Study of the quality and antibacterial activity of some honeys

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Abstract

Honey is a sweet substance that bees make from the nectar of flowers or honeydew, having varying chemical composition and properties. The aim of this research is screening the antimicrobial effects of eight different honey samples from Algeria . Samples of honey were collected from different areas of Tizi-Ouzou. Samples of honey have been studied for their physicochemical and pollen parameters and their antimicrobial effect on *Escherichia coli*, *Staphylococcus aureus* , *Pseudomonas aeruginosa* , *Staphylococcus epidermidis*, *Listeria innocua* *Enterococcus faecalis* *Bacillus cereus* .The analyzes carried out on the 8 samples of honey reveal a water content of 16.80 to 22%, an acidic pH of 3.70 to 4.04, an electrical conductivity of 0.26 to 0.85×10^{-4} S / cm and a hydroxymethylfurfural level between 16 to 38.03 mg / kg. These results show that these samples are of good quality compared to international standards. The pollen analysis allowed the identification of different botanical families. According to our results all the honey samples have effectively high antibacterial activity against studied strains.

Introduction

Honey is sweet, viscous syrup produced by the honey bee (*Apis mellifera*). It is probably the first natural sweetener ever discovered, and is currently used as a nutritious food supplement and medicinal agent (Olas 2020).It is a very complex biological compound with very wide diversity, giving it a multitude of properties, both nutritionally as therapeutically (BELHAJ et al. 2015) mainly due to its chemical composition, in particular polyphenols and flavonoids (da Silva et al. 2016)(da Silva et al. 2020).

The impact of infectious diseases continues to grow around the world. This is usually due to the phenomenon of antibiotic resistance. For this reason, recent studies are interested in the therapeutic virtues of certain natural products which do not generally have side effects. Honey is one of these most coveted products because of its inhibitory and therapeutic properties, honey is subject to a number of speculations as to its origin and its physico-chemical qualities Algerian consumer does not manage to distinguish between an authentic product and another falsified because of the absence of official structures that control the quality of local products. These allow us to identify the properties of the different honeys harvested in several regions of Tiziouzu .

1. Material And Methods

1.1. Honey samples

Our study focused on 8 samples of honey, of different geographical origins distributed in the wilaya of Tizi-Ouzou (Sidi naamane, Tizi rached, Freha and Azeffoun) code respectively);A code has been assigned to each sample, in order to facilitate their handling during the various analyzes in the laboratory. H1 to H8 Assumed floral origin : Eucalyptus for H1, Orange tree for H2, acacia for H5 ,lavender for H6 ,all flowers for other samples .The honey samples were collected in sterile 50mL falcon tubes and stored in the freezer at -20°C until analysis (water content, pH and free acidity, electrical conductivity; Hydroxy-Methyl-Furfural or simply HMF) and pollen analyzes.

1.2. Microbial strains

The evaluation of the antimicrobial activity of honey was carried out by diffusion through discs in the presence of the microbial strains. Provided by the microbiology laboratory of the University of Hassiba Ben bouali Chlef: *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa*, *Escherichia coli* (ATCC25922), *Staphylococcus epidermidis*, *Listeria innocua*, *Enterococcus faecalis* and *Bacillus cereus*

The choice of these strains is based on their wall differences (Gram + and Gram-), as well as their presence in the digestive tract or on the skin.

1.3. Antibacterial Activity

Antibacterial activity was tested against a panel pathogenic microorganisms. The disc diffusion method, known as the Kirby-Bauer method, was used to determine the antibacterial activities of samples honey

1.4. phenols and flavonoids evaluation

The total phenolic content was estimated using the spectrometric method of Folin-Ciocalteu' as described by Beretta et al. (2005). The total flavonoid content was determined according to the methodology described by Meda et al. (2005) and Ahn et al. (2007).

