

Physico-chemical study and quality control of some types of Moroccan honey

S. Mehanned^{(a)*}, A. Dehbi^(b), R. El moustaine^(a), A. Zaid^(a), M. Chahboun^(c), F. Souid^(d), M. Boukili^(e)

^(a) Management team and developing natural resources, laboratory of environmental and health, Faculty of Science of Meknes, Department of Biology

^(b) University Moulay Ismail, BP 11 201 Zitoune Meknès, Morocco. Moulay Ismail University, Faculty of Sciences, Department of Chemistry, Analytical Chemistry and Environment, P. B. 11201, Zitoune, Meknes, Morocco.

^(c) Science Laboratory and health technology, Institute Superior of the Science of Health, University Hassan1 Settat, Morocco.

^(d) Applied Hydrosiences Research Unit, Higher Institute of Water Sciences and techniques, University of Gabès, campus University, 6072, Tunisia.

^(e) Laboratory of Environment and health, department of biology, Moulay Ismail University, faculty of science BP 11201 Zitoune, Meknes, Morocco.

Abstract

Honey is a very complex biological compound of great diversity, giving it a multitude of properties, both nutritionally and therapeutically. In order to determine the physico-chemical characteristics, three types of samples were collected from the region of Morocco. These are zandaz honey and black honey from the Bouyblane region, multi-flower honey from the Atlas region, and commercial honey called "Achefae". These honeys are analyzed for determination of pH, electrical conductivity, ash content, density, free acidity, hydroxymethylfurfural content, color intensity, and phenolic compounds. These physico-chemical parameters studied are the most frequently used as indicators of the quality and stability of honey and having a great influence on its organoleptic properties. The results showed that there were differences from one sample of honey to another and that they all meet international standards.

* Corresponding author:

smahane_mehanned@hotmail.com

Received 30 Jun 2022,

Revised 03 Sept 2022,

Accepted 12 Set 2022.

Keywords: Atlas, Achefae, Bouyblane, Black, Honey, Zandaz.

1. Introduction

Known since ancient times, honey is the natural sweet substance produced by honey bees from nectar and/or honeydew [1]. It is one of the most popular foodstuffs for humans because of its flavors, aromas and energetic virtues [2]. In 2017, global honey production was around 1.9 million tons; China being the main producer country followed by Turkey and Iran [3]. In Morocco honey production plays an important socio-economic role. It is a means of combating poverty and a source of supply for the domestic market. On the ecological level, the bee is decisive, even vital, in terms of the pollination of plant species, which ensures biological diversity and the sustainability of plant species [4]. Honey is defined as the food produced by honey bees from the nectar of flowers or certain secretions from living parts of plants. Indeed, they forage, transform, and combine with clean materials, store and leave to ripen on the shelves of the hive. This is an advantage over synthetic products, as it is difficult for the insects to develop resistance [5]. This commodity may be fluid, thick or crystallized [6], with a uniform ripeness level and ready for consumption [7]. In addition it is a living product which undergoes over time a number of modifications leading to the loss of its essential qualities. However, honey is characterized by a certain group of substances which are always present but in variable quantities depending on the source, water, carbohydrates, proteins or nitrogenous substances, organic acids, lactones, mineral substances, trace elements, vitamins, lipids, pollutants such as lead, cadmium and hydroxymethylfurfural [8]. From a nutritional point of view, honey is first category food, with high energy value and having certain therapeutic properties. Thus, our study aims to characterize the physicochemical quality of some varieties of honey from different Moroccan regions.

2. Materials and methods

2.1. Sampling

Our study focused on four honey samples: mono floral honey from the zandaze plant, multi-flower honey from the Bouyblane region, multi-flower honey from the Atlas region, and commercial honey called "Achefae" are collected during the winter season 2019 (Table 1). The samples were packaged in airtight glass jars and kept at 4 °C until analysis. All analyzes were performed in two repetitions.

Table 1. Honey classified according to their floral origins and their origins.

