

Characterization and Chemometrics Based-Approach to Classify Some Algerian Blossom honeys

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Received: April 28, 2014 / Published: July 20, 2014.

Abstract: Physicochemical characterization of 82 Algerian honeys, collected between 2005 and 2010, from different botanical and geographical origins were analyzed. The studied parameters were: water content, pH, free acidity (FA), electrical conductivity (EC), ash content, hydroxymethylfurfuraldehyde (HMF), proline content, specific rotatory power and color. Most of the measured parameters had showed values in the range of the international standards, with a particular richness in proline and ash content. Chemometrics-based approach reveals that the discriminated groups were *Citrus*, *Ziziphus* and forest even with over represented groups like *Eucalyptus*. Principle component analysis (PCA) enabled to extract three principal components explaining nearly 65% of total variance, PC₁ and PC₂ were related to botanical origin whereas PC₃ to honey age. Analysis of variance showed that the studied variables were almost different depending on botanical, geographical origin and season. The current study also shows the presence of diverse honey varieties in Algeria. The collected data will contribute to the creation of products with protected geographical or/and botanical origins.

Key words: Algeria, blossom honey, quality, classification, chemometrics.

1. Introduction

Honey is an unprocessed natural food and sweetening agent used by humans [1]. It can be considered as a dietary supplement as it contains some important nutrients (sugars, α -tocopherol and ascorbic acid), and different flavonoids and phenolic compounds conferring it antioxidant and antibacterial activities [2-4]. Chemically, it is a mixture of sugars (70%-80%) and water (10%-20%) containing a large number of minor components mainly proteins, amino acids, aliphatic acid, salts, lipids and flavoring components as well as pollen grains [5-7].

This bee product with a complex matrix is influenced by geographical origins, soil and climate, post extraction treatments and storage conditions [8]; however, the botanical origin is largely responsible for

its flavor giving it the particular aroma and sweet taste touch that determines the selection of this food by consumer.

For many years, physico-chemical results was not enough to differentiate botanical origins honey samples because of their great variability, and the verification was based on pollen analysis which show many weaknesses [9]; that is why the current approach takes into account their combination with sensory analysis.

Bogdanov et al. [10] and several authors use chemometrics as an alternative solution to solve analytical problems in honey sector, whereas efforts, reagents and time saving [11-14], this science which extract information from chemical systems by data-driven means, using methods frequently employed in core data-analytic disciplines such as multivariate statistics, applied mathematics, and computer science; and which can give a lot of

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interesting explanations to collected data.

All over the world, countless studies have been conducted on honey, however, little is known about Algerian ones. Few studies have been found since 80's including pollen analysis [15] and physicochemical characterization of restricted regional interest [16-18], in which they report some melliferous plants and give an idea on honey quality, however, sample sizes and sampling zones were limited; although Algeria is characterized by a remarkable vegetation diversity due to its geographical configuration represented by Mediterranean coast, narrow coast plain limited by Tellian Atlas Chain Mountain, high plain zones limited by Saharan Atlas Chain Mountain and finally the desert (5/6 of total land). The climate from north to south is Mediterranean, semi arid, arid and Saharan.

Because of this heterogeneous climate and geographical repartition, several plant communities are present; Ricciardelli D'albore [19] reported the most known melliferous species giving monofloral honeys, namely *Anthyllis lotoides*, *Erica umbellata*, *Asphodelus*, *Taraxacum*, *Cichorium*, *Eriobotrya*, *Satureja*, *Salvia*, *Sesamum*, *Punica*, *Euphorbia*, *Persea*, *Gossypium*, *Musa*, *Agave*, *Erica multiflora*, *Peganum*, *Citrus*, *Eucalyptus*, *Rosmarinus officinalis* and *Trifolium repens*.

The recent growing interest in apiculture in the country, have encouraged several development programs which led to increased honey production. The mentioned fact has generated a need to characterize these honeys and to try to classify them using chemometric approach which enables us to establish relationships between botanical origins, production regions, harvesting seasons and physicochemical factors.

