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Korrespondenzadresse:
abelouahab.diafat@univ-bba.dz

¹⁾ Laboratory of characterization and valorization of the natural product Faculty of Nature and Life Science, University Elbachir Elibrahimi, Bordj Bou Arreridj, Algeria. ²⁾ Electrochemistry and Materials Laboratory, Ferhat Abbas University, Sétif, Algeria

Microbiological quality, physicochemical properties, pollen analyses and mineral contents of honeys from Bordj Bou Arreridj region (Algeria)

Mikrobiologische Qualität, physikalisch-chemische Eigenschaften, Pollenanalysen und Mineralstoffgehalt von Honigen aus der Region Bordj Bou Arreridj (Algerien)

Abdelouahab Diafat¹⁾, Hammoudi Mekhalfi¹⁾, Mounira Dehiri¹⁾, Abdelmalek Meribai¹⁾, Farid Bouaziz¹⁾, Ahmed Bahloul²⁾

Summary

The present study aimed to characterize 30 honey samples from Bordj Bou Arreridj region (Algeria) in respect to their floral origins, physicochemical parameters, mineral composition and microbial safety. Mean values obtained for physicochemical parameters were: pH 4.11, 17.17% moisture, 0.0061% ash, 370.57 $\mu\text{S cm}^{-1}$ electrical conductivity, 21.98 meq/kg free acidity, and 9.703 mg/kg HMF. The mineral content was determined by atomic absorption spectrometry. The mean values obtained were (mg/kg): Fe, 7.5714; Mg, 37.68; Na, 186.63; Zn, 3.86; Pb, 0.4869×10^{-3} ; Cd, 267×10^{-3} . Aerobic mesophiles, fecal coliforms and sulphite-reducing clostridia were the microbial contaminants of interest studied. Microbiologically, the honey quality was considered good and all samples showed to be negative in respect to safety parameters. The results obtained for physicochemical characteristics of Bordj Bou Arreridj honey indicate a good quality level, adequate processing, good maturity and freshness.

Keywords: Bordj Bou Arreridj honey, pollen analysis, physicochemical analysis, mineral content, microbial contaminants

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Introduction

Honey is a sweet natural substance, produced by bees, from the nectar of flowers, or honeydew collected from plants (Silva et al., 2009). Honey mainly contains simple sugars or monosaccharides, Proteins, flavour and aroma, phenolic compounds (phenolic acids and flavonoids), free amino acids, organics acids, vitamins and minerals constitute minor components of honeys (González-Miret et al., 2005). Honey varies greatly in quality all over the world. This is largely assessed on the basis of colour, flavour and density. Honey composition is influenced by the plant species, climate, environmental conditions and the contribution of the beekeeper (Anklam E., 1998; Azeredo et al., 2003), it is used for its nutritional and therapeutic properties.

The bee is in permanent contact with our natural environment. This is polluted by various emissions resulting mainly from human activity. Three types of contaminants are controlled: pesticides, colony treatment products and heavy metals. the bee can be used as a biological indicator of environmental pollution. Several pollutants can be found in pollen grains and nectar collected by bees.

Bordj Bou Arreridj region is located in the east of Algeria, being one of the most important region of honey production in this country, due to its climatic and edaphic conditions and plants diversity. The detailed characterization of the different honey type's existent in Algeria is important, once it will allow the establishment of technical specifications, avoiding occurrence of adulterations. Due to adulteration possibility, honey quality must be analytically controlled with the aim of guaranteeing its speculation. According to bibliographical research, no study has been carried out on chemical contamination by heavy metals, and microbiological in the region of Bordj Bou Arreridj; It is in this precise context that the objective of our study is situated. Which consists of carrying out a physico-chemical and microbiological analysis. For this purpose, pollen analysis was performed and physicochemical characteristics (pH, moisture, ash content, electrical conductivity, free, acidity, and hydroxymethylfurfural), mineral contents (Fe, Na, Zn, Pb, Cd and Mg) and the microbial contaminants were evaluated.

Materials and methods

Sample collection

30 samples of honey from different regions of bordj bou arreridj were collected. The samples taken under aseptic conditions are placed in sterile bottles at a temperature of 4°C until analysis.

