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Physicochemical analysis of some honeys from humid regions in North East Algeria

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ABSTRACT

This study is a physicochemical analysis (moisture, pH, electrical conductivity, ash, free acidity, lactonic acidity, total acidity, protein, sugars, HMF and density) of twenty one honey samples from humid regions of North Eastern Algeria. The main objective is to determine their quality. The comparison of obtained results with international standards showed that nineteen analyzed honeys have good quality (low HMF content, lower acidity than the standard, low water content and low quantity of proteins). All analyzed honeys have a nectar source this is confirmed by ash content, pH and electrical conductivity. Statistical studies have shown that the first five components explain 81.05% of the variability on the honey and the honey samples analyzed divided into six groups at similarity 48.

KEY WORDS: Honey, Physicochemical analysis, humid regions, Multivariate statistical, Moisture.

1. INTRODUCTION

Honey is the natural sweet substance produced by honeybees from the nectar of flowers or secretions of living parts of plants. Bees collect nectar with specific substances and store it in honeycombs (Codex Alimentaire, 1981). The chemical composition of honey varies from one sample to another, depending on the plants visited by bees (Bertoncelj, 2007). The physico-chemical composition of honey determines its quality and its botanical origin. Honey has a relatively high density which ranges from 1.40 to 1.45 g/cm³ (Bogdanov, 1995). It is related to the water content that ranges between 15 and 20g/100g of honey. On the other hand, there is a link between the water content and the yeast content (Stephen, 1946). Honey electrical conductivity is related to the mineral content (Jean Prost, 1987). Honey acidity is due to the presence of acids in honey and most of them are added by the bees (Echigo, 1974). Flowers honeys have mostly low pH values (3.3 to 4.6). Honeydew honeys have higher pH values in average; this is due to their higher content of buffering salts (Bogdanov, 1995). Approximately, 85-99% of the honey dry matter consists of sugars (Gonnet, 1982). The fresh honey contains no or only trace of HMF (usually below than 3 mg / kg). During storage, the HMF is formed more or less rapidly from sugar (especially fructose) by the effect of acids and depending on the pH value and temperature of the honey (Afnor, 1990).

In the North-East of Algeria, honey production knows a significant growth thanks to the richness in honey species and the support of the agriculture ministry. Several studies have been done to evaluate the quality of honey (Ouchemoukh, 2007; Cheffrour, 2009; Makhloufi, 2010; Amri and Ladjama, 2013; Haouam, 2016).

The aim of this work is to evaluate the physicochemical characteristics (moisture, pH, electrical conductivity, ash, free acidity, lactonic acidity, total acidity, protein content, sugars, HMF and density) of twenty one honeys samples from different humid regions in the North-East of Algeria.

2. MATERIALS AND METHODS

Honey samples: Twenty one natural honey samples from some humid regions of Northeastern Algeria were collected. All the samples were stored at -18 ° C in plastic jars for further analysis. Regions of study are shown in Table.1.

Physicochemical parameters: Moisture was determined by refractometry reading at 20°C (AOAC, 1995). The refractive index (RI) is measured than the corresponding moisture percentage is obtained from the Chatway Table. Ash, electrical conductivity, free, lactonic and total acidity and pH were determined according to AOAC methods (1990). Total sugars were determined using a special refractometer (Carl-Zeiss Jena refractometer) reading at 20°C.

Protein content was determined by the method of Bradford (1976). A volume of 0.1ml of protein extract was added to 5ml of Coomassie Brillant Blue. After 2 min of incubation, the quantity of protein was estimated at 595nm in relation to Bovine Serum Albumine standard cuve.

HMF was measured by spectrophotometric method (AOAC, 1990), using Jenway 6305 spectrophotometer. Density was determined according to Bgdanov (1995), by dividing the weight bottle (10ml) filled with honey by the weight of the same bottle, filled with distilled water.

