CHARACTERIZATION OF HONEYS FROM ALGERIA ACCORDING TO CLIMATIC ORIGIN BASED ON PHYSICOCHEMICAL PROPERTIES.

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Abstract

Description of the subject: Honey is studied in different countries and regions because of the interest of consumers in its benefits for health, natural origin, and quality; this quality is linked with floral and geographic origin which is associate with climatic conditions. We focused on this aspect to assess quality of Algerian honey.
Objective: The aim of the present study was to characterize the honey collected in the different regions all along the North of Algeria with humid, subhumid, arid and semi-arid climate, in order to determine the existing correlations between abiotic factors including hydroxymethylfrufural (HMF) linked to the quality of honey and climate effect.
Methods: Six standard physico-chemical parameters were determined according to the international legislation (Electrical conductivity, moisture, Brix degree, pH, free acidity and HMF).
Results: The results obtained were in agreement with international regulations for 89% of samples. Then, results were analyzed by principal component analysis applying forward selection. The analysis allowed us to show climatic origin impact on quality of honey samples analysed.
Conclusion: Algerian beekeepers and authorities will have to work for the labeling of honeys, starting possibly by that of the arid regions of Ain oussara and Djelfa, according to a geographical controlled appellation of origin, the climate of these regions allows having a honey with low moisture content and therefore more stable and keeps its organoleptic properties longer.
Keywords:honey; climatic origin; physicochemical; quality; Algeria.

CARACTÉRISATION DES MIELS D'ALGÉRIE SELON L'ORIGINE CLIMATIQUE SUR LA BASE DES PROPRIÉTÉS PHYSICOCHIMIQUES

Résumé

Description du sujet : Le miel est étudié dans différents pays et régions en raison de l'intérêt des consommateurs pour ses bienfaits pour la santé, son origine origine naturelle et sa qualité ; cette qualité est liée à l'origine florale et géographique qui est associée aux conditions climatiques.Nous nous sommes intéressés à cet aspect pour évaluer la qualité du miel algérien.
Objectifs : Le but de la présente étude était de caractériser le miel récolté dans les différentes régions du nord de l'Algérie à climat humide, subhumide, aride et semi-aride, afin de déterminer les corrélations existantes entre les facteurs abiotiques dont l'hydroxyméthylfrufural (HMF) liés à la qualité du miel et l'effet du climat.
Méthodes : Six paramètres physico-chimiques standard ont été déterminés conformément à la législation internationale (conductivité électrique, taux d'humidité, degré Brix, pH, acidité libre et HMF).
Résultats : Les résultats obtenus sont en accord avec les réglementations internationales pour 89% des échantillons. Ensuite, les résultats ont été soumis à une analyse en composantes principales en appliquant une sélection directe. L'analyse nous a permis de montrer l'impact de l'origine climatique sur la qualité des échantillons de miel analysés.
Conclusion : Les apiculteurs et les autorités algériennes devront travailler sur la labellisation des miels, à commencer éventuellement par celui des régions arides d'Ain Oussara et de Djelfa, selon une appellation d'origine contrôlée géographiquement, le climat de ces régions permet d'avoir un miel à faible taux d'humidité et donc plus stable et pourra ainsiconserversa qualité plus longtemps.
Mots clés: Miel ; origine climatique; physicochimique; qualité; Algérie.

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INTRODUCTION

In Algeria, honey represents an important element given its therapeutic effects and its nutritional benefits. Algeria Produces between 4000 and 6000 Tons of honey per year [1]. Algerian beekeepers who have always been careful to preserve and defend the natural qualities of honey hope to find other markets than the local one to sell their excess production. But to succeed, they must first comply with international guidelines and regulations that impose standards of quality now unavoidable. In Algeria different types of honey are harvested. In the north, near coasts characterized by Mediterranea climate we can found all flowers honeys, citrus, Eucalyptus, Rosemary, Thyme; further south other types of honey are harvested like Spurge , Ziziphus , Thistle ,Peganum Harmala , This diversity is probably due to climatic biodiversity and botanical wealth [2]. Honey is composed majority by monosaccharides and minority by components such as aminoacids, enzymes, vitamins and minerals. Precise chemical composition and physical properties of natural honeys differ according to the plant species on which the bees forage [2] , furthermore by geographic area, soil, differences in climatic and vegetations are important factors that can affect the various properties of honey [3, 4, 5]. To assess honey quality, The international Codex Alimentarius and the European standards suggest two parameters, the Hydroxymethylfurfural (HMF), the main measurable evaluation criterion of the honey quality and water content [6,7].Two parameters are also generally assessed because they are related to quality, free acidity which, with a high humidity level, promotes the appearance of HMF [8]; as well the pH and the electrical conductivity, two parameters allowing the distinction between nectar honeys and honeydews' [5].