Therefore, the present study aims to contribute to investigating local honeys and to increasing the visibility of Algerian melliferous diversity by collecting data that will support the current certification process, when the global requirements is awareness of agronomic actors on the benefits of

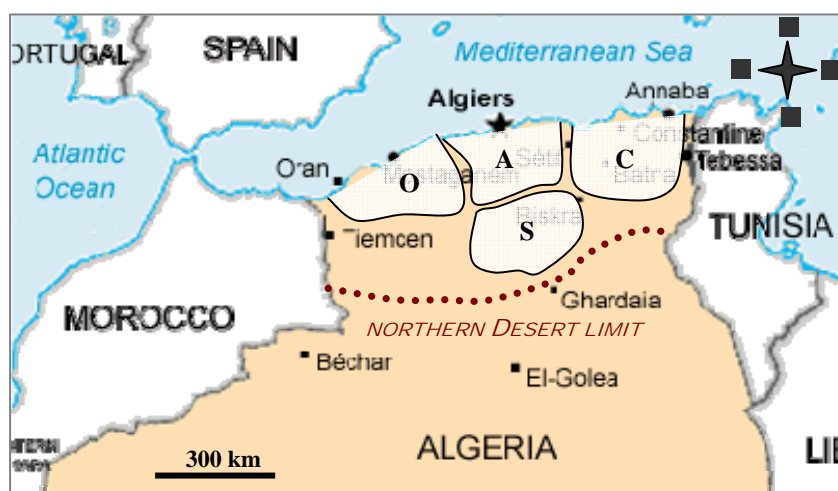
beekeeping as a pillar of sustainable agriculture and as a guarantee of biodiversity and life preservation.

2. Materials and Methods

2.1 Samples

This study was carried out on 82 monofloral and multifloral blossom honey samples (250-500 g/sample) harvested in Algeria, which were collected from different zones of the territory to include all melliferous regions, represented by 20 departments (Alger, Blida, Bouira, Médéa, Oran, Chlef, Boumerdes, Tizi ouzou, Tipasa, Béjaïa, Skikda, La Kale, Jijel, Constantine, Soukahrass, Batna, M'sila, Djelfa, Laghouat and Biskra) (Fig. 1) with different botanical origins. The most melliferous zones from which the samples have been collected were: Algiers (North Center), l'Oranie (North West), le Constantinois (North East) and South region aiming to highlight the differences between plain, valley, coast, mountain and steppe floral resources. The samples were obtained from professional and amatory apiarists and stored at 4 °C and protected from light until analysis. The process of collecting and analysis of samples have been carried out over five years (2005-2010) in order to have a better idea on the diversity and the constancy of honey production. The necessary information about supposed botanical origin, production season and honey age were registered.

The samples were divided into different groups according to their botanical and geographical origin as well as season. Thus, for botanical classification, different floral groups were stated, namely *Citrus spp.* ($n = 7$), *Eucalyptus spp.* ($n = 12$), *Ziziphus spp.* ($n = 7$), forest blossom ($n = 6$), multifloral ($n = 32$), others ($n = 18$); the last group includes minor represented honeys like *Asphodelus*, *Rosmarinus*, *Lavendula*, *Carduus*, *Peganum harmala*, *Daucus carota* L., *Arbutus* and *Brassica*. For geographical origin, they were divided into three groups, plain, mountain and steppe, and for seasons it was summer, spring and autumn.



A: Algérois ($n = 42$), O: Oranie ($n = 5$), S: Steppe ($n = 26$), C: Constantinois ($n = 10$)

Fig. 1 Geographical repartition of honey samples.

2.2 Methods

The physical and chemical analyses were performed according to the harmonized methods of the international honey commission (IHC) [20]. Botanical origin was verified by melissopalynology [19].

Water content was estimated using an ABBE refractometer (Atago Nar-1T liquid, Japan) and the results were relived as refracting index and reported on Chataway table to be converted in percent.

Electrical conductivity (EC) was determined in a 20% (w/v) honey solution using a conductimeter (JENWAY 4510, Bibby Scientifics, UK). The results were expressed as $\mu\text{S}/\text{cm}$.