Pollen analysis

The botanical origin of the samples was determined using the techniques described by Lutier et al. (1993). Five grams of honey were dissolved in 20 ml of warmed distilled water, not above 40°C. The solution was centrifuged for 10 min at 3000 rpm and the supernatant was drawn off. The sediment was dispersed again with 10 ml of distilled water and centrifuged again. The sediment was put on a slide and spread out. After drying, the sediment was covered with a liquefied Kaiser's glycerin-gelatin and a cover glass. The slides were examined microscopically at 45×, using a bright-field microscope (Optika, B-500 TPL, Italy).

Physicochemical characteristics

Honey were analyzed according to methods previously reported for pH, moisture, ash content, electrical conductivity, free acidity, hydroxyl methyl furfural, (AOAC, 1990).

pH and free acidity

The pH was measured by an Inolab pH-meter, with a precision of ± 0.002 pH units, as a solution of 10% (W/V) in distilled water. Free acidity was determined by titrimetric method; the addition of 0.05 M NaOH was stopped at pH 8,5.

Moisture content

The simplest and most reproducible technique for measuring the humidity level in honey is refractometry (Bogdanov, 2002). The water content is a value which is determined from the refractive index (RI) of the honey by referring to a standard table (Chataway table).

Ash

Ash content was measured by calcinations, 5g of each honey sample was weighed (M0), then the samples are placed in a furnace at 600°C/8h until clear or white ashes are obtained (M1).

Hydroxymethylfurfural content (HMF)

The Winkler method (Winkler, 1955) was used to determine the HMF content in honey samples: 5g of each sample was treated with a clarifying agent (Carrez solution). The volume was completed to 50ml and the solution was filtered. The absorbance of the filtered solution was measured at 284 and 336nm against an aliquot treated with NaHSO₃.

Electrical conductivity

Electrical conductivity of a honey solution at 20% (dry matter basis) in distilled water was measured at 20°C in a WTW, GmbH conductimeter, and the results were expressed as mS.cm⁻¹.

Insoluble solids

The gravimetric method was used for the determination of the insoluble solids according to the Bogdanov (2002) method. Honey samples (10 g) were diluted with the minimum amount of water at 40°C. Then the solution is transferred to test tube and the electrode of pH-meter is plunged in the solution and without ceasing agitation The solution of hydroxide of sodium NaOH (0.1 N) is added drop by drop by the pipette until obtaining a pH ranging between (7 to 9) so as to preventing that the proteins which are constituent normal honey do not clog the filter. The solution is filtered on cellulose ester membrane of a diameter of 25mm and porosity of 5µm. to porous previously weighed crucibles. After this procedure, the samples were flushed with distilled water at 80°C to remove all sugars. The porous crucibles were placed in an oven at 135°C for 1 hour and then cooled and weighed. Results expressed in percentages.

Analysis of honey color intensity

The color of honey was determined by spectrophotometry. Honey samples were heated to 50°C and the color was determined by spectrophotometer by measuring the absorbance of a 50% (W/V) honey solution. Absorbance is calculated from the difference between A450–A720 nm. Honeys are classified according to the Pfund balance after absorbance conversion according to White, (1984).

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Determination of mineral elements

Ash obtained by calcinations was dissolved by 5ml of nitric acid 0.1M, and the mixture was heated until dryness. the residue was then, taken up in 10ml of nitric acid and the mixture was made up to 25ml with distilled water, sodium and magnesium were determined by atomic absorption spectrometry, using an air/acetylene flame. Quantitative determination of the elements by atomic absorption spectrometry was carried out after calibrating the instrument, using Fe (1 to 4ppm), Na (10 to 40ppm), Zn (1 to 40ppm) and Mg (10 to 60ppm), Pb (1 to 4ppm), Cd (1 to 4ppm).

Microbial contamination

5g of each honey sample were homogenized into 90mL with peptone water solvent. Decimal dilutions were made into the same solvent. Aerobic mesophilic bacteria were counted onto standard plate count agar (PCA) and incubated at 30°C for 48h (NP-788:2002). Moulds and yeasts counts followed the protocol of ISO 21527-2:2008. Microbial counts were expressed as colony-forming units per gram of honey (cfu/g).

Enumeration of total mesophilic aerobic flora (FAMT) Microorganisms can grow in a non-selective nutrient medium. incubated at 30°C for 72 hours. Appear as colonies of different size and shape. Enumeration of total and faecal coliforms This research was carried out on BCPL broth (Bromo Cresol Purple Lactose), enrichment medium for total coliforms; by inoculation of 01ml from dilutions 10⁻¹, 10⁻² and 10⁻³. The reading was carried out after 48 hours of incubation at 37°C.