Producers, consumers, food industry and authorities must be interested in correct labeling or origin, in traceability and honeys quality. The labeling on the basis of regional and/ or floral origins is an added value which would protect at a time the consumers and the beekeepers [3] and increase its commercial value. Many studies have been done in order to characterize honeys in different regions of the world [3, 9–14]. Available literature on the properties and qualities of Algerien honey largely focused on samples obtained in different regions of the country [15–22]. However, little study on the impact of climatic factors including humidity on the quality of honey. In this context we oriented our research towards a physico-chemical characterization of the honey collected in the different regions all along Northern Algeria. The latter is characterized by humid, sub-humid, semi-arid and arid climate, with the objective of determining the existing correlations between abiotic factors including hydroxymethylfrufural (HMF) and the diversity of the quality of honey in Algeria and to find out if the climatic origin had an impact on the quality of honey.

MATERIALS AND METHODS

1. Honey Samples

Sixty five (65) samples from different botanical origin , with a weight of 250g are acquired from beekeepers in different regions of Algeria during the period for the years 2016 and 2017. In order to find out whether the climatic origin has an impact on the quality of the honeys, the samples are divided into 2 groups according the geographical (Fig. 1) and climate (Tab. 1) of the acquisition regions. Group 1 with honeys from humid and sub humid regions and group 2 from semiarid and arid regions. The regions are classified according to climate according data free acceded on line [23,24].The climatic data were used to define the type of climate by using Martonne aridity index: $P/T+10$ (P: Average annual pluviometry, T: Average annual temperature) [25] .

- Group 1 : honey samples from humid and sub-humid regions
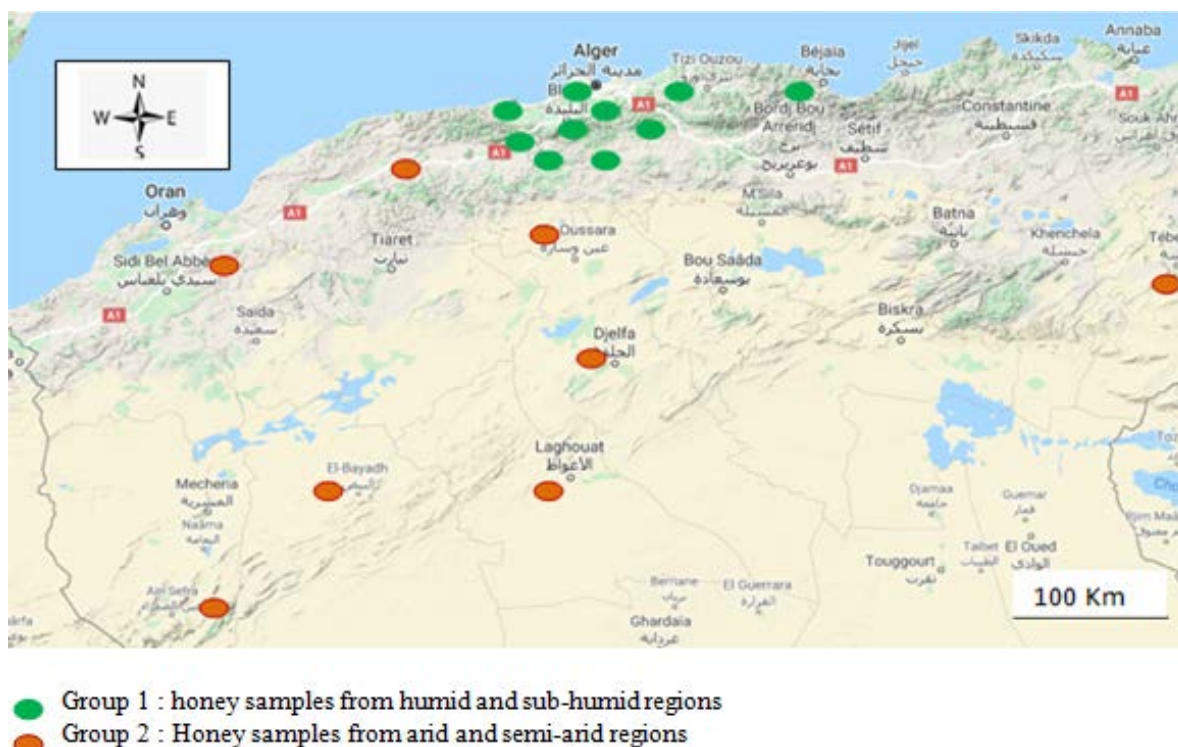- Group 2 : Honey samples from arid and semi-arid regions

