Determination of Some Major and Minor Elements in the East of Morocco Honeys Through Inductively Coupled Plasma Optical Emission Spectrometry

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Abstract

Forty two honeys collected in the East of Morocco, were studied to determine the presence of the following minerals, Ca, Cd, Cu, Fe, K, Mg, Mn, Pb, Se and Zn. The honey samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP OES) technique. The elements with the highest frequency were K, Ca, Mg, Fe, Zn and Mn. Cadmium and Pb, the two most dangerous heavy metals for human health were detected in all samples. If the levels of Cd were below to the maximum residue limit of the European standards, the levels of Pb were not normal in some samples.

Key words: Mineral content, honey, ICP-OES, east of Morocco.

1. Introduction

The research concerning the determination of mineral content of honeys is increasing during the last years. The goal, of the same studies, is very variable:
Honey offer a potential as a dietary supplement and shows therapeutic features so, its important to know the levels of trace elements that are essential to health.
The concentrations of inorganic species present in honey vary according to the resources in the soil and to the kind of plants from witch the bees took nectar, so it can establish the geographic origin of honey. The need for finding reliable marker compounds to characterize a certain type of honey and show adulteration in this substance is obvious [1], some mineral elements could have this character.
Some over, several studies have indicated that honey can be used as a biological indicator of environmental pollution, especially for heavy metals [2]. Honey bees have access to an area of about 50 Km2. Therefore, they represent an excellent system of small samplers [3].

Different analytical methods have been developed to determine many major and trace elements, with atomic absorption spectrometry [4], [5], [6], through inductively coupled plasma optical emission spectrometry [7], [8], [9], [10], [11], or by total X-ray fluorescence spectroscopy [12].
Many methods are used for preparation of honey samples: wet digestion using acids, high temperature dry oxidation [11], dissolution using ultrasonication, or microwave assisted digestion [13].
The aim of this study is a contribution to the mineral characterization of honeys produced in the east of Morocco.

2. Materials and methods

2.1. Honey samples

Honey samples collected in ten localities in the east of Morocco between 2002 and 2004, represent several Moroccan sorts: Honeydew, unifloral and multifloral Honeys.

2.2. Apparatus

A Jobin-Yvon Ultima ICP optical emission spectrometer was used for metal determination. The operating conditions in the ICP-OES measurements and the quantification limits of the minerals studied were respectively summarized in table 1 and 2.

Table 1. ICP OES operating conditions

Operation conditions	
Power (W)	1
Sample flow rate (l/ min)	1200
Plasma gas flow rate (l/min)	0.2
Nebulizer gas flow rate(l/min)	12
Nebulizer pressure (bar)	0.02
Elements wavelengths	Ca (422.673), Cd (228.802) Cu (213.598), Fe (262.167) K (766.490), Ng (382.935) Nn (259.373), PB (220.353) SE (196.026), Zn (334.502)

Table 2. Quantification limits of the minerals studied

Mineral	Ca	Cd	Cu	Fe	K	Mg	Mn	Pb	Se	Zn
LQ (ppb)	6	0.36	0.8	0.8	6	0.12	0.2	6	4.4	0.8

2.3. Reagents and solutions

Deionized water of 18 MΩ cm^{-1} resistivity obtained from a Milli-Q system (Millipore), was used to prepare all solutions. Nitric acid 0.1M-analytical grade (69.5%, Riedel, SIGMA ALDRICH). Spex plasma standard (1000 mg l^{-1}) was used to prepare all element reference solutions.

2.4. Procedures

Pre-treatment sample

We used a procedure of wet digestion witch simplifies the analysis and minimizes the risk of contamination or losses that can occur when dry ashing is adopted as a pretreatment sample. One gram of honey sample was transferred into 50 ml conical flask; Nitric acid (0.1 M, 2 ml) was added, and the mixture was stirred on a heating plate to almost complete dryness. Then 10 ml of the same acid was added and the mixture was made up to 25 ml with distilled water.

The ranges of standard concentrations used were:

For Ca, Mg and Fe: 0- 2ppm - 4ppm - 6ppm

For Pb, Se, Mn, Zn and Cu 0- 0.2ppm - 0.4ppm - 0.8ppm

For Cd: 0-0.01ppm - 0.02ppm - 0.03ppm

For K: 0-15ppm - 30ppm - 40ppm - 60ppm

Data analysis

To reduce the size of the data matrix obtained, a multivariate statistical analysis (PCA) was performed. Obtained data were also evaluated with statistical cluster analysis.