Ash content was measured after incinerating 5 g of sample at $600\text{ }^\circ\text{C}$, and it was expressed in percent.

Diluted honey solutions (10% w/v) were analyzed for pH and free acidity (FA) by a JENWAY pH meter glass electrode and by titrating to pH 8.3, respectively. Titration volume was converted in milli-equivalent free acids by kg of honey.

Hydroxymethyl furfural (HMF) was determined by the following White's method, in which the diluted honey was treated with clarifying agents (Carrez solutions I and II) in order to prevent HMF breakdown. The absorbance of the filtered solution was measured at 284 nm and 336 nm against an aliquot treated with

bisulfite solution (0.2%) using a UNICAM UV/visible spectrophotometer. Color values were determined using Lovibond honey comparator and were reported in millimeters Pfund unit.

Proline content was determined by spectrometric method. Briefly, formic acid and ninhydrine were added to a 5% honey solution in celled tubes, placed in boiling bath for 15 min then in $70\text{ }^\circ\text{C}$ bath for 10 min, 2-propanol/water solution (50/50) was added and left cooling for 45 min. Finally, the absorbance was measured at 510 nm.

Specific rotatory power ($[\alpha]_{20}^D$) was determined using a POLATRONIC polarimeter (SCHMIDT, Germany) with a sodium lamp.

The sugar solution was prepared one day prior to the measurement by adding the carrez solutions and filtered, and then the rotatory angle was measured. Values were expressed as specific rotatory power.

2.3 Statistical Analysis

Statistical analysis was performed with a statistical package for social science (SPSS 9.0) software. First descriptive statistics of honey samples by floral origin were calculated. Then pair wise correlations between variables were done and, finally, principle component analysis (PCA) and one-way analysis of variance (Duncan test) was established. Prior to performing

PCA, the suitability of data for factor analysis was assessed by the calculation of the determinant, the Bartlett sphericity and the KMO adequacy tests.

3. Results and Discussion

3.1 Description of Samples and General Quality Analyze

Among the pollen analysis, there were in our case only 45.1% of monoflorals honeys, represented in order of importance by *Eucalyptus spp.*, *Citrus spp.* and *Ziziphus spp.* with a total percentage of 31.7%. *Asphodelus*, *Rosmarinus*, *Lavendula*, *Carduus*, *Peganum harmala*, *Daucus carota L.*, *Arbutus* and *Brassica spp.* honeys were underrepresented but could be an interesting indication to melliferous resource potency like investigated by Ricchiardelli D'albore [19].

Table 1 shows pair wise correlation ($P < 0.01$ and $P < 0.05$). Strong correlation was found between EC and

ash content, allowing as to eliminate one of the two parameters to avoid redundancy like raised by Terrab et al. [21]. Modest ones were observed between EC, color, FA and proline and water content and HMF were both weakly correlated with FA and pH at 1 and 5% levels, indicating that their amount is largely independent of chemical composition of honeys, concurring with Esriche et al. [22] study, where HMF apparition was found to be dependent on samples thermal history.

Referring to codex quality criteria [23], Table 2 shows clearly that studied samples, generally, present a good degree of maturity ($M 16.5\% \pm 1.5\%$) and a favorable aptitude to storage ($pH 4.06 \pm 0.60$; $HMF 13.2 \text{ mg/kg} \pm 16.8 \text{ mg/kg}$) with particular richness in proline ($624 \text{ ppm} \pm 303 \text{ ppm}$) and attractive dark amber colors ($110 \text{ mm Pfund} \pm 26 \text{ mm Pfund}$; $CE 455 \mu\text{S/cm} \pm 167 \mu\text{S/cm}$). Great values of standard deviations of some criteria indicate that there may be

Table 1 Correlation matrix of honey samples (n = 82).