Figure 1 : Geographical and climatic origins of the honey samples
Google (n.d.) [Google Maps, Map data ©2020. Algeria] [26].

Table 1: Distribution of honey samples origin regions according to climatic classification

Group	Climate classification	Geographic region	Botanical origin
Gr1	Humid and sub-humid	Blida, Boumerdes, Lakhdaria ,Chiffa, Chrea, Hadjout,Tipaza,Algiers, Khemismeliana , Bejaia,	All flowers (3)*,Asphodel(1)*,camphor (1)*,Citrus (9)*,Eucalyptus (2)*,Forest (2)*,mountain(9)*,rosemary (4)*,spurge(3)*,(Thyme (3)*
Gr2	Semiarid and arid	Al Bayadh, Ain Oussera, Djelfa, Ainsefra ,Laghouat, Sidibelabes, Chlef, Tebessa,	All flowers (2)*,*peganum harmala* (3), mountain (1)*, rosemary (2)*, Spurge (9)*,Thistle (3)*,*Ziziphus* (8)*

*(Number of samples for each type of honey)

2. Methods

All the samples were kept away from light and at room temperature in glass vials until analyzed. The studied parameters in this study are: electrical conductivity, water content and degrees Brix, pH, free acidity and Hydroxymethylfurfural (HMF). These analyses are performed in the laboratory of the Technical Institute of Breeding ITELV (Ministry of Agriculture). All assays were performed in duplicate. The electrical conductivity was measured at 20°C in a solution of 20% using a conductivity meter CORNING (Harmonized Method of the European Commission, 2009) [7].

Moisture (or water content) was defined with a hand refractometer featuring automatic temperature compensation, honey tester Meopta (MeoptaPřerov, Czech Republic) with the method established by the Codex Alimentarius Commission [6]. The results are expressed in percentage (Grams of water in 100g of honey). This same refractometer permitted us to read the Brix rate as described by the ISO's norm 2173: 2003[27]. The degree Brix represents the percentage of soluble solids that are contained in a mixture. It is a value that is nearly equal the percentage of sugar that is present in a liquid product. Its commonly write Bx [28].

pH measuring was carried out potentiometrically at 20°C in a honey solution of 15% (p/v) freshly made of distilled water [29]. The total free acidity was measured by titrimetric method using NaOH 0.1 M, in accordance with the harmonized method of the European Commission 2002, the results are expressed in milli equivalent. HMF rate was measured according to White's method [29] thanks to a single-beam spectrophotometer CECIL 3041, functioning in a range including the wavelengths 284 and 336 nm. The results are expressed in milligrams HMF per 1kg of honey. Hydrochloric acid (HCl) was purchased from Sigma-Aldrich (Steinheim, Germany) Sodium bi-sulfite ($NaHSO_3$) 0.05g/25ml from Cheminova ; Hexacyanoferrate de potassium (15g/100ml) and zinc Acetate (30g/100ml) from Biochem Chemopharma.

3. Statistical analysis

The principal component analysis (PCA) was carried out on the physicochemical variables after auto-scaling the variables and the data corresponding to each variable were analyzed by one-factor analysis of variance (ANOVA). All the statistical data analyses were carried out with STATISTICA solution Version 7 and Statistical Package for Social Science (SPSS 21).