Statistical analysis: The statistical analysis was conducted by Minitab 16 and XL stat studying the multivariate analysis data type Principal Components (ACP), with a correlation circle and Ward dendrogram to determine the parameters that are closer to each other.

Table.1. Geographical origins of honeys samples from Algerian humid regions

Samples	State	Geographic origin	Samples	State	Geographic origin
H1	Annaba	Seraidi	H12	Skikda	Jbel ben welben
H2	Annaba	Asfour	H13	Skikda	Skikda
H3	Annaba	berrahal	H14	Skikda	Tabet saleh
H4	Annaba	Sidi ammar	H15	Skikda	El hadaik
H5	Annaba	Ben mhidi	H16	Skikda	Azzaba
H6	El-Taref	Jbel bni saleh	H17	Skikda	El kol
H7	El-Taref	Ain khiar	H18	Bejaia	Kheratta
H8	El-Taref	Ain el karma	H19	Bejaia	Borj mira
H9	El-Taref	Boutheldja	H20	Jijel	Tahir
H10	El-Taref	Bougous	H21	Jijel	Jijel
H11	El-Taref	Laysoum			

3. RESULTS AND DISCUSSION

Physicochemical parameters: The results of physicochemical analyses of honey are summarized in Table.2.

Table.2. Results of some physicochemical parameters (mean±SD)

Physicochemical parameter	Units	Minimum	Maximum
Moisture	g/100g	14,47±0,11	23±0,5
pH	pH units	3,15±0,02	4,50±0,15
Electrical conductivity	S/Cm	$1x10^{-4}$ ±0,0	$3,9x10^{-4}$±0,03
Ash	g/100g	0,02±0,03	0,53±0,12
Free acidity	meq/Kg	10,16±0,28	28,03±0,35
Lactonic acidity	meq/Kg	2,38±0,09	8,55±0,09
Total acidity	meq/Kg	15,46±0,15	34,27±0,35
Proteins	mg/g	0,11±0,02	2,85±0,04
Total sugars	%	75,46±0,28	83,63±0,05
HMF	mg/Kg	0,5±0,04	4,75±0,55
Density	-	1,32±0,01	1,55±0,06

Moisture is a parameter related to weather conditions, to the harvest season and to the maturity (Nanda, 2003). This is the most important physicochemical parameter for the study of conservation and stability of foods in general (Cano, 2001). The obtained results show that the moisture of the studied samples varied between 14.47 and 23.05g / 100g. These results show that all analyzed honeys comply with the standards proposed by the codex, except for samples H18 and H6 which have moisture levels higher than 21%. These honeys are susceptible to fermentation (Gonnet, 1982).

The pH values range from 3.15 to 4.5. The pH is very important during the extraction and storage of honey, because it influences the composition and the stability of the product (Terrab, 2004). The results show that all analyzed honeys are from a nectar source, the pH of the flower honeys vary between 3.5 and 4.5 while honeydew honeys have higher pH values ranging from 4.5 to 5.5 (Gonnet, 1986).

The ash content is a parameter used to determine the botanical origin (floral, honey or mixed) (Terrab, 2004). The maximum ash content in flower honeys is equal to 0.6% (Terrab, 2004). The ash content in the analyzed honey samples varies between 0.02 and 0.53 g / 100g, therefore these honeys have a nectariferous origin.

Honey electrical conductivity is a parameter related to minerals content, organic acids and proteins. It varies according to the botanical origin (Terrab, 2003), the electrical conductivity values of the nectar honeys range from $1x10^{-4}$ to $5x10^{-4}$ Siemens / cm, while those of melliferous origin have values between $10x10^{-4}$-$15x10^{-4}$ Siemens / cm (Alphandery, 1992). The electrical conductivity is considered as one of the best parameters to differentiate between honeys from different origins (Krause, 1991). The analyzed samples showed electrical conductivity values-ranging from $1x10^{-4}$ to $3x\ 10^{-4}$ Siemens/cm. According to these results, it can be concluded that all analyzed honeys have a nectar source. These results are in accordance with those obtained by Ouchemoukh, (2007) working on honey from the Bejaia region that is located in eastern Algeria.