	M	pH	FA	Ash	EC	$[\alpha]_{20}^D$	Color	HMF	Proline
M	1.000								
pH	-0.277**	1.000							
FA	0.396**	-0.400**	1.000						
Ash	0.133	0.061	0.464**	1.000					
EC	0.208	0.084	0.447**	0.851**	1.000				
$[\alpha]_{20}^D$	-0.236	0.382**	0.292	-0.192	-0.050	1.000			
Color	0.165	-0.160	0.385**	0.385**	0.522**	-0.074	1.000		
HMF	0.115	-0.195*	0.075	-0.074	-0.126	-0.096	0.091	1.000	
Proline	0.132	-0.192	0.455**	0.419**	0.384**	-0.144	0.406**	0.145	1.000

Determinant = 0.004188; **statistically different ($P < 0.01$); *statistically different ($P < 0.05$); $[\alpha]_{20}^D$: specific rotatory power.

Table 2 Descriptive statistics of honey samples classified by floral origin.

	<i>Citrus</i> (n = 7)		<i>Eucalyptus</i> (n = 12)		<i>Ziziphus</i> (n = 7)		Forest blossom (n = 6)		Multifloral (n = 32)		Others (n = 18)		All samples (n = 82)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
M (%)	16.7	0.6	16.5	1.1	15.0	0.6	17.7	0.8	16.5	0.8	16.4	0.8	16.5	1.2
pH	3.88 ^a	0.49	3.90 ^a	0.33	5.01 ^b	0.50	3.89 ^a	0.14	3.92 ^a	0.37	4.19	0.36	4.06	0.60
FA (meq/kg)	15.9 ^a	5.6	18.9 ^{ab}	4.6	13.1 ^a	2.9	28.0 ^c	4.9	27.1 ^{bc}	7.5	20.8	5.2	22.4	8.7
EC (Ms/cm)	163 ^a	28	501 ^{bc}	46	502 ^b	58	612 ^c	112	481 ^b	131	412	91	455	167
$[\alpha]_{20}^D$ (mL/g-dm)	-11.1 ^a	1.4	-12.6 ^a	5.0	-0.7 ^b	4.4	-11.4 ^a	4.2	-11.4 ^a	3.7	-7.2	6.8	-9.8	7.6
Color (mm Pfund)	63 ^a	19	108 ^b	16	103 ^b	10	137 ^c	3	110 ^b	22	112	21	110	26
HMF (mg/kg)	11.7	5.6	12.5	7.8	4.8	4.3	11.4	9.9	17.6	10.7	9.2	9.4	13.2	16.8
Proline (ppm)	269 ^a	75	689 ^c	148	521 ^{abc}	121	478 ^{ab}	44	758 ^{bc}	325	478	103	624	303

M: water content; FA: free acidity; EC: electric conductivity; HMF: hydroxymethyl-2-furaldehyde; $[\alpha]_{20}^D$: specific rotatory power; different letters in the row means values are statistically different ($P < 0.05$), Duncan test.

big differences between honeys, due to other considerations, hence the fragmentation of results depending on botanical origin, region and season were assessed in Tables 2-4, respectively.

Region and season based classification showed non-homogenous groups, but we observed that honeys from semi arid zones (steppe) were less moist ($16.0\% \pm 1.1\%$), less acid ($\text{pH } 4.56 \pm 0.57$) and presents a particular rotatory power ($-3.8 \text{ mL/g}\cdot\text{dm} \pm 8.16 \text{ mL/g}\cdot\text{dm}$). Mountain and plain groups do not present significant differences, only in water content values estimated to be $16.4\% \pm 1.4\%$ for the first and $17.1\% \pm 0.9\%$ for the second.

Autumn honeys showed the greatest water content with $17.3\% \pm 0.9\%$, corresponding to season water levels. Indeed, Chirife et al. [24] affirm that the amount of water content in honeys is a function of the factors involved in ripening, including weather conditions, nectar original water content and storage

conditions. This fact explains well the results reported in region classification especially when we know that 94.4% of semi-arid honeys and 68% of mountain blossom honeys were produced during summer, while 50% of plain ones were produced during spring (in our sampling).