The faecal coliforms were counted on the VRBG agar, by spreading 01ml, from the positive BCPL tubes, after incubation for 48 hours at +44°C.

Escherichia coli research From a BCPL positive tube, inoculate a 10ml tube containing indole-free peptone water with 1ml. Incubate at 44°C for 24h. After incubation, add a few drops of KOVACS reagent to the tube.

The search for faecal streptococci, Rothe medium was used. By inoculation of 1ml of the stock solution and dilutions) and the inoculation of the positive tubes in the confirmation medium: Eva Litsky. The reading was carried out after 24 hours of incubation at 37°C.

TABLE 1: Distribution data for physicochemical parameters in Bordj Bou Arreridj (Algeria) honey samples.

| Honey samples | pH | Free acidity (meq/kg) | Lactonic acidity (meq/kg) | Total acidity (meq/kg) | Electrical conductivity (µS cm ⁻¹) | Moisture (%) | color (mm Pfund) | insoluble matter (mg/100g) | Ash (%) | Hydroxymethyl furfural HMF (mg/kg) |
|-----------------|------|-----------------------|---------------------------|------------------------|--|--------------|------------------|----------------------------|---------|------------------------------------|
| H ₁ | 4,0 | 17,5 | 482,45 | 49,95 | 485,13 | 19,4 | 405,54 | 18 | 0,019 | 13,88 |
| H ₂ | 3,7 | 21 | 477,29 | 49,29 | 201,52 | 15,2 | 208,92 | 19 | 0,017 | 7,140 |
| H ₃ | 4 | 20,5 | 479,39 | 49,89 | 482,39 | 14,8 | 400,22 | 18 | 0,003 | 8,016 |
| H ₄ | 3,8 | 26 | 473,94 | 49,94 | 463,24 | 18 | 457,44 | 17,5 | 0,185 | 22,83 |
| H ₅ | 3,9 | 19 | 480,96 | 49,96 | 318,25 | 17,4 | 253,84 | 17 | 0,057 | 12,47 |
| H ₆ | 3,6 | 25 | 474,93 | 49,93 | 389,38 | 18 | 237,87 | 18 | 0,475 | 9,393 |
| H ₇ | 4,0 | 35 | 464,94 | 49,94 | 391,2 | 18 | 851,68 | 21 | 0,023 | 15 |
| H ₈ | 4,81 | 22 | 477,94 | 49,94 | 297,27 | 16 | 334,35 | 19 | 0,005 | 9,73 |
| H ₉ | 4,5 | 19,5 | 480,45 | 49,90 | 375,7 | 18 | 655,73 | 16,5 | 0,029 | 15,62 |
| H ₁₀ | 4,06 | 29 | 470,93 | 49,93 | 207,91 | 17,2 | 188,63 | 20,5 | 0,014 | 10,32 |
| H ₁₁ | 4,69 | 20 | 474,94 | 49,94 | 337,4 | 19 | 205,6 | 18,5 | 0,018 | 16,16 |
| H ₁₂ | 4,02 | 18,5 | 481,44 | 49,94 | 351,99 | 16 | 213,91 | 22,5 | 0,004 | 0,134 |
| H ₁₃ | 3,84 | 24,5 | 475,46 | 49,96 | 271,74 | 15,4 | 129,74 | 20 | 0,004 | 20,34 |
| H ₁₄ | 4,13 | 24,5 | 475,44 | 49,94 | 245,3 | 16,8 | 222,56 | 21 | 0,013 | 9,588 |
| H ₁₅ | 4 | 25 | 475,42 | 50,42 | 243,47 | 15,2 | 454,78 | 20 | 0,009 | 11,90 |
| H ₁₆ | 4,71 | 16 | 480,90 | 49,9 | 325,54 | 18,4 | 140,39 | 14,5 | 0,039 | 8,173 |
| H ₁₇ | 4,26 | 22 | 477,94 | 49,94 | 580,88 | 17,2 | 531,97 | 20 | 0,048 | 5,082 |
| H ₁₈ | 3,92 | 19,5 | 480,45 | 49,95 | 258,06 | 18,8 | 429,17 | 12 | 0,013 | 0,157 |
| H ₁₉ | 3,61 | 17,5 | 482,44 | 49,94 | 163,23 | 13 | 732,58 | 13,5 | 0,005 | 0,538 |
| H ₂₀ | 4,97 | 18 | 482,39 | 50,39 | 235,27 | 15,6 | 403,55 | 15,5 | 0,010 | 5,164 |
| H ₂₁ | 3,89 | 19 | 480,96 | 49,96 | 234,35 | 19,2 | 318,05 | 12,5 | 0,014 | 12,49 |
| H ₂₂ | 4,63 | 19,5 | 480,46 | 49,96 | 223,41 | 14 | 473,08 | 12 | 0,013 | 4,19 |
| H ₂₃ | 3,61 | 26 | 437,94 | 46,94 | 507,13 | 22,2 | 233,88 | 15,5 | 0,169 | 9,917 |
| H ₂₄ | 4,8 | 20 | 481,25 | 50,25 | 660,21 | 18 | 356,97 | 11 | 0,018 | 7,073 |
| H ₂₅ | 4,02 | 18,5 | 482,55 | 50,05 | 424,03 | 16,8 | 425,17 | 11 | 0,008 | 13,60 |
| H ₂₆ | 3,67 | 20 | 480,85 | 50,85 | 203,35 | 16,4 | 665,04 | 9,5 | 0,061 | 8,420 |
| H ₂₇ | 3,84 | 30 | 471,65 | 50,65 | 434,03 | 16,8 | 223,9 | 8 | 0,019 | 10,89 |
| H ₂₈ | 4,15 | 22,5 | 478,35 | 50,85 | 539,84 | 15,6 | 646,71 | 11,5 | 0,53 | 8,353 |
| H ₂₉ | 3,92 | 22 | 475,54 | 49,54 | 375,70 | 21,6 | 233,88 | 17,5 | 0,001 | 7,230 |
| H ₃₀ | 4,1 | 22 | 479,25 | 50,25 | 890,39 | 17,2 | 414,19 | 14,5 | 0,021 | 7,230 |
| Medium | 4,11 | 21,98 | 476,62 | 49,94 | 370,57 | 17,17 | 381,64 | 16,16 | 0,061 | 9,703 |