RESULTS AND DISCUSSION

Means, ranges, and the standard deviation of physico-chemical results detected in honey samples are given in table2.

1. Electrical conductivity (EC)

The electrical conductivity (EC) is an indicator for honey origin; Codex Alimentarius Committee [6] and European Commission [7] indicate that the botanical origin of honeys is often classified into two classes: blossom and honeydew, a liquid thick and viscous constituted by the liquid excrements of homopterous richer in nitrogen, in organic acids, minerals and complex sugars [30]. EC must not exceed 0.8 mS / cm for the honeys of nectar and not less than 0.8 mS / cm for the honeys of honeydew [31]. The measurement of this parameter on our samples showed that 98% have an electrical conductivity lower than 0.8 mS / cm (64 samples) which means that almost all honeys are produced from nectar,

and only 2% (1 single sample) of the Bejaia region described like having floral origin of Eucalyptus can be considered honeydew honey, the minimum value observed in samples from the two groups are close respectively 0.06 and 0.07 mS/cm, same with the mean (0.24 mS/cm in group 1 and 0.26 mS/cm in group 2) which joins the results described by Draiaia and Zerrouk [17, 21] on Algerian honeys. Difference in electrical conductivity was not significant between the Two groups (Fig. 2a). The electrical conductivity of honey is closely related to the ash content and acidity of honey, reflecting the presence of ions, organic acids and proteins [3], even though there is a significant difference in electrical conductivity due to hive types and locations [14].

2. Moisture content

Moisture content, is a parameter that is linked to climatic conditions, honey's maturation degree and extraction and storage. In addition, moisture content affects honey's color, its flavor, viscosity and density [3, 32] .The honeys containing 19% of humidity are likely to ferment easily [3]. Our study allowed us to see that 100% of honeys analysed were conform to the Codex standards and have a moisture level lower than 20%. The average of the calculated moisture level is 16.91% for honeys from group 1and 15.80% from group 2 (Fig 2b), with a significant difference between group 1 and 2 (*p*=0.000). The minimum 14% is observed on the sample n° 2, spurge honey of the region of Al Bayadh with arid climate. The maximum of 19.5% is observed in group 1, in humid region. Bogdanov et *al.* [31] stated that harvesting of honey with high moisture content, or subsequent addition of water can result in honey fermentation and spoilage. Those low levels allows a good conservation of honey. Our results join what is described by Draiaia [17] ; (13% (minimum) and 20.13% (maximum); and other studies [15, 16] and confirms the results obtained from honeys from arid regions [19] and thus a significant influence of the climatic and geographical conditions on the quality of honey, the fact that leads us to think about how to guarantee a low level of humidity in the honeys, in wet climatic conditions. The moisture level varies also according to the honey type [22], a verdict that is confirmed in our study.The ideal in terms of quality is a humidity level lower than 18% [31].