Sample	Botanical origin	Location	Year of production
Honey 1	Multifleurs	Bouyblane region	2014
Honey 2	Zandaz	Bouyblane region	2014
Honey 3	Commercial	-----	2014
Honey 4	Multi-flowers	Bouyblane region	2014

2.2. Physico-chemical analysis of honey

2.2.1. Determination of ash content

The ash content is based on the incineration of honey in an oven, 5 g to 10 g of honey is added with a few drops of olive oil and the whole is heated at 350-400 °C for one hour [9]. These measures were expressed as a percentage (%) (Figure1).



Figure 1. Appearance of the honey ash studied.

2.2.2. Conductivity

The measurement of the electrical conductivity of each honey sample is carried out using a conductivity meter. The technique is based on the measurement of the electrical resistance at 20 ° C.

2.2.3. pH and acidity

The pH is measured using a pH meter calibrated by standard solutions on a 10% honey solution (Figure 2). The free acidity is obtained by neutralizing 25 ml of this solution with NaOH (0.05 N). The acidity of the lactones is obtained by adding an excess NaOH (10 ml) to the honey solution and titration back with sulfuric acid (0.05 N) [10].



Figure 2. Diagram of the titration of the Samples studied by NaOH.

2.2.4. Density

The principle is based on the determination of honey by the use of pycnometer. A pycnometer of 25 ml is weighed empty and after having been filled with honey until the gauge line. The density is obtained by dividing the density of honey to that of distilled water under the same conditions (Figure 3).



Figure 3. Diagram of sample pycnometers of honey studied.

2.2.5. Hydroxymethylfurfural (HMF)

The amount of hydroxymethylfurfural (HMF) was obtained with the method of Bogdanov et al. [11]. The principle is based on reading the absorbance of HMF at a wavelength of 284 nm and then at 336 nm using a Jenway-type UV-visible spectrophotometer (Figure 4).



Figure 4. HMF appearance of the three honeys studied

2.2.6. Color intensity

The measurement of the intensity of the color aims to determine the different pigments responsible for the color of honey (carotenoids, flavonoids, etc.), the honey samples were diluted with distilled water to 50% (m / v) and hydrolyzed at 50 ° C. Then, the absorbance of the solution thus obtained is measured using a spectrophotometer at 450 nm and 720 nm [12].

2.2.7. Phenol content

The phenol level was determined by the Folin-Ciocalteu method. The phenols react with this reagent, forming a blue complex which absorbs at 765 nm. Indeed, the folin-Ciocalteu reagent is a mixture of phosphotungstic acid (H₃PMo₁₂O₄₀) which is reduced, during the oxidation of phenols, to a mixture of blue oxides of tungsten (W₈O₂₃) and molybdenum (Mo₈O₂₃).

2.2.8. Determination of flavonoids

The principle of the flavonoid assay method results in the formation of a flavonoid-metal complex such as aluminium which is used in its form of aluminium chloride (AlCl₃) which forms yellowish complexes with the atoms of oxygen present on carbons 4 and 5 of flavonoids [13].

3. Resultants and discussion

The physico-chemical characteristics (pH, acidity, density, ash content, electrical conductivity, HMF, color intensity, phenol content, assay of flavonoids) of the five types of honey studied are summarized in Table 2.

3.1. Free acidity and pH

Honeys from nectar have a pH between 3.5 and 4.5 and those from honeydew are between 5 and 5.5 [14]. The pH of our samples oscillates between 3.1 and 4.09, so they are all acidic. We can say that they correspond to nectar honeys, a low pH for honey, predetermines a fragile product for preservation, against honey with a pH = 5 or 5.5 keeps better and longer. According to our results all the honeys studied were of an acidic nature, these values are similar to those found for Doukani et al. (2014) [1], and those reported for other samples of honeys from India, the Brazil, Spain and Turkey, which have a pH between 3.49 and 4.70 [15], and honey's Algerians (3.72 - 4.85), Iranians (4.1 - 5.5), Malaysians (3.22 - 4.03), Ethiopians (3.79 - 4.20), Indians (4.114 ± 0, 02), Egyptian (4.415 ± 0.09), Saudi (4.460 ± 0.02) and Kashmiris (4.637 ± 0.03) [16-22]. The fermentation of honey causes an increase in the acidity of honey, a strong acidity promotes the degradation of hexones in HMF which depreciates the quality of honey, the free acid contents of honeys vary between 17, 5 and 23.7 meq / Kg , this indicates the absence of undesirable fermentation.