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For research sulphite-reducing Clostridia after a thermal shock, of 20ml of the stock solution, at 80°C. for approximately 10min, then rapid cooling, under a stream of cold water. The volume of the heated honey solution is sterile poured into a tube containing liver meat agar (VF), previously melted and added with two additives (iron alum and sodium sulphite), and incubated at 37°C. Readings were taken after 24, 48 and 72 hours.

Detection of staphylococcus aureus 1ml of the stock solution and dilutions 10^{-2} , 10^{-3} , were inoculated, in Giolliti Cantoni broths, after incubation for 24 hours at 30°C, 1ml of each tube, is kept under super cooling, homogenization has was performed on Chapman Stone Agar. Counting is carried out after 24 hours of incubation at 30°C.

The search for salmonella, inoculation of a series of tubes containing Tetrathionate solution. From decimal dilutions 10^{-1} , 10^{-2} , 10^{-3} , aseptically transfer of 1ml to each of the three. After incubation at 37°C/24h. Confirmation from positive tubes (toning, turbidity of the medium) on agar, incubation at 44°C/24 h.

TABLE 2: Distribution data for cationic mineral content in Bordj Bou Arreridj honey samples.

| Samples | Fe (PPM) | Mg (PPM) | Na (PPM) | Zn (PPM) | Pb (PPb) | Cd (PPb) |
|-----------------|----------|----------|----------|----------|----------|----------|
| H ₁ | 4,623 | 41,2 | 14,8 | 9,1 | 0,0107 | 32,1 |
| H ₂ | 2,3495 | 31,2 | 21,25 | 1,25 | 0,136 | 31,2 |
| H ₃ | 3,941 | 33,35 | 17,05 | 3,8 | 5,2255 | 43,3 |
| H ₄ | 5,76 | 46,75 | 32,9 | 5,85 | 0,0885 | 23,25 |
| H ₅ | 5,5325 | 42,6 | 22,35 | 2,15 | 0,103 | 17 |
| H ₆ | 7,276 | 113,15 | 15,9 | 5,6 | 1,0845 | 16,35 |
| H ₇ | 15,3855 | 178,5 | 21,26 | 3,3 | 0,107 | 12,15 |
| H ₈ | 7,655 | 35,1 | 4,25 | 1,65 | 0,107 | 40,8 |
| H ₉ | 18,6445 | 63,2 | 40,45 | 7,95 | 0,163 | 7,25 |
| H ₁₀ | 5,1535 | 27,15 | 14,85 | 1,8 | 0,299 | 12,7 |
| H ₁₁ | 3,7895 | 24,15 | 17,05 | 2,25 | 0,536 | 84,9 |
| H ₁₂ | 4,2445 | 11,48 | 26,6 | 3,8 | 0,3915 | 57,63 |
| H ₁₃ | 4,6223 | 57 | 22,35 | 1,05 | 0,0825 | 57,45 |
| H ₁₄ | 4,6223 | 17,95 | 19,15 | 3,15 | 0,2555 | 13,45 |
| H ₁₅ | 4,8505 | 25,95 | 26,6 | 1,6 | 0,5255 | 6,75 |
| H ₁₆ | 4,699 | 25,4 | 16 | 1,75 | 0,4535 | 11,05 |
| H ₁₇ | 12,2025 | 50,15 | 20,2 | 4,7 | 0,569 | 16,35 |
| H ₁₈ | 4,2445 | 47,3 | 9,55 | 2,05 | 0,5465 | 94,35 |