3. Results and discussion

Determined levels of elements in samples of honey are given in table 3. Results were expressed as mg of metal per Kg of honey

Table 3. Results obtained for the determination of the minerals studied in honey samples

Element	Mean	Range	Standard deviation
Ca	102.92	22.32-228.57	53.555
Cd	0.0058	0.0013-0.0249	0.005
Cu	1.329	0.51-4.75	1.061
Fe	13.52	0.88-207.65	38.173
K	511.28	30..65-5097.91	808.310
Mg	26.83	5.02-62.80	16.057
Mn	1.412	0.080-9.76	1.928
Pb	0.347	0.036-1.88	0.239
Zn	0.319	0.04-2.74	0.401
Se	2.27	0.26-17.39	2.558

The results of the 10 minerals are very variables regarding botanical and geographical origin of honey samples. Honeydew sample (7) had the highest total content of minerals studied with 5288.66 mg/Kg. Potassium account for 76 % of the total minerals quantified. The same result was reported by many authors who consider K to be the most abundant in honey [4], [7], [6], [9]. The levels of selenium were more important than those found in Spanish honeys [9] and American ones [14].

Cadmium levels in the 42 honey samples collected in the east region of Morocco were found below the maximum allowed according to the current European standards for other foods. There is no specific MRL value for honey, but value of 0.1 mg/Kg has been suggested for the EU [15]. The Cadmium content with average of 0.005 mg/Kg found in this study, were lowest than those reported by many authors about honey samples from different parts of world [16], [4], [17], [9], [11].

The other heavy metal studied is present with high levels in many samples (Table 3). Honey standards of many contaminants like Pb are not defined at the moment. So it is necessary to explorate from rules related to other foods [18]. The concentrations of Pb in our samples (0.036-1.88) were higher than the LMR values for many foods (Table 4) and also than the concentrations reported by many studies: 0.1-0.85ppm [19]; 0.048 mg/Kg [20]; 0.08 mg/Kg [9]; 0.02-0.1 mg/Kg; [11]; 0.04-0.1 mg/Kg [16]. This is probably due to the proximity of a lead mine. Many studies report that the content of elements reflects the environmental contamination of the locality witch honeys come from [20], [21], [22].

H I E R A R C H I C A L   C L U S T E R   A N A L Y S I S

Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine

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     C A S E      0         5        10        15        20        25
   Label     Num  +---------+---------+---------+---------+---------+

  Guafaït    26
  Laâyoune   27
  Touissit   16
  Touissit   15
  figuigbo   31
  Boukhwal   33
  Cap de l   32
  Tzayast    10
  genfouda   42
  Touissit   18
  Berkane    36
  Laâyoune   29
  Touissit   17
  Tafoural    2
  Berkane    11
  Fezouane   13
  berkane    37
  Fezouane   14
  Elouinat   23
  Guercif    35
  Guafaït    22
  taourirt   40
  Chouihiy    8
  Laâyoune    4
  Laâyoune   28
  Taourirt   34
  Mestegme    6
  mestegme   39
  Chouihiy    9
  Guafaït    24
  bouarfa    41
  Touissit   19
  Debdou     20
  Taourirt   38
  Debdou     21
  Guafaït    25
  Mestegme    5
  S.Bouhri    1
  Fezouane   12
  Ain Sfa     3
  figuigbo   30
  Debdou      7
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Fig.1 Diagram of honey samples clustered according to element composition.

If we admit the usefulness of honey as a geographical and botanical bioindicator of contamination with metals, the area where the honey samples with high levels of Pb were produced are contaminated. Although more extended investigation are necessary.

Table 4.Maximum residue limits (LMR) for Pb (mg/Kg) [23]:

Juice of fruits	0.050
oils and fats	0.10
Wines	0.20
fruits	0.10
preparation for new-born babies	0.020
Milk	0.020

On the basis of results of cluster analysis (fig. 1), the honey samples could be divided into two different groups. The mean of total content of minerals were 416.63 and 1553.42 mg/Kg in the first and second group respectively. Honey samples of the second group are characterized by a dark brown color.