The free acidity values of the analyzed samples are between 10.16 and 28.03 meq/kg, so, we can say that all analyzed honeys comply with the required standards (50 meq / 100g honey) of the Codex Alimentarius (1998). The lactonic acidity is considered as a secondary acidity when the honey becomes alkaline (Gonnet, 1982). The values obtained in our study are comprised between 2.38 and 8.55 meq/ kg. The total acidity is the sum of free and lactonic acidity, it varies according to the harvest season (De Rodriguez, 2004).

Proteins: honey contains less than 5mg / g protein (Anklam, 1998) and the pollen is the main source of honey protein (Baume, 2004). The obtained results vary between 0.11 and 2.85 mg / g, these values are low than those obtained by Ouchemoukh, (2007) who found values ranging from 3.7 to 9.4 mg / g in Bejaia honeys.

Sugars: according to Gonnet (1982), sugars represent the greater part of the honey dry matter (95 to 99%). Flowers honeys contain a small amount of monosaccharides compared to nectar honeys. The results of our study vary between 75.46 and 83.63%.

HMF: The freshly harvested honey contains almost no HMF. However, during storage at high temperature, this value increases (Bogdanov, 1995; Mendes, 1988). The amount of HMF tolerated in a honey should be always less than 10 mg / kg of honey. Although, the law tolerates up to 40ppm and 60ppm according to the European Union and the Codex Alimentarius respectively (Bogdanov, 1999).

The results of the studied honeys are between 0.5 and 4.75 mg / kg so; we can say that all the studied honeys are conform to the standard required by the codex. The obtained values are low than those found in other studies carried on Moroccans honeys (Terrab, 2002); on Irland honey (Downey, 2005) and on Portuguese honey (Mendes, 1998)

Density: the obtained values are comprised between 1.32 and 1.55. Chefrour (2009), found that the density values of some Algerian honeys range from 1.37 to 1.5

Statistical analyses: Correlation Matrix: Table.3 shows that there is a positive correlation between total acidity and free acidity (r = 0.98). An average positive correlation is observed between the lactone acidity and water content (r = 0.49) and between protein and ash content (r = 0.4). Sugar content is negatively correlated with water content (r = -0.76) and the lactone acidity (r = -0.45)

Table.3. Matrix correlations between different variables of honey samples

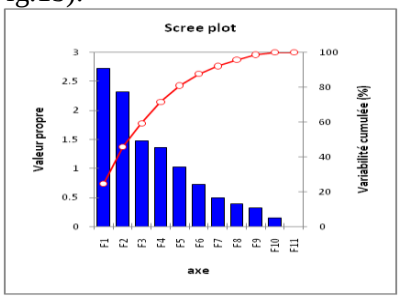
Variables	Moisture	pH	Electrical conductivity	Ash	Free acidity	Lactonic acidity	Total acidity	Proteins	Total sugars	HMF	Density
Moisture	1										
pH	-0.20	1									
Electrical conductivity	-0.12	0.01	1								
Ash	-0.29	-0.11	0.26	1							
Free acidity	-0.18	0.14	0.01	-0.19	1						
Lactonic acidity	0.49	-0.29	-0.18	-0.36	-0.30	1					
Total acidity	-0.09	0.09	-0.02	-0.27	0.98	-0. 12	1				
Proteins	0.10	-0.13	-0.26	0.17	-0.21	0.10	-0.20	1			
Total sugars	-0.7	0.04	0.26	0.40	0.14	-0.45	0.06	0.07	1		
HMF	0.18	0.09	-0.17	-0.19	0.14	0.06	0.16	0.33	-0.02	1	
Density	0.19	-0.01	0.36	0.07	0.30	-0.10	0.29	0.02	-0.19	0.11	1

Principal Component Analysis (PCA) : According to the graph (Fig.1a), we note that only the first five components are greater than 1. From Table.4, we see that the first five components explain 81.05% of the variability on honey.