| H ₁₉ | 1,895 | 17,65 | 4,25 | 4,55 | 0,3935 | 20,45 |
| H ₂₀ | 7,3515 | 28,3 | 21,25 | 2,35 | 0,75 | 42,35 |
| H ₂₁ | 23,6465 | 42,9 | 14,8 | 10,75 | 0,0555 | 8,7 |
| H ₂₂ | 6,594 | 42,45 | 15,9 | 3,85 | 0,235 | 27,45 |
| H ₂₃ | 4,8505 | 14 | 19,15 | 3,25 | 0,0765 | 14,1 |
| H ₂₄ | 5,9875 | 57,25 | 7,4 | 6 | 0,07 | 5,45 |
| H ₂₅ | 9,777 | 30,15 | 21,25 | 5,55 | 0,062 | 28,1 |
| H ₂₆ | 2,7285 | 13,85 | 17,85 | 1,9 | 1,3955 | 17,25 |
| H ₂₇ | 10,3835 | 4,9 | 16,95 | 2,65 | 0,2245 | 12,2 |
| H ₂₈ | 7,503 | 6,9 | 14,85 | 6,1 | 0,0455 | 90,7 |
| H ₂₉ | 6,518 | 0,35 | 23,4 | 1,65 | 0,563 | 83,3 |
| H ₃₀ | 20,312 | 0,15 | 21,35 | 4,4 | 0,0535 | 23,05 |
| Medium | 7,5714 | 37,68 | 16,97 | 3,86 | 0,4869 | 23,19 |

Results and discussion

The floral origin of honey samples were determined by microscopy. Pollen analyses data indicate that 100% of honey samples were multifloral.

The results obtained for the physico-chemical parameters are presented in (Table 1). PH is a useful index to control the quality of honey. The pH of honey varies with storage conditions, extraction methods, which also influence texture, shelf life and stability.

The pH values of our samples vary between 3.61 and 4.97 with an average of 4.11. These results are mentioned in (Table 1), The normal pH level varies between 3 and 6 (Bogdanov et al., 1997). So all honeys are acidic. Our results agree with the results reported by Silva et al (2009). Who found pH values between 3.45 to 4.70 and reports that the acidity of honey is due to the presence of organic acids, in particular gluconic acid and inorganic ions. Thus honeys from nectar have a pH between 3.5 and 4.5, on the other hand those preventing honeydews are between 5 and 5.5.

The pH values of the analysed honey samples ranged from 3.61 to 4.97 (mean value=4.11). These values are in accordance with acceptable range for honey (Bogdanov, 1999), and similar to those obtained with others Algerian honeys (Ouchemoukh et al., 2007).

Percent moisture in the analyzed honeys ranged from 13 to 22,2% (mean value=17,17%). Several factors influence the water content of honey such as the degree of maturity reached in the hive, the harvest season, and climatic factors. the maximum rate of water in honey is regulated for safety against fermentation. All the samples (28 of the 30 samples analysed) contain less than 20% water, the maximum amount allowed by international legislation.