This can be attributing to the differentiation between nectar honey and honeydew honey, the content of minerals could complete other useful parameters to differentiate between the two types of honey like electrolytic conductivity, color, quercitol concentrations and ash content [24 [25] [12] [26].

Honey samples coming from areas of rosemary, orange and eucalyptus showed lower mineral content than those originating from forest or multi-flower source (fig.2).

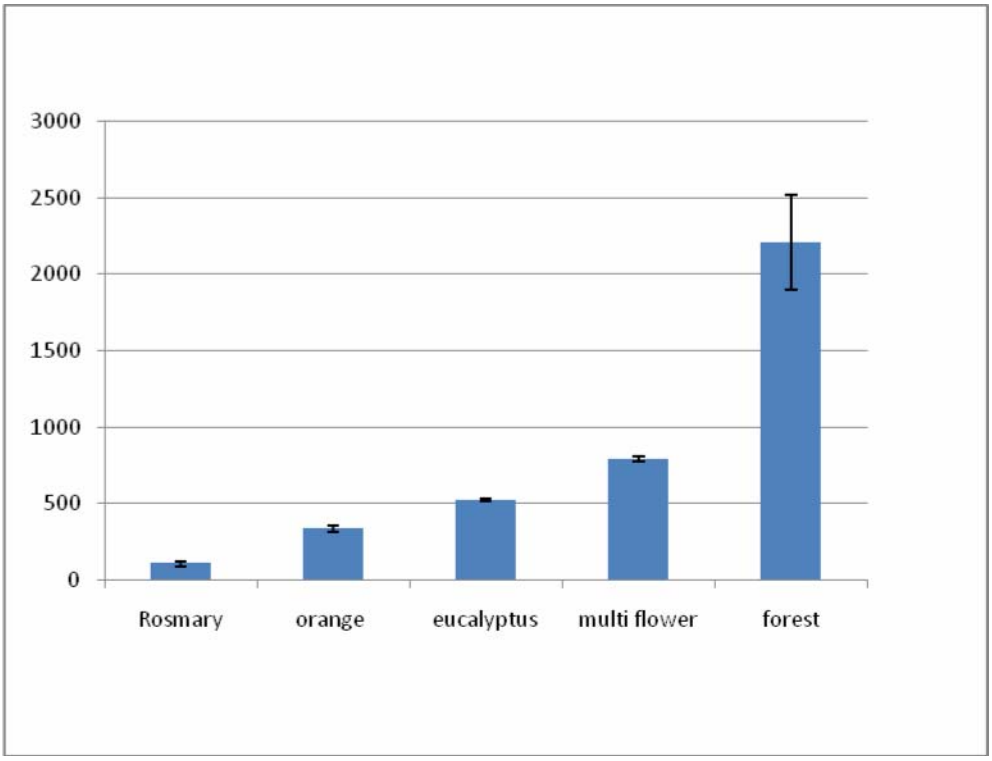

Fig. 2 Distribution of the average of mineral content according to the botanical origin of honey samples. The multivariate analysis performed from the results indicated the 10 variables analyzed, which explain the 100% data variability. Distribution along PC1 would be mainly dependent on the concentration of Mg (Ca, K and Mn), while distribution along PC2 would be mainly dependent on Fe (Zn, Cu, Cd and Se). The factorial map PC1-PC2 explains 82.7% of total data variability (30.689% and 52.0% respectively) (fig. 3).