1.5. Antioxidant activity

The antioxidant activity of the extracts was evaluated based on hydrogen-donating or radical-scavenging ability using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). An amount (0.1 mL) of phenolic extracts was added to 3 mL of 0.04% methanolic solution of DPPH. The mixture was mixed thoroughly and incubated in dark at room temperature for 60 min. The decrease in absorbance was then measured at 517 nm, against methanol as a blank.

2. Results And Discussion

The most important physico-chemical parameters were measured for the honey samples, namely: water content, electrical conductivity, pH, free acidity and 5-hydroxyméthylfurfural (HMF), and the results are summarized in Table 1.

Table 1
physico-chemical analyzes of the tested honey samples

Code	pH	Water content (%)	Brix level (%)	Electrical conductivity $E C \times 10^{-4}$ S/cm)	Ash content (%)	Free acidity (meq/kg)	HMF (mg/kg)
H1	4.04±0,004	16.80±0.55	86.88±0.67	0.591±0.002	0.26±0.006	31.01±0.2	16.04±0.55
H2	3.70±0.011	17.50±0.32	85.72±0.56	0,260±0.006	0,56±0.009	26.22±0.2	38.48±0.05
H3	3.73±0.022	17.6±0.18	80.22±0.39	0.268±0.008	0.40±0.005	25.03±0.2	19.50±0.11
H4	3.92±0.001	22±0.09	83.98±0.74	0.852±0.003	0.36±0.002	47.12±0.2	17.11±0.80
H5	4.32±0.05	16.8±0.42	84.92±0.42	0.650±0.004	0.46±0.003	23.00±0,2	35.00±0.55
H6	3.66±0.01	17.19±0.2	84,81±0,46	0.514±0.008	0.31±0.007	19.35±0.1	31.21±0.50
H7	4.09±0.007	18.01±0.79	85.89±0,77	0.363±0.009	0.29±0.008	22.44±0.3	38.03±0.85
H8	4.32±0.05	19.33±0.40	81.45±0.44	0.591±0.007	0.44±0.001	23.28±0.3	35.77±0.50

2.1. Water Content

The water content of the samples varies from 16.80 to 22% (Table 1); honey Samples show values that are within the range recommended by The international Codex Alimentarius Standard for honey (Alimentarius 2001), and which does not exceed 20%; This confirms that the risk of fermentation is very low in these samples.

According to Gonnet (1982), honeys are in good conservation because they represent water contents of less than 18%.

Unlike the H4 sample collected in the Azeffoun region which represents the highest water content (22%). This value exceeds the normal limits recommended by The international Codex Alimentarius Standard for honey (Alimentarius 2001) and the European committee (Fallico et al. 2006). This can be explained by harvesting the honey before it is fully ripe in the cells, or by extracting this honey in a humid place or in an area where the humidity is very high. (BELHAJ et al. 2015). Since honey is very hygroscopic.

2.2. Electrical Conductivity

The values of the electrical conductivity obtained are between 0,260 and $0,852 \times 10^{-4}$ S/cm. (Table 1). Samples represent values less than 0,8 μ S/cm which shows that they are honeys of nectar-bearing origin. On the other hand, honey H4 which represents a conductivity of $0,852 \times 10^{-4}$ S/cm is considered a mixed origin honey where the nectar is dominant.

According to Gonnet (1982), nectar honeys have a conductivity between 1×10^{-4} and 5×10^{-4} S/cm, and the honeydew honeys between 10×10^{-4} et 15×10^{-4} S/cm. The median values correspond to natural mixtures of the

two origins.(Acquarone, 2007).The measurement of electrical conductivity is very important because it makes it easy to distinguish honeydew honeys from those of nectar-bearing origin, the former having a higher conductivity than the latter(Bogdanov et al. 1999).

