020). Due to its multiple properties, honey is used in many cases, either internally as a remedy or externally as an ingredient in beauty products or for food preservation (Halimi, 2018).

The quality of honeys around the world depends on many biological, climatic, and ecological factors (Haderbache, 2021); the floral origin gives specific characteristics to a honey; the conditions of its elaboration always remain unique; and therefore, at each honey flow, a new product is created.

Scientific research has elucidated a lot of information on the composition of honey and attempted to answer questions about its quality and composition; as a result, honey will always be a natural heritage to be valued and preserved.

Thus, the aim of this study is to characterize same honey samples produced in two localities in north-east Algeria (Souk-Ahras and Tebessa) in order to determine their physicochemical parameters and their pollen spectrum.

Methodology

Ten (10) honey samples were collected between 2019 and 2020 from sites located in two regions of Souk-Ahras (seven samples: S1, S2, S3, S4, S5, S6, S7) and Tebessa (three samples: T1, T2, T3) (Table 1). The botanical origin was confirmed by the pollen analysis performed for this study.

Physicochemical Analysis

Water content: The water content was determined by the refractive index of the honey, referring to the table of Chataway (AOAC, 1995). The honey sample was placed on the refractometer's prism, two (02) readings were taken at 20o C, and the average allows the water content to be determined according to standards.

Density determination: The density was determined by dividing the weight of the density bottle (10 ml) filled with honey by the weight of the same bottle filled with distilled water according to the following formula: D = M / M' Where :

M: Mass of the honey's volume; M': Mass of the same volume of distilled water.

Determination of ashes (mineral matter): The content of total ashes was measured by incinerating 5 g of honey samples in a muffle furnace (Nabertherm) at a temperature of 550°C for 4–5 hours (AOAC, 1995; Codex Alimentarius, 2001). The amount of crude ash is obtained from the formula: C% = (M1-M2)/M0 ×100%.

C%: crude ash content; M0: mass in grams of the empty incineration capsule; M1: mass in grams of the incineration capsule and the sample before incineration, M2: mass in grams of the incineration capsule and the ash after incineration.

Determination of hydrogen potential pH: The pH was evaluated in a 20% (m/v) aqueous solution (AOAC, 1995) using a pH meter.

Determination of electrical conductivity: The determination of electrical conductivity is based on Voirol's method of using a conductivity meter. The measurements were carried out at 20° C in an aqueous solution at 20% (m/v) compared to the dry matter of honey (AOAC, 1995).

Determination of free acidity: The free acidity was determined in a titrimetric way. Titration at pH 8.5 with 0.05 m NaOH added determines the free acidity (Bogdanov, 1997). Free acidity = 1000 × V ×x N/M

Which: V: the volume in ml of NaOH used to neutralize the solution; N: the normality of NaOH; M: the weight of the honey (10 g).

Protein dosage: The dosage of proteins was made according to the Bradford method (1976), which uses the brilliant blue of Coomassie (BBC) and the serum albumin of bovine (BSA) 1 mg/mL standards. The dosage was determined with a calibration range. 1 gram of honey was dissolved in 1 ml of distilled water; after shaking, 100µL was taken, and 4 ml of BSA was added. Shaking was done after 5 minutes. The absorbance (OD) was measured using a spectrophotometer at a wavelength of 595 nm against a blank (100 l distilled water and 4 ml BBC).

Determination of Hydroxy-Methyl-Furfural HMF: The determination of HMF content was performed according to the Winkler method (Bogdanov et al., 1995). The principle was based on the measurement of the absorbance of this molecule by spectrophotometry, set at a wavelength of 550 nm, in the presence of barbituric acid used in aqueous solution at 0.5% and paratoluidine used in isopropanol solution at 10%. The HMF content was expressed in mg for 1 kg of honey.

Quantitative and Qualitative Pollen Analysis

The quantitative melissopalynological study consists of determining the pollen richness by the number of microscopic elements per weight unit of honey, by counting the number of pollen grains contained in 10 g of honey. The method of Layka (1989) was used by placing 10 g of honey in a test tube, the honey was mixed with the help of a spatula, then liquefied in a water bath at a temperature of 40 °C for 10 minutes; then 5 mg of this liquefied honey are spread between the slide and lamella. The preparation was licked with the nail varnish to avoid any contamination or alteration, and the microscopic observations at X40 and X100 magnification were released.

