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STUDY OF THE QUALITY (PHYSIOCHEMICAL PARAMETER) OF HONEY CARRIED OUT IN THE ANNABA REGION (NORTH-EASTERN ALGERIA)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author AB designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author BF managed the analyses of study and author NA manages the literature searches of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The most widely used honey bees for beekeeping are European races of Apis mellifera, species of honey bees that also originate from Africa and the Middle East.
Honey is a product largely known and used in our country. However, in Algeria honey is consumed in great quantity. This natural product is not sowing with a quality control and the consumer is exposed at the risks of health. In the Européenne and American scale there is an international commission of quality control and which definite standards of quality of honey. In this context we proposed to analyze some varieties of honey collected in the Algerian East in the bee-keeping occupies a place of choice in agriculture and two imported varieties. In addition Algeria imports each year of the considerable quantities of honey.
This research task thus made it possible to study certain properties Physicochemical (pH, moisture, conductivity, HMF, an acidity). These allow us to identify the different properties of the honeys harvested in the Annaba region.

Keywords: Honey; physiochemical parameter; *Apis mellifera*.

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1. INTRODUCTION

Bees play a role in pollination throughout the ecosystem. They are regarded as the world's primary pollinators [1,2]. Furthermore; They are important for sustainable agriculture since they produce honey and other natural items [3,4]. The bee obtains all of its necessities from its surroundings, including pollen, nectar, and water. As a result, the quality of the honey it produces reflects the natural circumstances of the locations where the hive is found [5].

Honey is an aqueous, sweet, and acidic solution that will degrade over time, undergo transformations, and adjust its physico-chemical properties [6,7]. Honey is used to treat wounds, burns, respiratory inflammations, and many other conditions due to its antiseptic, healing, and anti-inflammatory properties [8,9].

The majority of wilayas in Algeria have a very rich beekeeping culture about 30.000 tons of honey are produced annually. It falls short of what is required for local consumption [10].

The marketing of honey continues to be unregulated and, as a result, the consumer's health is not protected in the lack of clear-cut legislation on both the one hand and the other.

Currently, there are several theories in Algeria about the origin and physico-chemical properties of honey.In contrast to the European Union and the United States of America, honey marketing is not regulated by law, hence there is no standard in place.

Our work fits inside this framework. The foundation of it is physico-chemical analysis. These enable us to determine the caliber and other physico-chemical characteristics of the honey gathered in the Annaba region.

2. MATERIALS AND METHODS

2.1 Study Region

In the northeastern region of Algeria is the city of Annaba. It is regarded as one of Algeria's most significant industrial areas.

The Annaba region also has a variety of relief and natural resources due to its location on a coastal plain and on the eastern edge of the Edough, a mountainous massif that rises to a height of 1008 m.

It has a Mediterranean climate, which is hot and dry in the summer and humid and moderate in the winter. The Annaba region has a wide selection of melliferous plants. More than one honey harvest can be realized each year due to this huge wealth (Wikipedia).

2.2 Method of Sample Collection

- The honey samples were mechanically extracted between January and April, and their classification and labeling by codes included.
- The honey's geographic origin.
- Its alleged botanical origin.

Fig. 1. Geographical location of the wilaya of Annaba (Google map)

Fig. 2. Uncapping

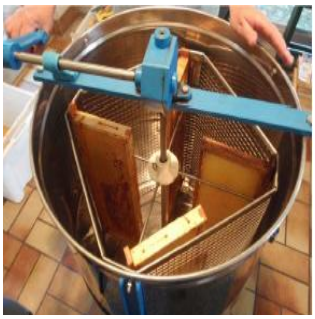

Fig. 3. Centrifugation

Fig. 4. At the exit of the extractor

Fig. 5. Filtration

Table 1. Presentation of the analyzed samples

Samples	Floral origin	Area
Honey 1	Thyme	Annaba
Honey 2	Wild	Annaba
Honey 3	Pegunum Harmal	Annaba
Honey 4	Eucalyptus	Annaba

2.3 Physico-chemical Analyses

2.3.1 Determination of water content (Chataway methods)

The water content within honey is the quality criterion that determines the capacity of honey to remain stable and to resist possible deterioration (fermentation, crystallization).

The water content of honey samples is determined by the optical measurement of the refractive index at 20°C.

- **Index of Refraction (IR):** A refractometer that had been calibrated with a drop of distilled water was used to conduct the analysis of the refractive index. The refractometer's prism is then covered with a drop of honey using a spatula. During the stabilization of the displayed value, the IR is read.