Table 2: Summary of the experimental results classified by climatic group

|	1st group Humid and sub humid region				2nd group Arid and semi arid region			
	Mean	Mini	Max	S.D.	Mean	Mini	Max	S.D.
Electricalconductivity (mS/cm)	0.24	0.06	0.81	0.15	0.26	0.07	0.58	0.14
Moisture (%)Codex limit:20%	16.92	15.00	19.50	1.20	15.80	14.00	18.00	1.00
Brix (%)	81.75	79.00	83.80	1.15	82.80	80.50	84.50	0.94
pH	3.06	2.83	2.53	0.31	3.81	2.53	4.99	0.45
Free Acidity (meq/Kg) Codex limit (50 meq/Kg)	36.97	10.00	350.00	55.80	24.18	6.00	50.00	9.69
5-Hydroxy-methyl-furfural mg/kg (HMF)Codex limit: 40 mg/kg	30.73	3.77	177.00	40.46	10.61	0.00	46.70	9.61
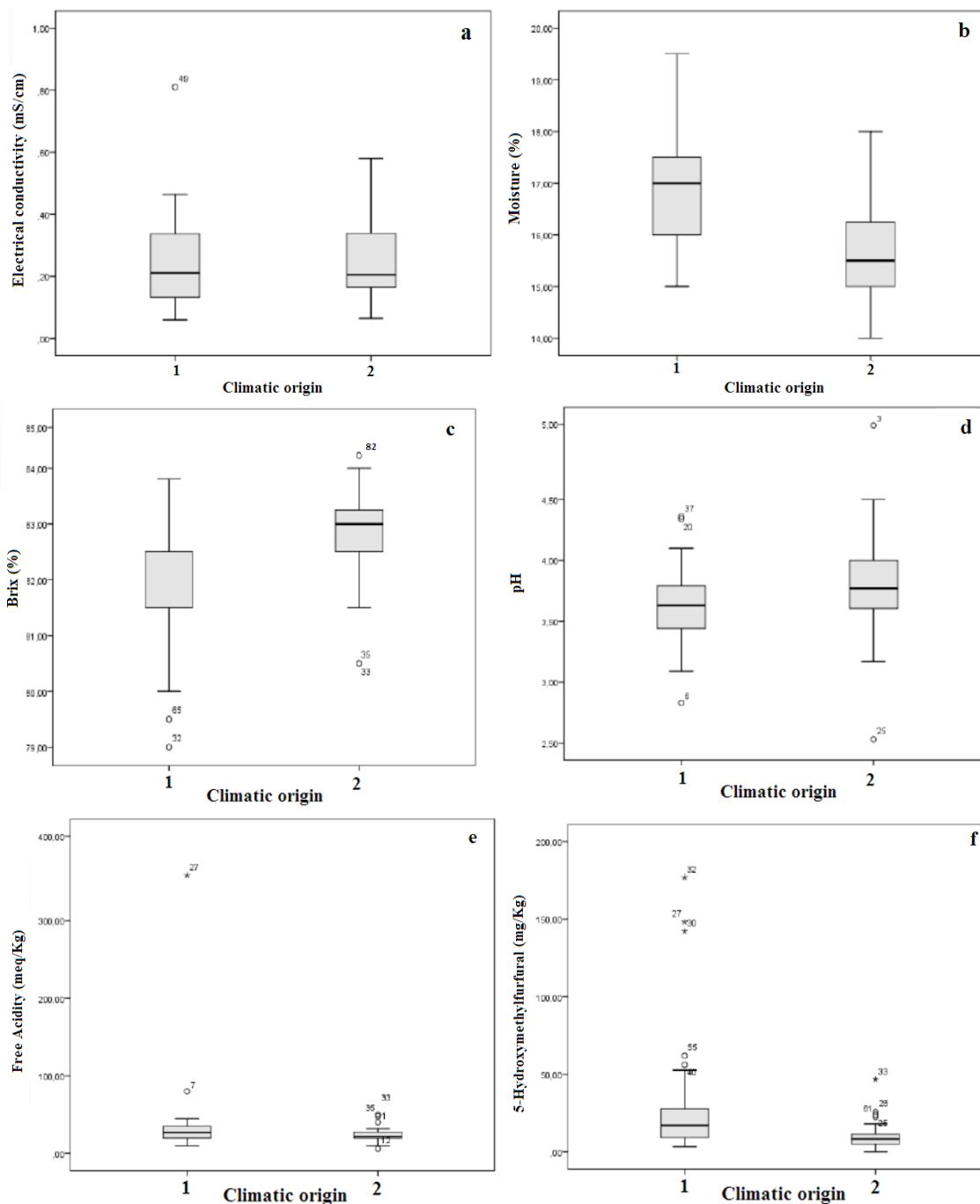
Figure 2 :Representation in box plots of studied honeys characterizing parameters based on origin.
1:humid and sub humid climate;2:arid and semiarid climate.(a):electrical conductivity;(b):moisture;(c):°Brix;(d) pH;(e):free acidity and (f):HMF.