The pollen analysis includes the counting and identification of pollen grains for each sample of the honey. The honey was classified according to the pollen representativeness of Maurizio's classes (1975): class I: < 20 000 grains (honey poor in pollen), class II: 20 000 < grains < 100 000 (honey moderately rich in pollen), class III: 100 000 < grains < 500 000 (honey rich in pollen), class IV: 500 000 < grains < 1 million (honey very rich in pollen), and class V : >1 million grains (honey extremely rich in pollen).

Table 1. Geographical origin, period of collection and, type of extraction of honey samples

Sample	Geographic origin		Collection period	Type of extraction
S_1	Souk-Ahras	Machrouha	September 2019	Electric extraction
S_2		Ain Senior (Rmalelhisan)	June 2020	Electric extraction
S_3		Ain Senior (Benisouiden)	July 2020	Electric extraction
S_4		Ain Senior	August 2020	Electric extraction
S_5		Machrouha	August 2020	Electric extraction
S_6		Ouled Moumen	August 2020	Electric extraction
S_7		Heddada	September 2020	Electric extraction
T_8	Tebessa	El Mrij	September 2019	Simple
T_9		Tebessa	August 2020	Simple
T_{10}		Bir El Atter	August 2020	Electric extraction

Table 2. Results of physicochemical parameters studied

Sample	Water content (%)	Density (g/ml)	Ash content (%)	pH	CE (µS/cm)	Free acidity (meq/kg)	Proteins (%)
S_1	25,00	1,3804	0,130	3,59	532,00	6,00	26,65
S_2	20,20	1,3904	0,089	3,78	455,00	7,25	27,71
S_3	20,40	1,5149	0,120	3,75	149,06	3,50	17,96
S_4	22,00	1,4173	0,093	3,81	336,66	4,00	10,23
S_5	20,60	1,6260	0,126	3,93	613,00	6,50	46,60
S_6	20,40	1,6538	0,054	3,50	217,00	8,25	26,29
S_7	24,00	1,5680	0,032	3,51	220,33	6,50	26,19
T_8	25,00	1,6097	0,038	3,70	589,00	8,00	30,18
T_9	20,60	1,5346	0,169	4,38	417,00	2,50	17,76
T_{10}	25,00	1,7810	0,088	3,61	621,33	8,00	26,14

Table 3. Pollen richness of the studied samples

Sample	Number of pollen grains /g	Class
S_1	2100	II
S_2	1000	I
S_3	12800	III
S_4	0	I
S_5	7800	II
S_6	1400	I
S_7	800	I
T_8	6000	II
T_9	800	I
T_{10}	0	I

Results and Discussion

Physicochemical Analysis

The table 2 summarizes results that were obtained.

Water content: The average values of the water content are illustrated in Table 2. They vary according to the floral origins of the honey, from 20.2% for the honey of Souk-Ahras (S2) to 25% for those of Tebessa (T8 and T10). These variations from honey to another have been reported by several authors (Belhadj et al., 2015; Mekious et al., 2015).

Density determination: The average value of the density of the analyzed honey was 1.5476 g/ml. The lowest density is recorded in the honey of Souk-Ahras (S1), while the highest value is observed in the honey of Tebessa T10 at 1.7810 g/ml.

Determination of ash (mineral matter): The rate of mineral matter found in the samples of the studied honey was between 0.032% for (S7) Souk-Ahras (Hadedda) and 0.156% for the sample (T9) Tebessa with an average rate of 0.0912%±0.055.

Determination of hydrogen potential pH: The pH of the studied honey tended toward acidity. It was between 3.5 for (S7) Souk-Ahras (Hadedda) and 4.38 for (T9) Tebessa, with an average of 3.738± 1.666. Our results are in agreement with those obtained by Amri (2016), who found that the measured pH varied between 3.46 and 5.49.

Determination of electrical conductivity: The values of electrical conductivity of the studied honey ranged from 149.06 µS/cm (S4) Souk-Ahras (Ain senior) to 621.3 µS/cm (T10) Tebessa (Bir El Atter) with an average of 469.034 µS/cm ±47.158. Our results are similar to those obtained by Amri (2016) who found values of electrical conductivity ranging from 120.5 µS/cm to 1137 µS/cm.

Determination of free acidity: According to the results obtained, we notice that the lowest value of free acidity of the 10 honey samples is observed in the honey of (T9) Tebessa with a value of 2.5 meq/kg, and the highest value is recorded in the honey of (S6) Souk-Ahras (Ouled Moumen) with a value of 8.25 meq/kg with an average of 6.05 meq/kg. Acidity is an important criterion of quality; it gives very important indications of the condition of honey (Doukani et al., 2014). The acidity of honey is due to the presence of gluconic acid from the transformation of glucose, which favors high water contents (Bogdanov et al., 2004).