3.2. Conductivity

It is a parameter used to differentiate between nectar and honeydew honeys. According to the codex alimentarius of the year (2001) [23] and the official journal of the European community [24], nectar honeys have a conductivity of less than 0.8 ms/cm, on the other hand, that of honeydew honeys is higher at 0.8 ms/cm. The samples analyzed have conductivity between 0.112 and 0.438 ms / cm, these values correspond well to that of the honeys produced from nectar [25]. Dark honeys contain higher levels of microelements than light honeys. In fact, light-colored commercial honey has a low conductivity (0.112 ms / cm while other darker honeys have higher conductivity and are also good current conductors) [26]. These results also show a big difference to those reported by Boussaid et al. (2014) on Tunisian honeys (0.39 to 0.89 mS / cm), and that of honey from French Polynesia which has a high electrical conductivity ranging from 0.756 to 1.535 mS/cm [27].

3.3. Ash rate

The variation in the ash content can be explained by the harvesting processes, beekeeping techniques and the materials collected by bees when looking for food on the flower [28] and mainly determined by the soil and the climate characteristics [29]. Ash content shows a measure of inorganic residue after charring of honey, while conductivity measures all organic and inorganic substances [30]. The ash content in the samples varies between 0.17 and 0.3%. These values are close to that found by Doukani 2014 [31] and Nada 2003 [32]. The ash values found were below 0.6% and these results are therefore in agreement with the limit authorized by the Codex Alimentarius for nectar honeys.

3.4. The density

The variations in the density of honey come mainly from variations in the water content. The more water rich in honey, the less dense it is [33], a honey harvested prematurely will have a low density. The density values of the analyzed honey samples vary between 1.418 and 1.42, it can be deduced from these results that the honeys are ripe and well stored.

3.5. HMF content

The production of HMF is a natural phenomenon whose process is slow at room temperature, the rate of HMF is therefore an important quality factor which makes it possible to locate the level of freshness of honey, it is the most important criterion and the most reliable for detecting overheated honey [34]. The HMF content is influenced by certain factors including the type of sugar and their concentration, temperature, acidity, and shelf life, its content is therefore a very good indicator of degradation. According to spectrophotometer analyses of the honey samples reveals HMF contents between 4.19 and 48.73 mg / Kg, which shows that these values comply with the standards set by the codex alimentarius. These honeys did not undergo heating and they were not stored in bad conditions, on the other hand to that found for Makhloufi (2001) [35], by analyzing samples of Algerian honeys found values varying between 9.6 and 157.4 mg / kg with an average of 42.6 mg / kg which have higher contents which means that the honeys have been heated. Also for Pakistani honeys, it was possible to obtain variable values ranging from 24.45 to 40.68 and from 316.86 to 516.26 mg/kg, respectively for fresh and marketed honeys [36], on the other hand Saudi and Indian honeys report very low values 3.78 and 3.87 - 4.64 mg / kg, respectively [37,38].

3.6. Color intensity

The color of honey can be correlated to its botanical origin; the higher the mineral content, the darker the color of the honey. It can vary depending on the floral origin and its composition, as it can be intensified by heating, aging and by light oxidation. The intensity of the color obtained for each of these honeys analyzed by spectrophotometry varies from light yellow to dark brown, which corresponds to absorbance values ranging from 0.303 to 1.68.