3. The Brix value (Brix)

The Brix degree values (Brix) is linked directly to sugar content, may be a reliable adulteration index [33]. The analyzed samples present a Brix from 79.0 which is observed in mountain honey from the region of Lakhdaria and the maximum at 84.5 in a honey of *Peganum harmala*, of the region of Ain Safra (average=82.2), 60% of the samples have a Brix between 82 and 83. The mean value of Brix is 81.8 for groupe 1 and 82.7 for group 2 (*p*=0.000) (Fig. 2c).We recorded Brix values which are likely to what has been described by Rebiai [15]. This parameter seems to be insignificant to discriminate honeys from different climate.

4. pH

Although there is no fixed limit of National and International standards for pH, this parameter is of significance in honey quality assessment [2, 31]. The pH is between 3.5 and 4.5 for nectar honeys and between 4.5 and 5.5 for honeydew honey due mainly to the fact that their mineral contents are higher than floral honey [31]. 92% of honeys have a pH lower than 4.5, only 2 samples have a pH greater than or equal to 4.5; Sample No. 3 and No. 72 both from *Ziziphus*honey from the regions of Djelfa and Ain Oussara which echoes the opinion of Mekious [19] ,who claimed that generally *Ziziphus* honey has a relatively high pH. We should, however, note that it does not concern the sample which the electrical conductivity has made us suppose it would be from honeydew (N° 65). For our samples, 50% of the *Ziziphus* honey has a pH between 4.4 and 4.9, which corresponds to the results recorded previously [16]. The minimum pH is 2.53 found on a *Ziziphus* honey of the region of Ain Oussara (N° 42) and 4.9 (maximum) in *Ziziphus* honey from region of Blida. The average pH for group 1 is 3.6 and 3.81 for group 2 (Fig. 2d). Honey pH is affected by extraction and storage conditions, and influences texture, stability and shelf life [9] and pH also plays a important role in antioxidant activity of honey [34].

5. Free Acidity

The Free Acidity, according to the Codex, should be beyond 50 milliequivalents by 1000g (meq/kg). 60 (95%) samples of honey have a free acidity rate lower than 50 meq/kg. With respect to free acidity, the honey samples analysed in this study compare favourably with honey samples from other geographical locations, as it has been described by Rebiai [18], Draiaia [17] and by Agbada [14].

The minimum is at 6meq/kg in the *Ziziphus* honey from the region of Ain Oussara, and the maximum is at 350 meq/kg in multi-flower honey from the region of Khemis Miliana group 1, very high value which supposes that this sample is of poor quality. Abu-Tarboush [35] relates these differences to the floral origin. The honeys of group 1 from the wet regions have a mean value free acidity of 36.90meq/kg, whereas the honeys in group 2 contain mean level 24.18 meq/kg (Fig. 2e). We can suppose that the climate has, thus, an impact on the acidity level. The free acidity is useful for the evaluation of the honey fermentation, authentication of single-flower honey and the differentiation between nectar honey and that one of honeydew. Moreover, this quality indicator can be affected by diverse factors, such as the floral origin or the harvest season [3, 35].

6. 5-Hydroxymethylfurfural(HMF)

HMF is considered as one of the most important parameters for the quality of honeys, it is a molecule that appears during the reaction of Maillard under conditions of acidity and during a heat treatment / or during a long storage [36], the maximal values (40mg/Kg for the tropical countries) are determined by the Codex standards [6]. According to our results, 89% are in the Codex standards <40mg/Kg. Two samples have a level equal to 0, indicating honeys that are freshly harvested and well conserved [29], which are *Ziziphus* honeys of Ain Oussara (from group 2), the HMF rate is between 20 and 40 for 20% of the samples; between 40 and 80 for 6% and 5% only have a rate that is considered as high for more than 80. These result join those described by Bendeddouche and Draiaia[16,17]. The maximum is detected at the sample 48, a multi-flower honey from the region of Lakhdaria (group 1) harvested in 2016, we may suppose that this honey has been much heated, or there has been fraudulency in the declared harvesting date [35] this sample presents a high moisture (19.5); another sample presents high levels also from group 1 and with a very high acidity (350 meq/Kg). Khalil [37] showed that the honeys issued from warm climates have a high HMF rate.It should be noted that in our study the HMF rate of honeys from group 1 are averagely higher (mean=30.72 mg/Kg), while in the group 2 (Fig. 2f) these values of HMF are rather low (mean=10.61 mg/Kg). Significant difference was recorded between the two groups (*p*<0.05).