2.3.2 Determination of pH (AOAC, 1996 methods)

A pH meter was used to take a direct reading of the pH (Fig. 6).

- **Procedures for calculating pH:**

  - Set the pH meter's calibration using two buffer solutions (pH = 4 and pH = 7).
  - 10g of the honey sample should be dissolved in 75ml of distilled water before being shaken with a magnetic bar.
  - Dry the electrode with Joseph paper after rinsing it with distilled water.
  - Insert the pH meter's electrodes into the liquid.
  - The pH meter's screen is where the reading is taken.

2.3.3 Determination of the free acidity (AOAC, 1996 methods)

Through titration with sodium hydroxide (NaOH) solution, the free acidity was determined.

- **The procedures for calculating free acidity:** Increase the preparation's sodium hydroxide concentration by 0.1N until the pH is 8.30

(this point must be reached within 120 seconds from the starting of titration).

- ❖ **A statement of the findings:** The amount of free acid per kilogram of honey is measured in milliequivalents or millimoles of acid.

Free acidity =V1 × 10

V1: Volume of NaOH necessary to have a pH of 8.30.

2.3.4 Determination of the electrical conductivity (Vorwohl methods)

A material's electrical conductivity measures how easily electric current can flow through it. S/cm, or Siemens per centimeter, is the unit of measurement.

- ❖ The following are the steps to determine electrical conductivity:
  - Weighing 5g of honey in dry matter value based on its initial water content
  - The honey is dissolved in distilled water, and the mixture is poured into a test tube until it has a volume of 25 ml.
  - After thoroughly homogenizing the mixture with a manual mixer, submerge the electrode of the conductivity meter in the sample solution and manually set the instrument's temperature to 20° C.The reading is taken directly after the cell is immersed in the solution.

2.3.5 Determination of Hydroxy-Methyl-Furfural (HMF) (Winkler technique)

The idea is based on measuring the absorbance of HMF with a spectrophotometer first at a wavelength of 284 nm and subsequently at a wavelength of 336 nm.

Table 2. Physico-chemical parameters analyzed

Parameters	Objectives
Water content	Determines the quality of the product, the conditions of conservation, its weight and its crystallization. It conditions the shelf life.
PH	To know the origin of honey: -For honeydew honey, it is between 4.5 and 5.5. -For the honey's nectar, it is between 3,5 and 4,5.
Acidity	Gives strong indicators of the honey's state.
Electrical conductivity	Allows the determination of the botanical origin of honey.
HMF : Hydroxyl-Methyl-Furfural	This is an important parameter that indicates the freshness of honey.

Fig. 6. Ph meter

❖ **The steps involved in determining the HMF:**

- Put 5g of honey in a 50ml beaker.
- Mix and add 0.5ml of the CAREZ II solution after mixing and dissolving 5g of honey in 25ml of distilled water (Fig. 7).
- The obtained solutions are put in 50 ml flasks and filled with distilled water till the gauge line.
- These solutions are filtered using filter paper, and the first 10 mL of the filtrate should be thrown away (Fig. 8).
- 5 ml of the filtrate from each sample is placed into two tubes:
  5 ml of water are added to the first tube and blended (sample solution).
  (Fig. 9). 5 ml of bisulphite solution (0.2%) are added and combined in the second tube (reference solution)
- The spectrophotometer's screen is used for the reading (Fig. 10).

The following formula is used to determine the HMF content:

HMF (mg/kg of honey) = (A284-A336 X 149, 7 X 5)/P

Results expressed as: The amount of HMF is measured in milligrams per kilogram of honey (mg/kg), where A284 represents absorbance at 284 nm, A336 represents absorbance at 336 nm, and 149,7 represents a constant.

3. RESULTS

The findings of the tested samples' water content are shown in Fig. 11.

These values are much below the 20% maximum limit advised by Codex Alimentarius [11].

The pH of the honey used in the study samples can be observed by looking at Fig. 12.

The obtained pH values range from 4.15 (M 2) to 4.73. (M3).

The results of our tested samples of honey's free acidity are reported in Fig. 13.
The tested honey samples have free acidity values ranging from 11 meq/kg to 23 meq/kg.

The outcomes of the examined honey samples' electrical conductivity are shown in Fig. 14.
This graph shows that the samples' electrical conductivity values range from 381.63 s/cm (M 1) to 590.96 s/cm (M 4).