3.7. Phenolic compounds

Phenolic compounds play a key role in the acquisition of therapeutic and organoleptic characteristics. Their content remains a good criterion for assessing the quality and healing properties of honey. The polyphenol contents found are between 26 and 72.4 mg / 100 g of honey and are close and proportional to their color. Indeed, a honey of dark color has a high rate in phenolic compounds compared to honey of light color, on the other hand commercial honey records the lowest content even its color is dark, and this can suggest that this honey is overheated or falsified. For the Atlas and bouyblane honeys, the polyphenol contents are important, which shows that these honeys will have strong antioxidant activities. Several studies have revealed the richness of honey by phenolic compounds. In fact, Slovenian researchers have found a content of 24.14 mg / 100 g in forest honey [39]. While another Italian study [40] showed a

percentage of polyphenols ranging from 3 mg / 100 g to 17.5 mg / 100 g, these values are lower than those found in the samples of our study. This difference in phenolic compounds can be explained by the botanical and geographical difference of the samples studied.

3.8. Flavonoids

The honeys used in this study have a flavonoid content between 8 and 30 mg / 100 g. Zandaz honey has the highest content while commercial honey has the lowest value (8 mg / 100 g). These values are in good correlation with the polyphenol contents. Apart from commercial honey, the other two will therefore have good healing properties. The results of this study indicated that the samples were of good chemical quality. They are comparable to the standards imposed by the food codex and the European Union standards. In addition, the honeys from the atlas and bouyblane (zandaz) are very rich in polyphenols and flavonoids. This gives them a more interesting antioxidant activity.

Table 2. Physico-chemical parameters of the five honey samples studied.

Sample	Bouyblane region Multi- flower	Zandaz bouyblane region	Commercial	Atlas region multi- flowers	Standard (EU project)
Conductivity ($\mu\text{s} / \text{cm}$)	0,378	0,43	0,11	0,38	< 800
% Ash	0,27	0,23	0,17	0,3	0,6 g/100 g
pH	4,05	4,09	4	3,12	3,5 < pH < 4,5
Acidity (meq /kg)	22,2	23,7	21	17,5	<50 meq/kg
HMF (mg/kg)	3,99	48,73	4,33	4,18	<60 mg/kg
Density	1,43	1,419	1,418	1,42	1,39 < PH < 1,44
Polyphenol concentration (mg/100 g)	11,2	30,8	8	12	variable
Concentration en flavonoïdes (mg/100 g)	50,6	72,4	28	60	variable

Conclusion

Quality honey must be a healthy product, extracted in good hygienic conditions, packaged correctly, and retaining all of its original properties for as long as possible. There are, in fact, a certain number of criteria on which this quality is based, namely the coloring, the water content, the sugars, the pH, the acidity and the level of hydroxy methyl furfural. All the quality parameters analyzed in this modest work are comparable to the standards imposed by the Codex Alimentarius and the European Union standards. The four honeys studied are therefore of good chemical quality. Atlas and Bouyblane (Zandaz) are very rich in polyphenols and flavonoids. This gives them a more interesting antioxidant activity.

References

- [1] K. Doukani, S.Tabak, A. Derriche, Z. Hacini, "Physico-chemical and phyto-chemical study of some types of Algerian honey", *Ecology-Environment Review* 10 (2014) 37-49.
- [2] O.Belhaj, J.Oumato, S.Zrira, "Physico-chemical study of some types of Moroccan honey", *Agronomic and Veterinary Institute Hassan II, Rabat, Morocco*, 3 (3) (2015) 71-75
- [3] FAOSTAT, "Production quantity of honey (natural) in 2017, Livestock Primary / World Regions / Production Quantity from pick lists", United Nations, Food and Agriculture Organization, Statistics Division (FAOSTAT). 2018. Retrieved 18 March 2019.
- [4] B. Bertrand, "Biodiversity hives", éditions du terran (2016).