Interest in the determination of HMF in foods is also related to its toxicity; ingestion, inhalation or absorption of the skin are the three routes of exposure to HMF [38]. HMF and its derivatives (5-chloromethyl and 5-sulfidemethylfurfural) are suspected of having genotoxic, cytotoxic, mutagenic and carcinogenic effects [39], however, we should point out that other studies have suggested that HMF is unlikely to pose a serious risk to human health [39]. Therefore, regardless of the type, 20 months are proposed as the 'best-before period once opened' for consummation of honey [40] .

7. *Influence of climatic origin on honey quality*

PCA was performed to explore experimental data, evaluate relevant variables and correlations, and produce bidimensional plots in which samples and variables are visualized in a particular plane that maximize the variance. Fig. 3 represents the analysis of the principal components of the abiotic variables of Algerian honeys harvested in several regions. PCA 1 with the inertia 44.65 % defines the physicochemical parameters (HMF,moisture content , free acidity and °Brix) . The moisture content is strongly positively correlated with free acidity and HMF.

which is consistent with what was previously described by Silva [33]. However, these variables are negatively correlated with the °Brix ; this has been described by Draiaia[17] , honeys containing a high amount of acidity are rich in HMF, the same phenomenon has been described on another matrix , balsamic vinegar [41]. On PCA2 with 25.70% inertia defines electrical conductivity positively correlated with pH. These results are similar with those of Fechner and *al.* [3].

From Anova in the present study allows us to say that parameters most influenced by climate origin are respectively moisture and Brix (p=0.000); HMF (p=0.012); pH (p=0.047); our study didn't showed significative influence of origin on other parameters electrical conductivity and free acidy. Similar results were described by Makhloufi et al.[42]. By combining the 2 legal parameters (HMF and humidity) we were allowed to distinguish 2 groups of climatic origin (Fig.4).We are not able to confirm by statistical analysis that change in pH and EC may be due to climatic origin. It is interesting to note that similar results have been reported in a study on Andalusian honey [43]. This could be explained by the fact that Honeys of studied regions are blends of flowers and honeydew honey.
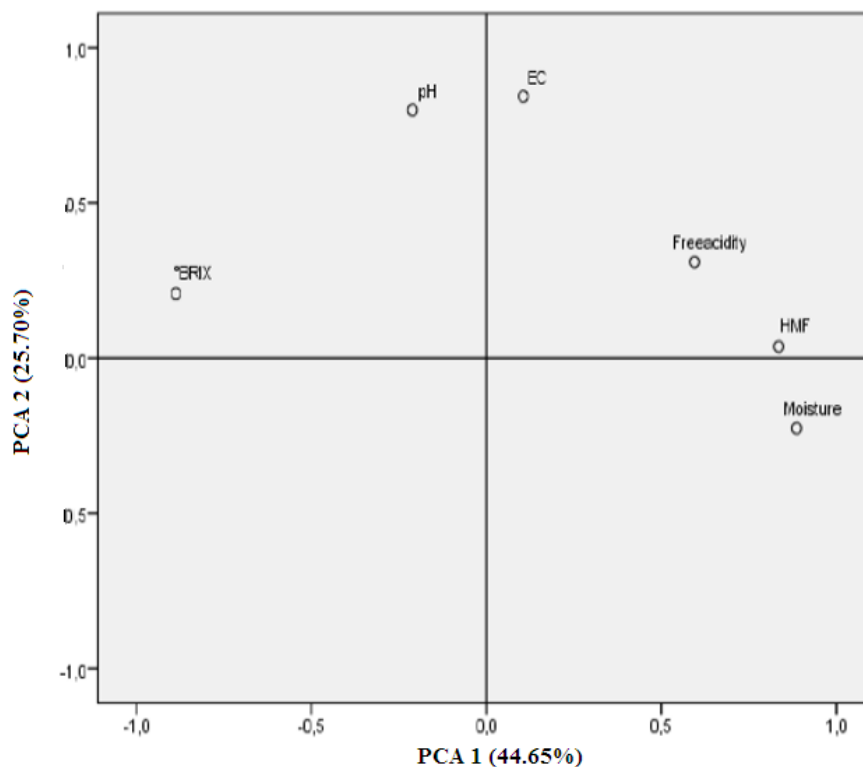