We conclude that region and season factors are, in general, not significant for discriminating between clusters, but show some influences on pH, rotatory power and water content in region classification, and on color, EC and water content in one season. We can deduce that the region factors have an effect on sugar composition and organic acids content while season present an effect on mineral composition and pigment components, like mentioned by Wang et al. [25].

The botanical origin-based classification was in general capable to discriminate between groups, it was true for all criteria ($P < 0.05$) excepting HMF. The

Table 3 Descriptive statistics of honey samples classified by region.

	Mountain (<i>n</i> = 33)				Plain (<i>n</i> = 26)				Steppe (<i>n</i> = 23)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
M (%)	14.0	19.2	16.4 ^{ab}	1.4	14.8	18.6	17.1 ^b	0.9	13.0	17.8	16.0 ^a	1.13
pH	3.46	5.50	3.95 ^a	0.52	3.22	5.22	3.71 ^a	0.42	3.83	5.95	4.56 ^b	0.57
FA (meq/kg)	8.8	39.6	25.6	7.9	8.8	39.6	21.8	8.7	6.6	44.0	19.1	11.0
EC ($\mu\text{S}/\text{cm}$)	143	773	499.9	148.0	108	745	390.5	181.1	117	968	478.3	180.1
$[\alpha]_{20}^D$ (mL/g·dm)	-25.0	-4.5	-11.6 ^a	5.4	-41.2	-8.0	-12.9 ^a	7.0	-16.5	19.0	-3.8 ^b	8.2
Color (mm Pfund)	71	140	113.2	25.5	18	140	100.2	34.4	10	135	101.2	28.7
HMF (mg/kg)	3.1	32.3	11.5	7.8	5.4	42.7	17.3	10.3	0.0	131.9	14.18	30.2
Proline (ppm)	177	1,291	589.7	270.1	175	1516	662.6	378.4	300	1,380	667.7	361.3

Different letters in the row means values are statistically different ($P < 0.05$), Duncan test.

Table 4 Descriptive statistics of honey samples classified by season.

	Summer (<i>n</i> = 46)				Spring (<i>n</i> = 26)				Autumn (<i>n</i> = 10)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
M (%)	13.0	19.0	16.2 ^a	1.2	14.4	19.2	16.8 ^{ab}	1.1	16.4	19.0	17.3 ^b	0.9
pH	3.49	5.97	4.13	0.61	3.22	5.22	3.95	0.57	3.42	3.84	3.58	0.15
FA (meq/kg)	6.6	44.0	22.4	9.4	8.8	39.0	21.8	9.1	19.8	39.6	27.1	7.1
EC ($\mu\text{S}/\text{cm}$)	117	968	494.3 ^{ab}	155.7	108	733	367.1 ^a	179.6	300	675	505.2 ^b	132.3
$[\alpha]_{20}^D$ (mL/g·dm)	-25.0	+19.0	-9.1	8.0	-41.2	-4.9	-11.8	7.3	-15.0	-8.0	-10.4	2.5
Color (mm Pfund)	10	140	111.8 ^b	27.3	18	139	89.3 ^a	28.5	115	140	131.5 ^b	10.5
HMF (mg/kg)	0.0	131.9	13.4	20.5	2.10	42.7	14.4	10.2	4.2	25.7	16.4	8.5
Proline (ppm)	300	1516	639.8	281.7	175	1337	572.7	398.8	500	1354	748.2	320.2

Different letters in the row means values are statistically different ($P < 0.05$), Duncan test.

significant differences were mainly due to forest blossom, *Citrus* and *Ziziphus* honeys. Forest blossom group was significantly different regarding to M, FA, EC, color and proline, while in *Ziziphus* group the difference was found for M, pH, rotator power and proline.