Ash content is a parameter used for the determination of the botanical origin (floral, mix or honeydew) (White, 1978). The results found (0,001–0,475%) are within the limit allowed for floral honeys (0.6%), indicating clearness of honey samples and possibly lack of adulterations with molasses (Mendes et al., 1998).

The electrical conductivity of honey is an important parameter for the classification of honey; it is related to the concentration of mineral substances, proteins and organic acids. This parameter varies according to the floral origin (Terrab et al., 2002). The results obtained for the honey samples studied vary between 163,23 and 890,39 $\mu\text{S cm}^{-1}$ (average= 370.57 $\mu\text{S cm}^{-1}$). These values are below the maximum limit indicated by European legislation for nectar honey (800 $\mu\text{S cm}^{-1}$) (EUD, 2002) (Table 1).

The values of the electrical conductivity that we obtained are between 163.23 and 890.39 $\mu\text{S/cm}$ with an average of 370.57 $\mu\text{S/cm}$. the results from this analysis are presented in (Table 1). Vorwohl (1964) identifies the relationship between the electrical conductivity of honeys and their floral origin. He states that electrical conductivity seems to be a characteristic of the plant species from which the honey comes. Honeys of the same floral origin have approximately the same conductivity, even if they come from different harvest years and geographical and climatic regions (Gonnet, 1986). Specifies that honeys from nectar have an EC between 100 to 500 $\mu\text{S/cm}$ and those from honeydew between 1000 to 1500 $\mu\text{S/cm}$, on the other hand the median values correspond to mixtures of nectar and honeydew.

Honey acidity is due to the presence of organic acids, mainly gluconic acid, in equilibrium with their corresponding lactones or internal esters, and to inorganic ions. Free acidity was within the limits of European legislations (below 50meq/kg), indicating the absence of undesirable fer-

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mentation. Free acidity varied between 17.0 and 51.5 meq/kg, with a mean value of 49.94 meq/kg. The results obtained for acidity were in agreement with data reported for other Algerian honeys (Ouchemoukh et al., 2007).

The values of the free acidity of our samples of honey vary between 16 and 35 meq/kg with an average of 21.98 meq/kg, it lower than 50 meq/Kg, the values of the acidity are mentioned in the (Table 1). These results agree with the values found by Silva et al., (2009).

HMF content is widely recognized as parameter of freshness for honey samples. Several factors influence the formation of HMF, such as storage conditions (e.g. temperature) and floral sources (Terrab et al., 2002; Fallico et al., 2004). The HMF level of our samples varies between 0.1347 and 22.83 mg/kg with an average of 9.462 mg/kg. The HMF content should not exceed 40 mg/kg in order to preserve the honey's dietary and therapeutic quality. It is well known that honey heating results in the formation of HMF, which is produced during acid-catalysed dehydration of hexoses, such as fructose and glucose (Belitz and Grosch., 1999).

Mineral elements

Mineral salts are very important elements of honey such as magnesium and sodium, although these elements are present in moderate amounts in the honey samples, with average contents of 37.68 and 16.97 ppm, respectively (Table 2).

The mineral content is an important index of possible environmental pollution and a potential indicator of geographical origin of honey (Anklam, 1998). The results of the cationic metals determined in Bordj Bou Arreridj region (Algeria) honey samples are summarized in (Table 2).

Honey is known to accumulate trace metals, cadmium, nickel, and lead which are known as toxic metals, and other essential metals like zinc, copper, manganese and chromium are important elements for human health and development (Hernández et al., 2005; Pohl, 2009). On the other hand, excessive consumption of these elements can cause chronic toxicity (Ashenef, 2014). This is a very important parameter for the biomonitoring of the environment where bees live. These bees come into contact not only with air but also with soil and water, the concentration of heavy

TABLE 3: Microbial analyses of honey sample.