2.3. pH

The pH is a measure which allows the determination of the floral origin of honey, of which the honeys from nectar have a pH between 3.5 and 4.5, whereas those from miellas are between 5 and 5.5 (Gonnet 1982).The pH values obtained range from 3.70 to 4.04 (Table 1). We can thus say that our samples are likely to have a nectariferous origin.(Acquarone et al. 2007)

2.4. Free Acidity

we notice that the values of the free acidity of the honeys vary from 25 to 47 méq /kg. (Table 1) ;According to the international standards of Codex Alimentarius (2001), the free acidity of honey should not exceed 50 méq /kg(Alimentarius 2001). The free acidity level of samples is quite far from this value, therefore they have the advantage of aging very slowly. Sample H4 has a value that is a bit high (47 méq /kg), this value can be explained by its aging. Therefore, we can say that the free acidity of our samples complies with the legislation in force., acidity is an important quality criterion, it gives important indications on the state of honey. (Bogdanov et al. 1999)

2.5. Hydroxymethylfurfural (Hmf) Rate

The presence of HMF in honey is indicative of more or less advanced degradation of the product, it therefore provides information on the state of freshness of honey. The HMF rate must remain very low for a guarantee of quality and proof of good conservation of the product.(Thrasyvoulou 1986)

According to Bogdanov et al. (2004), the amount of HMF tolerated in honey should always be very low and not exceed 40 mg / kg.

The quantitative results of HMF presented in (Table 1), confirm that samples which represent values of 16 and 17 mg / kg respectively, are genuine honeys. Sample H2 which has a value of 38 mg / kg is close to the maximum value (40 mg / kg), this may be due to its natural aging due to storage conditions (room temperature). Sample H3 and H7 represents a value of 38.48 and 38.03 mg / kg respectively, they exceeds the normal value, considered than old honeys, their shelf life is about 2 years.From these results, it can be concluded that H1 and H4 honeys comply According to the international standards of Codex Alimentarius (2001), while H2 and H3 honeys are no-compliant. (Alimentarius 2001).

2.6. Pollen Analyzes

The pollen analysis is mainly done for the purpose of confirming the floral source of the honeys tested. The results are summarized in Figure 1

Pollen analysis of sample H1 shows the presence of fourteen different taxa of nectar-bearing pollen with a total of 4850 grains.

According to the percentages of the pollen families, this honey is a multifloral containing eucalyptus, because usually eucalyptus honeys are over-represented and can only carry the mention "eucalyptus honey" from 90% of eucalyptus pollen) (Bobis et al. 2020).

Observation of sample H3 shows the presence of sixteen different taxa of nectar-bearing pollen with negligible traces of honeydew, with a total of 5740 grains.

According to the percentages of the pollen families, this honey is a Hedysarum monofloral. (Makhloufi et al. 2015) .

The observed slide of sample H4 is very rich in pollen, it presents a thick protein film with a mineral residue reminiscent of sand. The honey shows no signs of fermentation. Depending on the percentages and families of pollen present, this honey has the characteristics of a nectar / honeydew mixture. (Carmen Seijo, Jesús Aira, and Méndez 2003) (Sanz et al. 2005) The results of the pollen analysis allow us to identify the different types of pollen to confirm where to deny the multi floral origin of the studied honeys.

2.7. Evaluation Of Antimicrobial Activity

The results of antimicrobial activity as shown in Table 2 show that all the strains studied are more or less sensitive to the action of honey, regardless of their origin. These variations are in agreement with another study on the antimicrobial activity of honey (Sherlock et al. 2010) (Szweda 2017). In another study; the antimicrobial activities of both honey and this solution towards 21 types of bacteria and two types of fungi were examined .The kinds of antimicrobial substances (inhibines) in honey are discussed. Hydrogen peroxide is not the only inhibine in honey. In fact, inhibines in honey include many other substances. Two important classes of these inhibines are the flavonoids and the phenolic acids. Flavonoids have often been extracted from honey previously (Leyva-Jimenez et al. 2019). In conclusion of this part we can say that the best inhibitory activity towards *Escherichia coli* is exerted by the H8 with an inhibition zone of 17 mm, for *Staphylococcus aureus* the maximum inhibition diameter was observed with the H1 (16 mm). The zone of maximum inhibition for the *Pseudomonas aeruginosa* strain was observed with H8 (22mm). From the results of the evaluation of the antimicrobial activity obtained, it can be seen that all the bacterial strains tested are sensitive to the inhibitory action of samples honey with more or less pronounced effects.