Overall, *Ziziphus* class was distinguished in every respect. Thus, pH values were higher than common blossom honeys with 5.01 ± 0.5 , it show lowest water, FA and HMF contents with respectively $15.0\% \pm 0.6\%$, $13.1 \text{ meq/kg} \pm 2.9 \text{ meq/kg}$ and $4.8 \text{ mg/kg} \pm 4.3 \text{ mg/kg}$; a particular rotatory power values about $-0.7 \text{ mL/g}\cdot\text{dm} \pm 4.47 \text{ mL/g}\cdot\text{dm}$, in some cases, positive values indicating their particular sugars profile; Al Khalifa and Al Arify [26] report similar findings about Sidir aseer—another Rhamnaceae honeys (*Ziziphus spina-christi* L.). Proline values, a ripeness criterion, where 200 ppm has been used as a minimum level for honey authentication [27], can be a characterization indicator too, as reported by many other scientists (Sporns in Anklam [28]; Sabatini in Bogdanov et al. [10] and Oddo et al. [29]); it were in our case about $521 \text{ ppm} \pm 121 \text{ ppm}$, important value comparing to their results.

Forest blossom honeys showed the highest water content, FA, EC and color values, which are $17.7\% \pm 0.8\%$, $28 \text{ meq/kg} \pm 4.9 \text{ meq/kg}$, $612 \text{ mS/cm} \pm 112 \text{ mS/cm}$ and $137 \text{ mm Pfund} \pm 3 \text{ mm Pfund}$, respectively.

Citrus honeys gave intermediary values of water content of about $16.7\% \pm 0.6\%$. The lowest EC and color values with $163 \text{ }\mu\text{S/cm} \pm 28 \text{ }\mu\text{S/cm}$ and $63 \text{ mm Pfund} \pm 19 \text{ mm Pfund}$, respectively. EC, which is highly dependent on nectar source, can be a potential indicator of geographical origin as suggested by many authors [10, 25, 30, 31].

Citrus specific rotatory power $[\alpha]$ values presented a mean of about $-11.1 \text{ mL/g}\cdot\text{dm} \pm 1.4 \text{ mL/g}\cdot\text{dm}$ comparable to European *Citrus* honeys ($-13.5 \text{ mL/g}\cdot\text{dm} \pm 2.17 \text{ mL/g}\cdot\text{dm}$). It has also shown the lowest proline

contents comparing to other groups with a value of $269 \text{ ppm} \pm 75 \text{ ppm}$, but they are richer than Andalusian *Citrus* honeys reported by Serrano et al. [32], with mean values about $185.4 \text{ ppm} \pm 126.5 \text{ ppm}$.

Studied *Eucalyptus* samples were comparable to European honeys in most criteria [29, 33], with slight differences in color and proline content which are influenced by geographical situation of Algeria and important sun exposure of its flora inducing synthesis of secondary metabolites, generally, responsible of high pigmentations [34].

3.2 Contribution of the Different Criteria to Sample Discrimination

Table 1 clearly illustrates that all variables show significant correlation with at least one other variable. The determinant, the Bartlett sphericity and the KMO adequacy tests were in favor of PCA analysis.

To show the contribution of each parameter in the differentiation of samples, three components were extracted from PCA analysis of the data, describing 65.37% of the common variance (Fig. 2). First component PC1 explained 31.25% of data variance, positive loadings show mostly defined contribution by appearance honey elements (EC, color and proline). PC2 which accounts for 20.92% of total variance defined the contrast between two inversely correlated parameters profiles, positive loadings show M and FA contribution whereas negative loadings define pH and specific rotatory power $[\alpha]$ contribution, this component can be associated to taste honey attribute.

PC3 (13.21% of the data variance) is characterized by inverse correlation between HMF (positive loading) and pH (negative one), suggesting an antagonistic effect between these two variables related to honey age, noticed fact because aging promote HMF accumulation and honeys acidification [35].

After a global data analyze, we can affirm that all studied criteria are pertinent in honey classification and in the discrimination between samples, excepting