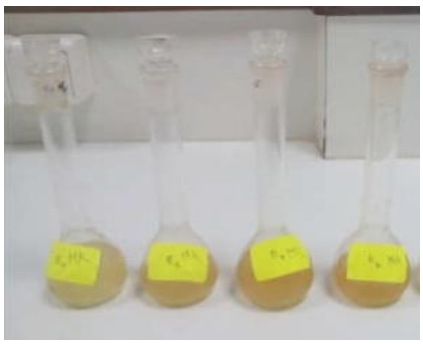

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Figs. 7-10. Instruments used for the study

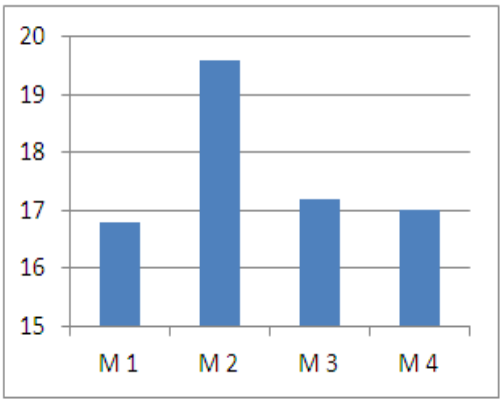

Fig. 11. The moisture content of the samples

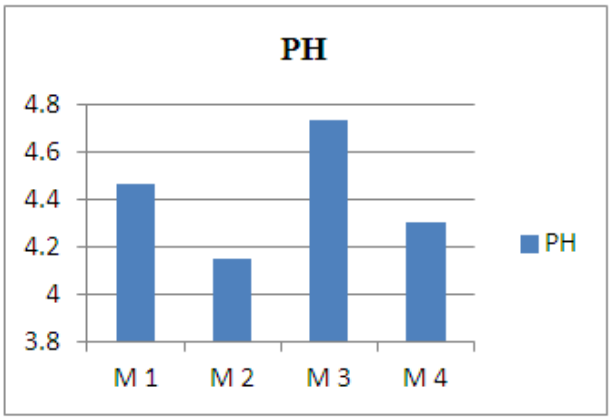

Fig. 12. pH of honey samples

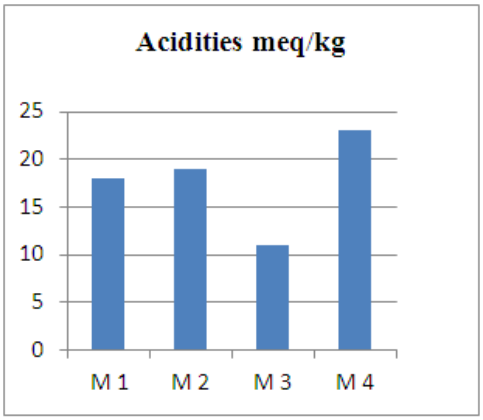

Fig. 13. Acidities of honey samples

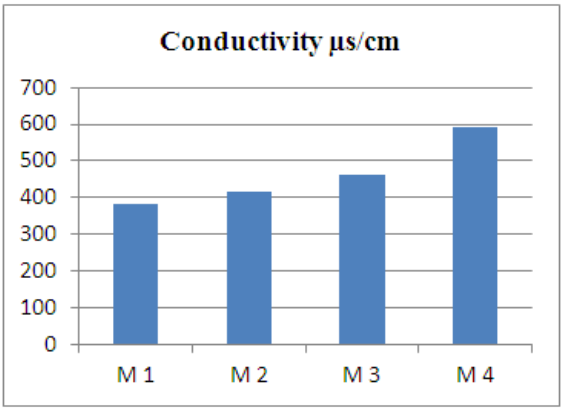

Fig. 14. Electrical conductivity of analyzed honeys

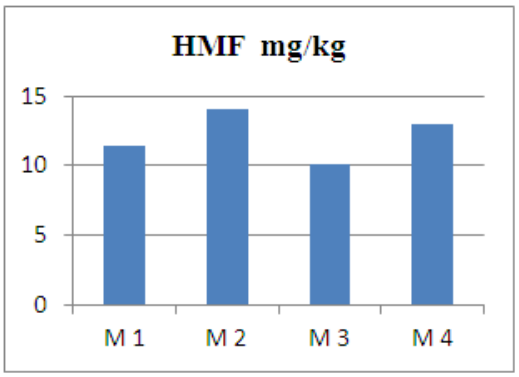

Fig. 15. HMF content of analyzed honeys

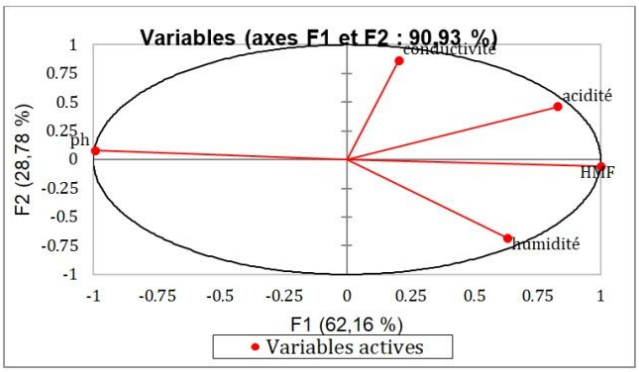

Fig. 16. Principale component analysais

Our honey samples' HMF concentration is described in Fig. 15.

The analysis of the data reveals that the HMF ranges from 10.10 mg/kg to 14 mg/kg. Honey's hydroxymethylfurfural content shouldn't be higher than 40 mg/kg. The HMF content should not, however, exceed 80 mg/kg for honeys with a declared provenance from tropical regions or nations that contain blends of these honeys .

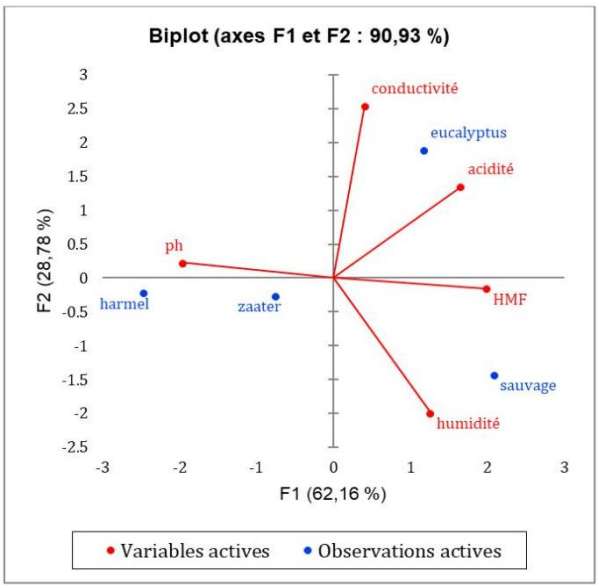

Fig. 17. Biplot showing variable and observational actives

Observation graph: The observation circle reading demonstrates a positive relationship between the physico-chemical variables HMF and moisture, as well as electrical conductivity and acidity.

HMF, on the other hand, has a negative correlation with pH but no correlation with electrical conductivity.

While the HMF is unrelated to the electrical conductivity, the pH has a negative correlation with it.

The graph of the observations shows that the wild honey and the eucalyptus honey, which is an antiseptic honey for the respiratory and urinary tracts, affections affecting the respiratory cavity and the urinary tree as a whole, are closer in terms of the majority of the studied physico-chemical parameters, particularly the HMF and free acidity [12,13].