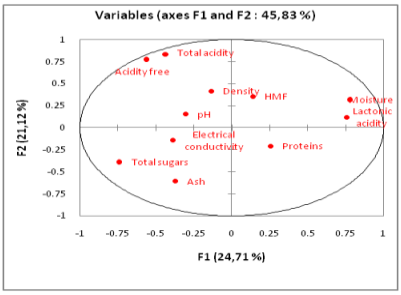
Table.4. Eigen values of the first five components

	PC1	PC2	PC3	PC4	PC5
Eigen values	2.71	2.32	1.48	1.36	1.02
Variance%	24.70	21.12	13.46	12.40	9.36
Cumulative%	24.70	45.82	59.29	71.69	81.05

The first two axes explain 45.83% of the information, the first axis alone explains variability of 24.71%, this axis is strongly positively correlated with the water content and acidity lactone and strongly negatively correlated to the sugar content; the second axis also explains a significant proportion of variability in the range of (21.12%), this axis is correlated to the free acidity and total acidity and HMF, which that honey containing a high acidity are rich in HMF (Fig.1b).



a



b

Figure.1. Principal Component Analysis (PCA)

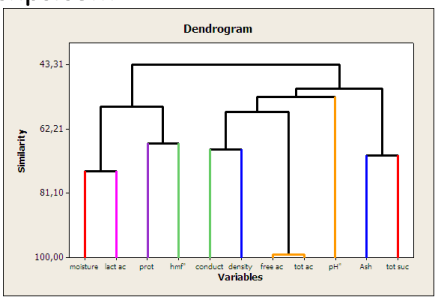


Figure.2. Dendrogram of Ward of physicochemical parameters

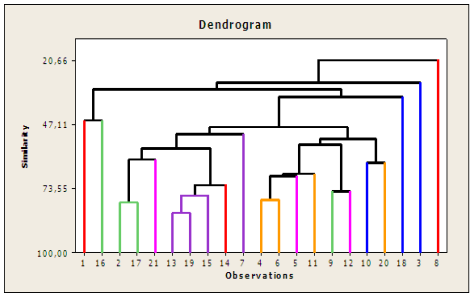


Figure.3. Dendrogram of Ward between the samples

Hierarchical Clustering (HC): The hierarchical clustering (HC) is a method of hierarchy construction; we realized this classification between honeys samples and between variables.

Fig.2, shows Ward dendrogram and the distance of the correlation coefficient between the variables. It shows two distinct groups of variables according to their degree of correlation, the first group includes variables moisture, lactonic acidity, proteins and HMF and the second group comprises electrical conductivity, density, free acidity, total acidity, pH, ash content and total sugars.

Fig.3, shows the relationship between the samples of the studied honeys. At the similarity level 48, the dendrogram shows six groups: group 1: code 8 (H8), group 2: code 3 (H3), group 3: code 18 (H18), group 4: codes 20, 10, 9, 11, 5, 6, 4 (H20, H10, H9, H11, H5, H6, H4), group 5: codes 7, 14, 15, 19, 13, 21, 17, 2 (H7, H14, H15, H19, H13, H21, H17, H2) and the sixth group of H1 and H16 (code 1 and 16).

4. CONCLUSION

In this work we have studied some physicochemical parameters of twenty one honey samples from humid regions in North Eastern Algeria. The obtained results allowed us to better know our honeys and determine their quality. The studied parameters show that the majority analyzed honeys have a nectar source. The levels of HMF and total acidity showed that all the studied samples are consistent with the food codex standard. The pH and water content show that all analyzed samples can be stored for a long period. Statistical analysis showed a positive correlation between total acidity and free acidity. This study is to be continued by other researches to study the botanical origin of these honeys and their antibacterial effect.

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