Figure 3 : PCA loadings of honeys parameters on the first and second principal components.
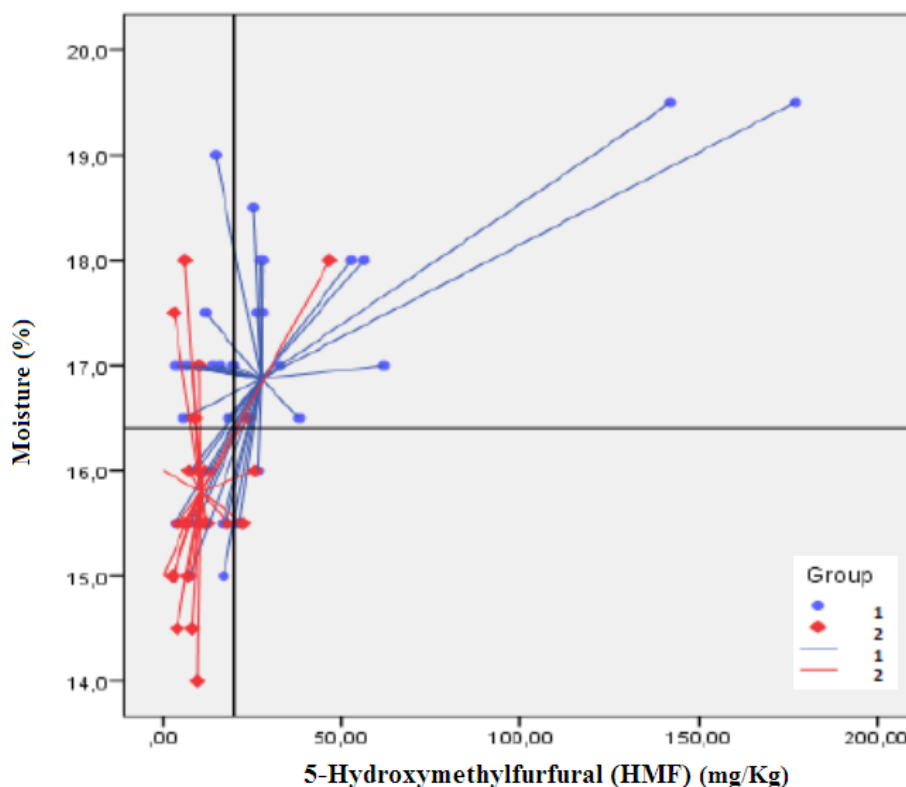EC: electrical conductivity; HMF: 5-hydroxymethylfurfural.

Figure 4:Dispersion of the samples according to the HMF and moisture according to climatic origin.
1:humid and sub-humid region; Group2:Arid and semi-arid region.

CONCLUSION

This study allows us to confirm the quality of Algerian honey. In fact, 89% of honey samples analyzed were Codex-compliant. The parameters that will need more attention are moisture, HMF and free acidity levels. Honeys that have shown better quality are honeys classified in group 2 from steppe and pre-Saharan regions, with arid and semi-arid climate. Algerian beekeepers and authorities will have to work for the labeling of honeys, starting possibly by that of the arid regions of Ain Oussara and Djelfa, according to a geographical controlled appellation of origin, the climate of these regions allows having a honey with low moisture content and therefore more stable and keeps its organoleptic properties longer. The study of our honey samples allows us to mount a fragility of the honeys in the humid regions and higher moisture and HMF levels. These honeys, which are still very popular, such as the citrus honey of the Mitidja will have to be better studied to have a better mastery over time of their quality. The necessity to adopt national legislation is imposed mainly by the absence of provisions regarding the characteristics of honey, the declaration of the geographical origin of the product, the natural deviation of different types of honey and the quality rating of domestic honey. To the establishment of a quality honey label in, Algeria will bring to this noble product an added value and an international recognition essential to an export promotion by raising the awareness of beekeepers wishing to value the fruit of their work to the international standards the honey they produce and the respect of good beekeeping practices, packaging and storage of the finished product.

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