Determination of proteins: The protein content ranges from 0.102% (S3) Souk-Ahras (Ain senior) to 0.465% (S1) Souk-Ahras (Machrouha).The protein content varies with the number of pollen grains in the honey, with an average of 31.737% ± 1.771. Our results are similar to those obtained by Draiaia (2015), who found protein content values varying between 0.107 and 1.279. Other studies have shown that honey samples are rich in protein (Bucekova et al., 2020; Bucekova and Majtan, 2016).

Determination of Hydroxy-Methyl-Furfural HMF: The results obtained from the studied samples vary between 3.517 mg/kg (S3) for the Souk-Ahras region and 56.66 mg/kg for the site (T8) of Tebessa (El Mrij), with an average of 30.036 mg/Kg ± 2.904. Thrasyvoulou (1986) showed that pine honeys have low HMF values.

Quantitative and Qualitative Pollen Analysis

The pollen richness of the studied samples fluctuates from one honey to another. In Table 3, the honey is classified according to its botanical richness, referring to the classification established by Maurizio (1975).

- Class I: less than 2000 pollen grains per gram; this class includes 6 samples from the 10 analyzed (S2, S3, S6, S7, T9, and T10), with a percentage of 60% of samples.

- Class II: from 2000 to 10.000 pollen grains per gram. This class includes 3 samples (S1, S5, and T8) presenting 30% of the analyzed honey.
- Class III: from 10.000 to 50.000 pollen grains per gram. This category includes only one sample (S4) (10% of the studied honey).

Samples moderately rich (S1, S5, and T8) and rich (S4) in pollen species are mountain honey origin, comes from the regions of Machrouha, Ain Senior in Souk-Ahras, and Mrij in Tebessa, where the hives are installed mainly in meadows made up of spontaneous plants.

The results of Manamani et al. (2021), showed that the pollen richness varies between 0 and 499600 pollen grains per 1g of honey, belonging to 36 botanical families with 65 genera and 45 species were identified in the studied honey samples. According to Maurizio's classes (1939), 33% of samples belong to Class V, 7% to Class IV and Class III, Class II with 13%, and 40% in Class I.

The pollen spectrum indicates the existence of 27 botanical families, especially Fabaceae, Asteraceae, Apiaceae, and Brassicaceae, encompassing a total of 65 melliferous species visited by the foragers. From the 10 analyzed honey samples, six were monofloral; the rest were without apparent dominance of the melliferous type. The most frequent plant species were Eucalyptus (*Eucalyptus* sp.), Italian sainfoin (*Hedysarum coronarium*), and chickweed (*Lathyrus* sp.). Furthermore, the results obtained indicate that all honey samples (monofloral and polyfloral) show great variability in the number of pollen grains.

According to Manamani et al. (2021), a total of 108 melliferous plant species distributed in 40 families were recorded in Souk-Ahras region where Asteraceae, Rosaceae and, Fabaceae were the most represented families.

The abundance of the Asteraceae, Brassicaceae, and Fabaceae families in the analyzed honey is a good indicator of the importance of these families for beekeeping as honey plants (Makhloufi et al., 2015).

The bivariate statistical analysis of the 9 parameters studied by PSS (version 22) reveals a strong positive correlation between mineral matter and pH (r = + 74%), which is significant at p 0.05. As a result, strong negative correlations were found between free acidity and mineral matter on the one hand (r = - 70%) and pH on the other (r = - 65%).

For the correlations between the other parameters, they were not significant (Sig > 0.05).

Conclusion

This study allowed us to study eight (08) physicochemical parameters of honey collected in two localities in the Algerian Northeast region. The results indicate that the samples are of good chemical quality and meet international standards. The analysis of physicochemical parameters is a criterion of honey value, often used in quality control. These parameters depend on various factors, such as the harvesting season, the degree of maturity reached in the hive, climatic factors, botanical origin, harvesting technique, and also the species of bee.

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References

Amri A. 2016. Contribution à l'étude approfondie de Quelques miels produits en Algérie : Aspect physico-chimique et botanique (Biochimie appliquée en agroalimentaire et santé). PhD Dissertation. Departement of Biology, Annaba University, Algeria.

AOAC, 1995. Official Methods of Analysis, 15éme Ed. Arlington, VA. Association of Official Analytical chemists. London.

Belhaj O, Oumato J, Zrira S. 2015. Étude physico-chimique de quelques types de miels marocains. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 3(3):71-75.