| Honey samples | FTAM Eucaryotes UFC/g | FTAM Procaryotes UFC/g | Staphylococcus UFC/g | Streptococcus UFT/g | Total coliforms UFT/g | Fecal coliforms UFC/g | E. coli | Salmonella | CSR |
|-----------------|-----------------------|--------------------------|-------------------------|---------------------|-----------------------|------------------------|---------|------------|-----|
| H ₁ | IND | 0 | 0 | 0,3 | 4.0 | 0 | - | - | - |
| H ₂ | 0 | 0 | IND | 0,3 | 1.5 | 0 | - | - | - |
| H ₃ | 0 | 0 | IND | 0,3 | 2.5 | 0 | - | - | - |
| H ₄ | 0 | 90.9 x 10 ² | 0 | 140 | 140 | 0 | - | - | - |
| H ₅ | 0 | 0 | IND | 0 | 2.5 | IND | - | - | - |
| H ₆ | 0 | 0 | 0 | 110 | 140 | 0 | - | - | - |
| H ₇ | 0 | 400 | 0 | 0 | 4.5 | 0 | - | - | - |
| H ₈ | 0 | 74.54 | IND | 25 | 140 | 0 | - | - | - |
| H ₉ | 0 | 120 x 10 ² | 0 | 45 | 16 | IND | - | - | - |
| H ₁₀ | 0 | 0 | 30.18 x 10 ² | 0,3 | 1.1 | 0 | - | - | - |
| H ₁₁ | 0 | 0 | 0 | 0 | 4.0 | 21.6 x 10 ³ | - | - | - |
| H ₁₂ | 0 | 0 | 0 | 0,3 | 9.5 | 0 | - | - | - |
| H ₁₃ | 0 | 0 | 0 | 0,6 | 140 | 0 | - | - | - |
| H ₁₄ | 0 | 0 | IND | 0 | 25 | IND | - | - | - |
| H ₁₅ | 0 | 0 | 0 | 45 | 1.4 | 0 | - | - | - |
| H ₁₆ | 0 | 0 | 0 | 0,6 | 30 | 0 | - | - | - |
| H ₁₇ | 0 | 0 | 0 | 1,6 | 25 | 0 | - | - | - |
| H ₁₈ | 0 | 0 | 0 | 0 | 2.5 | 0 | - | - | - |
| H ₁₉ | 0 | 1181.8 | 0 | 0,3 | 140 | 0 | - | - | - |
| H ₂₀ | 0 | 0 | 0 | 0,3 | 16 | 0 | - | - | - |
| H ₂₁ | 0 | 0 | 0 | 0 | 140 | 0 | - | - | - |
| H ₂₂ | 310.10 ⁻² | 0 | 0 | 0,6 | 3.5 | 0 | - | - | - |
| H ₂₃ | 0 | 0 | 0 | 0,3 | 1.4 | 0 | - | - | - |
| H ₂₄ | 0 | IND | 0 | 140 | 30 | 0 | - | - | - |
| H ₂₅ | 0 | 400 | 0 | 0,7 | 45 | 0 | - | - | - |
| H ₂₆ | 0 | 2120 | 0 | 0,4 | 1.1 | 0 | - | - | - |
| H ₂₇ | 0 | 0 | 0 | 0 | 140 | 0 | - | - | - |
| H ₂₈ | 0 | 0 | 0 | 0,6 | 140 | 0 | - | - | - |
| H ₂₉ | 0 | 106.36 x 10 ² | 0 | 4 | 16 | 0 | - | - | - |
| H ₃₀ | 300 | 3672.7 | IND | 6,5 | 140 | 0 | - | - | - |
| % | 10 | 33 | 23 | 76 | 100 | 13 | 0 | 0 | 0 |

IND: uncountable

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metals in honey reflects their amount in the environment. Therefore, honey has been recognized as a biological indicator of environmental pollution (Silici et al., 2008).

Cadmium was detected in all honey samples ranging from 6,75–94,35PPB. The mean level of Cd in present studied honey samples 23,19PPB did not exceed the limit established by Codex Alimentarius Commission, 0,05 $\mu\text{g g}^{-1}$ (Codex Alimentarius Commission Standards, 2002), its less then obtained from Egypt, 0,01–0,5 $\mu\text{g g}^{-1}$ (Rashed & Soltan, 2004), and Turkey Central Anatolia, 0,09–0,24 $\mu\text{g g}^{-1}$ (Leblebici & Aksoy, 2008).

Lead is a very toxic heavy metal for humans and the environment, lead contamination may be due to the presence of highways, the presence of metal workshops, house construction tools and the extensive use of fertilizers. The lead level in honey from Bordj Bou Arreridj (Algeria) was detected in all of the analyzed samples ranging from 0,0107 to 5,22PPB with a mean 0,4869PPB. honey was lower than the mean concentration reported by Chudzinska, (2010) Poland, LD-9,20 $\mu\text{g g}^{-1}$, Turkey, 0,451 $\mu\text{g g}^{-1}$ (Özcan et al., 2006), Turkey central Anatolia, 0,02–1,50 $\mu\text{g g}^{-1}$ (Leblebici & Aksoy, 2008).