Although Zaater (Thyme) honey and Harmal honey, which is used to treat neurological symptoms prior to convulsions [14], are extremely similar in terms of quality parameter "pH," Zaater honey (Thyme) is a great antibacterial, anti-infectious, and physical and psychological stimulator [15]. They are not, however, the same as eucalyptus honey or wild honey.

4. CONCLUSION

The various honey samples gathered in the Annaba region for this investigation all satisfied the required physico-chemical standards.

The honey varieties under study are more stable against fermentation during storage according to the measurement of their water content. In comparison to the Spanish honey variety, the results obtained indicate that none of the tested honey varieties contain a moisture level more than 20%.

Since bacteria cannot grow in an acidic environment, honey's acidic pH increases its antibacterial action [16-18]. All of the honey samples analyzed had an acidic pH range of 3.50 to 5.5.

The tested honeys have free acidity concentrations ranging from 11,000 to 23,000 meq/kg. These results show that there was no fermentation in the samples.

All of the investigated honey types are nectar honeys, according to the results of the electrical conductivity measurements.

The hydroxymethylfurfural (HMF) quality standard is used to evaluate degradations brought on by heat and storage [19,20]. The results from these two factors indicate that the honey from our area is new honey.

The PCA analysis shows that the eucalyptus honey and the wild honey are close in their quality estimated by the studied physicochemical parameters, whereas the zaater honey (thyme), which is an excellent antiseptic, anti-infectious, and a physical and psychological stimulator [21] as well as the harmal honey, are close in their qualities primarily by the parameter of the pH, which explains their use in the traditional medicine to treat the same pathological symptoms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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