Table 2
Antibacterial activity of honey samples from tiziouzou in Algeria

Microorganisms	Inhibition zones (mm)*								
	H1	H 2	H3	H4	H5	H6	H 7	H 8	NE
<i>S.aureus</i> ATCC 29213	17	19	12	18	14	12	12	16	31
<i>Pseudomonas aeruginosa</i>	16	9	12	19	16	10	18	22	25
<i>Staphylococs epidermitidis</i>	12	14	10	18	10	19	25	26	30
<i>Listeria inocua</i>	18	24	38	34	34	32	12	14	20
<i>E.coli</i> ATCC 25922	10	15	13	16	9	9	10	17	18
<i>Enteococcus fecalis</i>	8	13	18	12	11	8	7	17	15
Bacillus cerius	25	18	16	15	21	22	8	8	22
NE neomycin *: inhibition zones includes 6 mm disk diameter, data are average of three measurements									

2.8. Evaluation of phenols, flavonoids and antioxidant activity

Table 3 shows that the content of polyphenols in honey varies considerably from 53 to 132 mg EAG/kg honey. (The lowest value was recorded in H2 honey (53 mg EAG/Kg honey) and the highest polyphenol concentration is obtained with H8 sample; the IC50 values of the honey samples studied ranged from 5.5 to 22.5 mg/ml. The lowest IC50 value indicates high free radical trapping capacity (Kanoun 2010). The highest IC50 value was 22.5mg/ml in the H7 sample. However, the lowest value in H5 sample, confirming the possibility that it contains the largest amount of free radical-accepting compounds and the greatest antioxidant potential.

Tableau 3: polyphénols and flavonoides cotents of the differents honey

Code	Total Polyphenols (mg GAE/ Kg)	Flavonoids (mg REE/kg)	Antioxidants (IC50 mg/ml)
H1	61±0.001	29.11±0.51	10.2
H2	53±0.001	19.32±0.51	7.5
H3	54±0.001	33.88±0.51	19
H4	73±0.005	9.3±0,0036	8.4
H5	102±0.005	32.55±0.66	5.5
H6	98±0.004	41.55±.,66	14.6
H7	108±0.004	26.55±0.66	22.5
H8	132±0,006	58.55±0,66	11.1

Conclusion

The different parameters studied show that all the honey samples comply with the standards proposed by the Codex Alimentarius Commission, that most of them are fresh honeys, according to their HMF content. But also that they are not falsified following the results of the pollen analysis. Regarding the antibacterial activity, the present study confirms that each honey analyzed exerts inhibition for the different tested bacteria.

Declarations

- **Consent for publication:**

Authors Consent for all data publication

- **Availability of data and materials:**

All data are available

- **Competing interests**

The authors declare that they have no conflicts of interest

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All costs of this scientific study are borne by chlef university and, INRAA (Baraki, Algeria),

- **Authors' contributions**

- Abdelkader Mezaini Is working as an Assistant Professor in the Department of Nutrition Laboratories, Chlef University ,Algeria . His research interests include measurement of the therapeutic potential of natural compounds in the management of diseases, Public health and Microbiology.

- Abdelhamid belhadj benziane is currently works in civil protection . He has published several meaningful research and review article, of international reputed journal and has presented his papers in many national and international conferences.
- Naima smaili is currently working as Co-Investigator on different Interdisciplinary research projects. The major research areas are investigating the therapeutic potential of different medicinal plant extracts.

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ethics approval and consent to participate

'Not applicable' for that section.

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Figures

Figure 1

Pollen analyzes of honey samples

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