Microbial contamination

Levels of microbial contamination of honey samples are presented in (Table 3). Levels of quantification for the commercial quality parameters (aerobic mesophiles) in the analyzed honey samples are generally lower than those reported by other authors. Iurlina and Fritz (2005) found higher levels of contamination for both aerobic mesophiles (average 244 cfu/g) counts. In respect to sanitary quality (fecal coliforms) and safety (sulphite-reducing clostridia and Salmonella), all our samples were negative. In contrast, Iurlina and Fritz (2005) detected coliform contamination in one tested sample. Finola et al. (2007) reported that 70% of 23 honey samples were contaminated with sulphite-reducing clostridia.

Honey quality could be compromised by hygienic practices during harvesting and extraction as well as storage time and conditions (Snowdon and Cliver, 1996).

The total mesophilic aerobic flora always informs us about the hygienic quality of honey. Indeed, the total mesophilic aerobic flora oscillates between 74.54 and 120x10² cfu/g with an average of 84 cfu/g) in nearly 33% of the samples. However, the other samples gave negative results. This is probably due to the richness of honey in antimicrobial compounds, or its low water activity (Aw) constitutes a hostile environment for the development of these microorganisms (Carvalho et al., 2006; Voidarou, 2011).

The search for microorganisms, indicators of faecal contamination origin, makes it possible to judge the hygienic state of a food product. These microorganisms can contaminate the honey during the handling required for packaging, carried out in poor hygienic conditions (Fléché et al., 1997). The average obtained from the various samples analyzed at 37°C in our study is 87.2 cfu/g with a maximum of 140 cfu/g and a minimum of 1.1 cfu/g. These results are in agreement with those reported by Adenekan et al. (2012).

Escherichia coli is an Enterobacteriaceae lactose +, gasogen, our results were all negative. Our results agree with the data shown by Miriam et al. (2005). The presence of staphylococci in food represents a risk to human health, because certain strains, mainly belonging to the Staphylococcus aureus species, produce heat-labile toxins, the ingestion of which causes staphylococcal food poisoning (Buyser, 1996). These microorganisms can contaminate

honey during harvest handling, carried out in poor hygienic conditions (Fléché et al., 1997). The search for Staphylococcus aureus shows that all the samples are negative. the search for Sulphite-Reducing Clostridia (CSR), in the honey samples, shows the absence of these germs, despite the fact that the medium was very selective for them, could suggest that these samples were not contaminated by the telluric germs, which excludes any exogenous contamination. Our results are in agreement with those reported by Gomes (2010). Faecal streptococci are indicators of faecal contamination. The results of our samples oscillate between (0 UFT/g) and (140 UFT/g) with an average of (17.43 UFT/g). It is highly probable that the contamination of the honey by these germs took place during the manipulations necessary for the extraction; in the packaging of honey (Kitambala, 1998). The results of salmonella research, shows that all the samples analyzed gave a negative result, which indicates that there is no contamination by these germs, or their absence due to the antimicrobial effect. Our results are in agreement with those reported by Gomes (2010).

Conclusions

Honeys from Bordj Bou Arreridj region present a good level of quality, once all analysed samples are in agreement with the European honey directive (EUD, 2002), indicating adequate processing, good maturity and freshness. Five samples did not fit within European standards relative to the diastase activity, reflecting inadequate sample manufacture and/or storage.

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Conflict of interest

The authors hereby declare that they have no known conflict of interests could have appeared to influence the work reported in this paper. The authors have no competing interests to declare.

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Address of corresponding author:

Abdelouahab Diafat
Laboratory of characterization and valorization
of the natural product Faculty of Nature and Life
Science
University Elbachir Elibrahimi
Bordj Bou Arreridj
Algeria
abdelouahab.diafat@univ-bba.dz

Kontakte

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Ravenstraße 45 · 31061 Alfeld (Leine)
Postfach 16 42 · 31046 Alfeld (Leine)
Telefon (0 51 81) 80 02-0
Telefax (0 51 81) 80 02-55
E-Mail info@p-d-ges.de