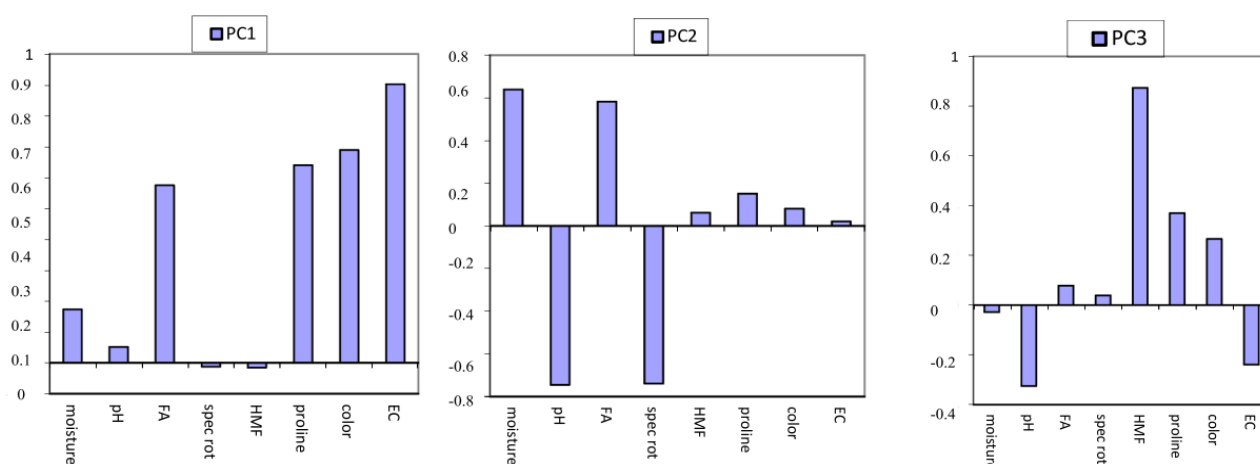


Fig. 2 Loadings of variables in the three principal components for honey samples.

HMF criterion which is certainly important for honey quality but not in clustering.

EC was found to have the highest classification power, this result is in accordance with Bogdanov classification [10] but in this study EC is pushed in the first rang followed by color, proline, $[\alpha]$, pH and FA.

When we observe the composition of PC, knowing that the color and proline are well correlated to EC (Table 1), we can conclude that this criterion can explain alone near 31% of the total variance.

The botanical origin-based classification could explain nearly 52% of the differences between samples followed by season classification (secondary factor) in relation with appearance criterion (PC1) and region classification related to taste attributes (PC2). This fact results from the greatest relationship between botanical and season origin than region.

With the combination of physicochemical characterization and statistical analyses, we were able to distinguish three honey groups, namely *Citrus*, *Ziziphus* and forest blossom, but we were not able to well discriminate *Eucalyptus* and multiflora honeys which seems similar.

The *Ziziphus* group can be discriminated from all other groups regarding water content, FA, pH and $[\alpha]$, in general, it shows good initial properties participating to its prolonged shelf life.

Forest blossom honeys seem to be the most fragile

samples, and can be differentiated by EC, color and proline content. *Citrus* cluster was the most distinguished group; it shows, together, appearance and taste attribute differences.

4. Conclusions

Beyond the fact that the studied samples were of good quality, with particular richness in proline and attractive amber colors, generally, darker than European ones; the studied criteria combined with chemometrics helped us to classify honey, especially when the botanical origin factor is considered, this does not decrease region and season approaches which can explain the variability of some parameters. PCA analyses also show that EC is the strongest parameter that can be used to discriminate between honey groups.

Citrus, *Ziziphus* and forest blossom groups were well distinguished, despite *Eucalyptus* and multiflora were the most represented groups. This fact confirms that chemometrics approach is well adapted for this purpose and being capable to pass through some practical difficulties like insuring equivalent sample numbers in all groups and the great parameters variability.

The present study reveals that *Ziziphus* honeys, harvested in semi arid Algerian zones, were outstanding and can be labeled as “controlled

botanical or geographical origins”; therefore, it will be interesting to focus our future investigations on it.

Moreover, the apiculture development, in our areas, depend hardly on scientific based-investigations and data collection allowing the understanding of honey production and promote this agricultural sector, that’s what we expect to do with this modest work.

Acknowledgments

This work was done with the help of Algerian apiarists who provided us honey samples, the pedagogic laboratories DTA department (FSI, Boumerdes) who allowed us to use their laboratory material and Mr. Abdelhak Mansouri for his precious advices.

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