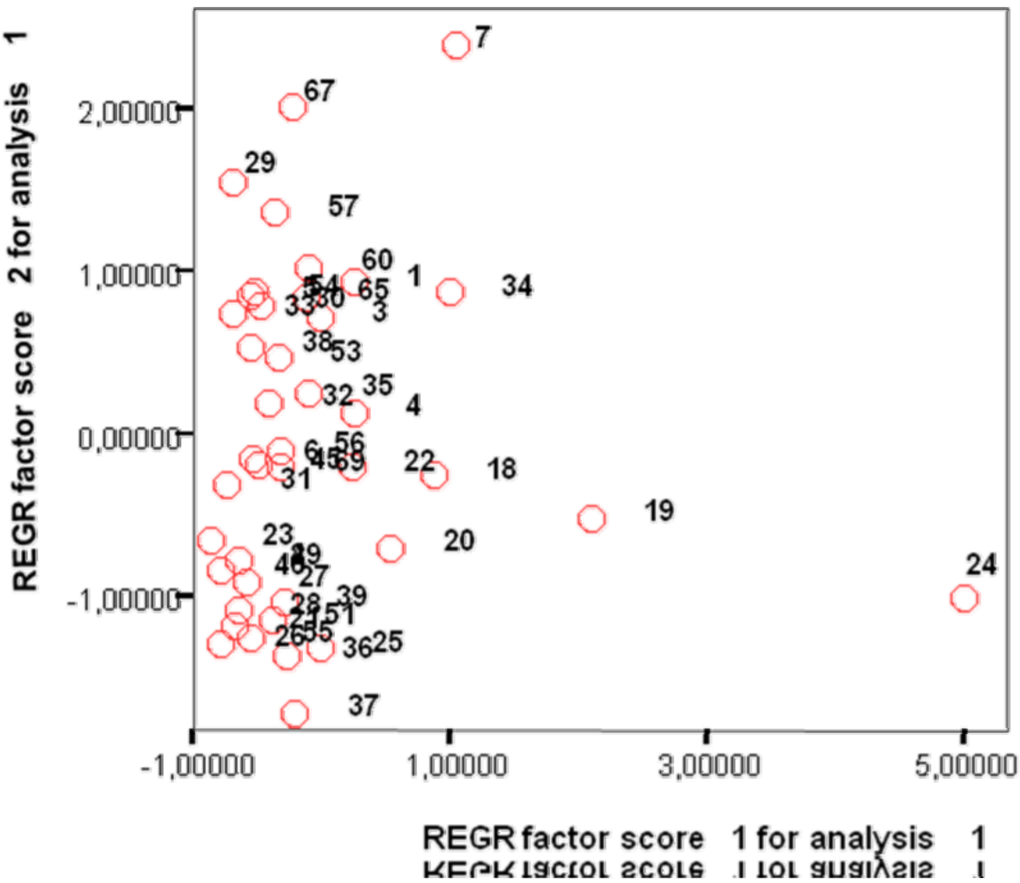

Fig.3 Factorial map PC1-PC2 for 42 honeys analyzed (PC: principal component). Distribution samples 24 and 19 had the highest Fe content (207.65 and 155.73mg/Kg respectively) and high total mineral content. Honey sample 7, 29 an 57 had the highest Mg content (62.8, 58.04 and 52.64 respectively). Honey samples 37, 36, 25, 26 and 21 had the low total mineral content and mineral levels on Mg. It appears closer to the interception axis.

4. Conclusion

Pb levels in the majority of honey samples collected in the east region of morocco were found to be higher than the permitted levels in many other foodstuffs. The results of the 10 minerals determined are more influenced by the botanical origin than the geographical parameter. It was possible to divide the samples into two groups, forest-honey and nectar honey.

References

[1] Alissandrakis, E., Tarantilis, P.A., Harizanis, P.C. & Polissiou, M., Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatographic-mass spectrometric analysis. Food chemistry, 100, (2007), 396-404.

[2] Podgorski, W., Kanoniuk, D., Honey as marker of environmental contamination with heavy metals. Annales universitatis Mariae Curie-Skodowska. Sectio EE Zootechnica, vol 22 (2004).

[3] Ponikvar, M., Snajder, J., & Sedej B., Honey as a bioindicator for environmental pollution with SO2. Apidologie 36 (2005), 403-409.

[4] Nanda, V., Sarkar, B.C., Sharma, H.K., & Bawa, A.S., Physic-chemical properties and estimation of mineral content in honey produced from different plants in northen India. Journal of food composition and analysis, 16(5) (2003), 613-619.

[5] Rodriguez, G. J.C., Barciela, G.J., Herrero L.C., Garcia, M.S., Pena C.R.M., Comparison of palladium, magnesium, nitrate and ammonium dihydrogen phosphate modifiers for lead determination in honey by electrothermal atomic absorption spectrometry. Food chemistry, *91 (*2004), 435-442.

[6] Sulbaran de ferrer, B., Ojeda de Rodriguez, G., Pena, J., Martinez, J., & Moran, M., Mineral content of the honey produced in Zulia state, Venezuela. Archivos latinoamericanos de nutricion, 54(3) (2004), 346-348.