Bogdanov S, Bieri K, Figar M, Figueiredo V, Iff D, Kanzig A, Stockli H, Zurcher K. 1995. Miel : Définition et Directives pour l'Analyse et l'Appréciation. In Livre Suisse des Denrées Alimentaires, OCFIM. 1-26.

Bogdanov S, Ruoff K, Persano Oddo L. 2004. Physicochemical methods for the characterisation of unifloral honeys: a review. Apidologie, 35:4-17. DOI: https://doi.org/10.1051/apido:2004047

Bogdanov S. 1997. Nature and origin of the antibacterial substances in honey. Lebensmittel-Wissenschaft und-Technologie, 30:748-753. DOI: https://doi.org/10.1006/fstl.1997.0259

Bradford MM. 1976. A rapid and sensitive for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72:248-254. DOI: https://doi.org/10.1016/0003-2697(76)90527-3

Bucekova M, Bugarova V, Godocikova J, Majtan J. 2020. Demanding New Honey Qualitative Standard Based on Antibacterial Activity. Foods, 9(9):1263. DOI: https://doi.org/10.3390/foods9091263

Bucekova M, Majtan J. 2016. The MRJP1 honey glycoprotein does not contribute to the overall antibacterial activity of natural honey. Eur. Food Res. Technol., 242:625–629. DOI:10.1007/s00217-016-2665-5

Codex Alimentarius, 2001. Revised codex standard for honey. Codex stan, 12, 1982. Revue, 12:1-7.

Draiaia R. 2015. Caractérisation physico-chimique et appellation botanique des miels Algériens (Cas des ruches Langstroth). PhD Dissertation. Departement of Biology, Annaba University, Algeria.

Halimi H. 2018. Etude mélissopalynologiques, physicochimique et antibactérienne de quelques échantillons de miels du Sud algérien. PhD Dissertation. Departement of Biology, Kasdi Merbah University, Ouargla, Algeria.

Layka S. 1989. Les méthodes de la palynologie appliquées à l'étude des Papavéracées. Thèse de Doctorat. Université de Montpellier France. 18-25.

Louveaux J, Maurizio A, Vorwohl G. 1978. Methods of melissopalynology. Bee World, 59(4): 139-157. DOI : https://doi.org/10.1080/0005772X.1978.11097714

Makhloufi C, Kerkvfiet J, Scheweizer P. 2015. Characterisation of some monofloral Algerian honeys by pollen analysis. Grana, 54 (4):156-166. DOI: https://doi.org/10.1080/00173134.2014.999116

Manamani M, Boughediri L, Bouzebda, A. 2021. Pollen spectrum, melliferous plant inventory and, physicochemical proprieties of selected honeys from the North-east Algerian. PONTE International Journal of Science and Research, 77(7). DOI: 10.21506/j.ponte.2021.7.3

Maurizio A. 1975. Microscopy of Honey. In: E, Crane. Honey: A comprehensive, survey, London Heinemann. 257p. ISBN: 9780434902705 et 0434902705 (Print).

Mekious S, Masseaux C, Daoud N, Belhadj S, Houmani Z. 2020. Caractéristiques Mélissopalynologiques et Contenu Phénolique du Miel de *Ziziphus lotus* d'Algérie. Agrobiologia, 10(2): 2220-2228.

Necib A, Kefti L, Draiaia R, Mohamadi N, Rezig S. 2022. Evaluation of Polyphenols, Vitamin C Contents and Antioxidant Activity of Two Types of Algerian Honey. Turkish Journal of Agriculture-Food Science and Technology, 10(12):2539-2544. DOI: https://doi.org/10.24925/turjaf.v10i12.2539-2544.5475

Tarapatskyy M, Sowa P, Zaguła G, Dzugan M, Puchalski C. 2021. Assessment of the botanical origin of polish honeys based on ˙ physicochemical properties and bioactive components with chemometric analysis. Molecules, 26(16):4801. DOI : https://doi.org/10.3390/molecules26164801

Thrasyvoulou AT. 1986. The Use of HMF and Diastase as Criteria of Quality of Greek Honey. J. Apic. Res., 25:186-195. DOI: https://doi.org/10.1080/00218839.1986.11100715

Tsavea E, Fotini-Paraskevi V, Elisavet S, Abdessamie K, Dimitrios K, Bucekova M, Grigorakis S, Godocikova J, Gotsiou P, Dimou M, Loupassaki M, Remoundou I, Tsadila C, Tilemachos G, Majtan J, Tananaki C, Alissandrakis E, Mossialos D. 2022. Physicochemical Characterization and Biological Properties of Pine Honey Produced across Greece. Foods, 11(7):943. DOI: https://doi.org/10.3390/foods11070943