- [5]. M. R. Ismaili, H. Ramzi , A. Fidah , M. Rahouti , B. Kabouchi, M. Aberchane “Chemical variability and acaricidal activity of *Rosmarinus officinalis* L. essential oils”, *Mor. J. Chem.* 7 N°4 (2019) 636-651.
- [6] M. BLANC, Doctoral thesis, Univ. Limoges, (2010)142 p.
- [7]. Lina Mahardiani, Nur Laeli Azizah , Endang Susilowati, Budi Hastuti “The Sinergy Effect of Essential Oils from Traditional Herbs and Medicines as Antibacterial Materials of Edible Coating on Fresh Fruit”, *Mor. J. Chem.* 10 N°2 (2022) 351-361. DOI: <https://doi.org/10.48317/IMIST.PRSM/morjchem-v10i2.32650>
- [8] J. Louveaux, “Honey technology”, *Ann. Bee* 2 (1959) 343-354.
- [9] R. Silva Luis , Videiraromeu, P. MonteiroAdreia , Valentaopatricia, B. Adrade Paula , “Honey from IUso Region (Portugal)/ physicochemical characteristics and mineral contents”, *Microchemical Journal*, 93 (1) (2009) 73-77
- [10] D. Lord, M.J. Scotter, A.D. Whittaker and R. Wood. “The determination of acidity, apparent reducing sugar and sucrose, hydroxymethylfurfural, mineral, moisture, water-insoluble solid contents of honey, collaborative study”, *J. Assoc. Publ. Anal.*, 26 (1988) 51-76.
- [11].S. BOGDANOV, P. VIT et V. KILCHENMANN, “Sugar profiles and conductivity of stingless bee honey from Venezuela”, *Apidologie*, 27 (1996) 445-450
- [12].S. Bogdanov, P. Martin, C. Lullmann, “Harmonised methods of the international honey commission”, *Swiss Bee Research center FAM, Liebefeld* (2002).
- [13].A. Arvouet-grand, B.Vennat, A. Pourrat et P. Legret, “Standardization of propolis extract and identification of constituents”, *J. Pharm. Belg.* 49 (6) (1994) 462-468 .
- [14].J. Elise Mbogning, F. Tchoumboue, M. Damesse, SanouSobze., AntonellaCanini, “Caractéristiques physico-chimiques des miels de la zone Soudano-guinéenne de l’Ouest et de l’Adamaoua Cameroun ; *Tropicultura*”, 29(3) (2012) 168-175.
- [15].S. Saxena, S. Gautam and A. Sharma , “Physical, biochemical and antioxidant properties of some Indian honeys”, *Food Chem*; 118 (2) (2010) 391-397.
- [16].A. Chakir, A. Romane, G.L. Marcazzan, P. Ferrazzi, “Physicochemical properties of some honeys produced from different plants in Morocco”, *Arabian Journal of Chemistry*, 9 (2016) 946-954.
- [17].R. Khalafi, S.A.H. Goli, M. Behjatian, “Characterization and classification of several monofloral Iranian honeys based on physicochemical properties and antioxidant activity”, *Int. J. Food Prop.*, 19 (5) (2016), 1065-1079.
- [18] D. Nabti, M. Achou, F.M.H. Braia, “Physicochemical study of some types of Algerian honeys”, *International Journal of Medical Research & Health Sciences*, 5 (9) (2016) 8-12.
- [19] H. Laredj, R. Waffa, “Microbiological and physicochemical characterization of honeys from the Tiaret Region of Algeria”, *Asian J. Pharm. Res., Health Care*, 9 (3) (2017) 85-91.
- [20] E.M. Mohammed Ahmed, A. Alfifi, A. Aalmudawi , M. Alfaifi , S.E.I. Elbehairi, A. Al-Bushnaqh, “Some physiochemical properties of acacia honey from different altitudes of the Asir. Region in Southern Saudi Arabia”, *Czech. J. Food Sci.*, 35 (4) (2017) 321-327.
- [21] M. Lewoyehu, M. Amare, “Comparative Assessment on Selected Physicochemical Parameters and Antioxidant and Antimicrobial Activities of Honey Samples from Selected Districts of the Amhara and Tigray Regions, Ethiopia”, *Int J Food Sci.*, Article ID 4101695, (2019) 10p.
- [22] K. Selvaraju, P. Vikram, JM. Soon, KT. Krishnan, A. Mohammed, “Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia”, *J Food Sci Technol*, 56(5) (2019) 2508–2521.
- [23] Codex Alimentarius; revised codex standard for honey; codex standard 12-1981. REV. (1987).Rev.2; (2001)1-7.
- [24] Journal official des communautés européennes.2002. Directive 2001 /110 /CE/47-52.
- [25] A. Chakir, A. Romane, G.L. Marcazzan, P. Ferrazzi, “Physicochemical properties of some honeys produced from different plants in Morocco; *Arabian Journal of Chemistry*”, 9(2) (2016) S946-S954.