[7] Terrab, A., Gonzalez, A.G., Diez, M.J., & Heredia , F.J., Mineral content and electrical conductivity of the honeys produced in north-west Morocco and their contribution to the characterization of unifloral honeys. Journal of the Science of food and Agriculture, 83 (2003), 637-643.

[8] Matei, M., Birghila, S., Dobrinas, S. & Capota, P., Determination of C vitamin and some essential trace elements (Ni, Mn, Fe, Cr) in bee products. Acta chimica slovenica, 51 (2004), 169-175.

[9] Terrab, A., Recamales, F.A., Gonzales-Miret, M.L., & Heredia F.J., Contribution to the study of avocado honeys by their mineral content using inductively coupled plasma optical emission spectrometry. *Food chemistry, 92*, (2005), 305-309.

[10] Nalda, M.J.N., Yagüe, J.L.B., Calva, J.C.D., Gomez, M.T.M., Classifying honeys from the Soria Province of Spain via multivariate analysis. Analytical and Bioanalytical Chemistry, 382 (2) (2005) 311-319.

[11] Fredes, C., & Montenegro, G. Heavy metals and trace elements contents in Chilean honey. *Ciencia e Investigacion AGRARIA* 33(1) (2006), 50-58.

[12] Golob, T., Dobersek, U., Kump, P., & Necemer, M., Determination of trace and minor elements in Slovenian honey by total reflexion X-ray fluorescence spectroscopy. *Food Chemistry, 91(4)* (2004), 593-600.

[13] Mendes T. M. F. F., Baccan S. N., Cadore S.,. Sample treatment procedures for the determination of mineral constituents in honey by inductively coupled plasma

optical Emission spectrometry. Journal of the Brazilian Chemical Society. 17 (1) (2006), 168-176.

[14] Iskandar, F. Y., Trace and minor elements in four commercial honey brands. Journal of Radioanalytical and Nuclear Chemistry. 201 (5) (1995), 401-408.

[15] Bogdanov, S., Haldimann, M., Lunginbül, W. and Gallmann, P., Minerals in honey: environmental, geographical and botanical aspects. Journal of agricultural research and bee world. 46(4) (2007), 269-275.

[16] Kunchev, S., Peneva, V., Marinova, M., Contents of heavy metals in bee honey. Veterinary medicine, 4 (3-4) (1999), 200-202.

[17] Rashed, M.N, Soltan, M.E., Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys. Journal of food composition and analysis, 17 (6) (2004), 725-735.

[18] Piro, R. and Mutinelli, F., The EU legislation for honey residue control. Apiacta 38, 15 2003.

[19] Demirezen, D. & Aksoy, A., DETERMINATION OF HEAVY METALS IN BEE HONEY USING BY INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES). Journal of Science, 18(4) (2005), 569-575.

[20] Przybylowski, P., & Wilezynska, A., Honey as an environmental marker. *Food chemistry, 74 (*2001), 289-291.

[21] Erbilir, F., & Erdogrul, O., Determination of heavy metals in honey in Khahramanmaras City, Turkey. *Environmental Monitoring and assessment, 109*, (2005), 181-187.

[22] Rodriguez, R., Pena C.R.M., Barciela, G.J., Garcia, M.S., Herrero L.C., Preliminary chemometric study on the use of honey as an environmental marker in Galicia (northwestern Spain). J. Agric. Food chemistry, 54 (19) (2006), 7206-12.

[23] RÈGLEMENT (CE) N° 1881/2006 THE COMMISSION of December 19th, 2006 carrying fixing of maximum contents for some contaminating in foodstuffs; (JO L 364/5 du 20/12/2006, p. 14)

[24] Soria, A.C., Gonzalez, M., Lorenzo, C., Castro, I., & Sanz, J., Estimation of the honeydew ratio in honey samples from their physicochemical data and from their volatile composition obtained by SPME and GC-MS. *Journal of agricultural and food chemistry, 85*, (2005), 817-824.

[25] Sanz, M.L., Sanz, J., Martinez-Castro, I., Gonzalez, M., & Lorenzo, C., A contribution to the differentiation between nectar honey and honeydew honey. *Food chemistry 91(2) (* 2005), 313-317.

[26] Lachman, J., Kolihova, D., Miholova, D., Kosata, J., Titara, D. and Kult, K.,. Analysis of monitoring honey components: possible use for the evaluation of honey quality. Food chemistry, 101(3), (2006), 973-979.