- [26].M. GONNET, Le miel ; composition, propriétés, conservation, INRA station expérimentale d'apiculture, (1982) 1-18.
- [27] K.M. Rogers, "Melissopalynologie et caractérisation physico-chimique des miels de la Polynésie Française", Lower Hutt (NZ): GNS Science. 171p. (GNS Science international consultancy report 2017/05F).
- [28] MS. Finola, MC. Lassagno. and J.M. Marioli, "Microbiological and chemical characterisation of honeys from central Argentina", Food Chem. 100(4) (2007) 1649-1653.
- [29].C. Acqarone, P. Buera, and B. Elizalde, "Pattern of PH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys", Food Chem., 101(2) (2007) 695-703.
- [30] A. Terrab, M.J. Diez and F.J. Heredia, "Palynological, Physicochemical and color characterization of Moroccan honeys: Orange (Citrus sp)honey", International Journal of Food Science and technology; 38(4) (2003) 387-394.
- [31] Schweitzer, Le monde des miellats. Revue l'abeille de France N°41, Laboratoire d'analyse et d'écologie Apicole (2004) 02.
- [32] V. Naadaa, B.C. Sarkara, H.K. Sharmaa. And A.S.J. Bawa, "Determination of some major and minor elements in the east of Morocco honeys through inductively coupled plasma optical emission spectrometry", Food Comp. Anal., 16 (2003) 613-619;
- [33] J. Louveaux, "Composition, Propriétés et technologie du miel. In : CHAUVIN R .Traité de biologie de l'abeille", Editions Masson et Cie, Paris, Tome 3 (1968) 277-324.
- [34] S. Bogdanov, K. Ruff and Persanooddol, "Physico chemical methods for the characterization of unifloral honey", A review Apidologie, 35 (2004) S4-S17.
- [35] C. Makhloufi, Master's thesis in Agronomy Algeria, University of Tiaret, (2001).
- [36] M. Sajid, M.Yamin, F. Asad, S.Yaqub, S. Ahmad, M. Ali, M.S. Mubarik, B. Ahmad, W. Ahmad, S. Qamer, "Comparative study of physio-chemical analysis of fresh and branded honeys from Pakistan", Saudi Journal of Biological Sciences, 27(1) (2020) 173-176.
- [37].A. Al-Ghamdi, A.M Seif Eldin, J.A. Mohammad, A. Nuru, "Comparison of physicochemical properties and effects of heating regimes on stored Apis mellifera and Apis flora honey", Saudi Journal of Biological Sciences, 26 (2019) 845-884.
- [38].S. Kivrak, I. Kivrak, E. Karababa, "Characterization of Turkish honeys regarding of physicochemical properties, and their adulteration analysis", Food Sci. Technol. (Campinas), 37 (1) (2017) 80–89.
- [39].N. Gheldof, N.J Engeseth, "Antioxidant capacity of honey from various floral sources based on the determination of oxygen radical absorbance and inhibition of in vitro lipoprotein oxidation in human serum samples", J Agric Food Chem, 50(10) (2020) 3050–3055.
- [40] DD. Schramm, M. Karim, HR. Schrader et al., "Honey with high levels of antioxidants can provide protection to healthy human subjects", J Agric Food Chem.2000; 51(6) (2003) 1732–1735.
-
- (2022) ; <https://revues.imist.ma/index.php/morjchem>