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Physicochemical and antimicrobial properties of Tunisian honeys: Honey inhibited the motility of bacteria

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Honey is a sweet flavourful product which has been consumed as a high nutritive value food. The present study aimed to characterize 12 samples of honeys collected from the North of Tunisia in respect to their floral origins. pH, hydroxyl methyl furfural, moisture content, ash, acidity, electrical conductivity, reducing sugars, apparent sucrose and total phenolic contents, were the parameters analysed in each sample. All honey samples analyzed were found to meet European Legislation Ec Directive (2001/110) for all parameters studied. Antimicrobial assays showed that most honey samples tested inhibited the proliferation of *Escherichia coli* and *Salmonella typhimurium*, and altered **the flagella driven motility of these two bacteria tested in swarming assay. We have also demonstrated that the most important antibacterial activity was observed for the honey originated from eucalyptus flower.**

Keys words: North Tunisia, Honey, antimicrobial effect, physicochemical parameters, bacterial motility.

INTRODUCTION

Honey is a complex mixture produced by honeybees from the nectar and also exudates from plants and it is consumed as a sweetener as well as for its therapeutic properties. It is essentially composed of a complex mixture of carbohydrates (of which fructose and glucose account for nearly 85 to 95%) and other minor substances, such as organic acids, amino acids, proteins, minerals, vitamins and lipids (White, 1975). The composition and properties of honey is dependent on floral origins utilized by the bees and climatic conditions of the area from which honey is harvested. Honey has been found, by some workers, to possess antibacterial activities where antibiotics were ineffective (Subramanyam, 1991). It has been reported to have an inhibitory effect to about 60 species of aerobic, anaerobic, gram- positive and gram-negative bacteria (Molan, 1992). Also, pure honey has been shown to be bactericidal to many pathogenic microorganisms including *Salmonella* spp, *Shigella* spp, *Esherichia coli*, *Vibrio cholera* and others gram-negative and gram-positive organisms (Radwan et al., 1984; Ibrahim, 1985). The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. Honey physicochemical quality criteria are well specified by EC Directive 2001/110 (EU, 2001). The criteria of interest are moisture content, electrical conductivity, pH, absorbance, ash content, reducing and non reducing sugars, free acidity and hydroxymethyl-

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Abbreviations: *E. coli*, *Esherichia coli*; **G,** gentamycin; **HMF,** hydroxymethylfurfural; **MBC,** minimal bactericidal concentration; **MIC,** minimal inhibitory concentration; ***S. typhi,*** *Salmonella typhimurium*.

Table 1. Floral origin and scientific name of Tunisian honeys used for this study.

Code	Areas (Tunisia)	Floral origin	Scientific name
S1	Jendouba	Eucalyptus flower	*Eucalyptus globulus labill*
S2	Jendouba	Multifloral	-
S3	Jendouba	Thyme	*Thymus vulgaris L.*
S4	Beja	Thyme	*Thymus vulgaris L.*
S5	Beja	Multifloral	-
S6	Beja	Eucalyptus flower	*Eucalyptus globulus labill*
S7	Kef	multifloral	-
S8	Kef	Eucalyptus flower	*Eucalyptus globulus labill*
S9	Kef	Thyme	*Thymus vulgaris L.*
S10	Bizerte	Eucalyptus flower	*Eucalyptus globulus labill*
S11	Bizerte	Multifloral	-
S12	Bizerte	Thyme	*Thymus vulgaris L.*

S, Sample of honey.

furfural (HMF), total phenolic and protein contents.

The present study was undertaken to investigate the physicochemical properties and antimicrobial activity (bacterial growth and motility) of Tunisian honey obtained from most popular honey-producing areas in the North of Tunisia in respect to floral nectar origin.

Results obtained indicate that the values of physicochemical parameters of 12 samples of honey are in standards. We have also demonstrated that honey samples tested exhibited a significant broad spectrum activity against *E. coli* and *Salmonella typhi* with inhibition zone ranging from 23 to 42 mm in diameter.

Finally, we have shown that honey samples alterated the flagella driven motility of *E. coli* and *S. typhi* in swarming assay.

MATERIALS AND METHODS

Honey samples

12 samples of honey were collected from different points of collection in the North of Tunisia (Jendouba, Beja, Kef and Bizerte) then left in room temperature until further analysis (Table 1).

Physicochemical analysis

Moisture content

The determination of moisture was ascertained by refractometry using an Abbe refractometer. All measurement were performed at 20°C after waiting for 5 min for equilibrium and obtaining the corresponding % moisture (g/100) from the refractive index of the honey sample using Wedmore table (Association of Analytical Communities, 1990).

Density

Density was obtained by calculating the ratio of the mass of a precise volume of honey and the mass of the same volume of distilled water.

Electrical conductivity

Electrical conductivity was determined by conductimetric assay (WTW Inolab conductivimeter), from a solution containing 10 g of honey in 75 ml of distilled water (Sancho et al., 1992).

Ash

In order to determine ash content of honey samples, 3 g of each sample were weighed in a Chinese crucible and put in an electric furnace at 640°C for 6 h. Ash was measured in triplicate and the mean values were expressed in g (%) (AOAC, 1990).

pH

Honey pH was measured with a combined pH glass electrode connected to pH meter basic in a 4% of honey solution prepared in distilled water. The instrument was calibrated with standard buffer solutions of pH 7 and 4 prior to measuring the pH of samples (Saxena et al., 2010).

Free acidity

Free acidity was determined using potentiometric titration (AOAC, 1990, Official Method 962.19). Honey samples were homogenized in a water bath and filtered through gauze, prior to analysis. 10 g of honey were then dissolved in 75 ml of distilled water, and alcoholic solution of phenolphthalein was added. The solution was then titrated with 0.1 N NaOH. Acidity (milliequivalent of acid per kg of honey) was determined as 10 times the volume of NaOH used in titration (Gomes et al., 2010).

Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural was determined using the standard method of AOAC (1990) Official Method 980.23. 5 g of honey were dissolved in 25 ml of distilled water, treated with a clarifying agent

(0.5 ml of Carrez I and 0.5 ml of Carrez II solutions) and volume made up to 50 ml. The solution was then filtered, and the first 10 ml were discarded. The absorbance of the filtered solution was measured at 284 and 336 nm against an aliquot of the filtered solution treated with $NaHSO_3$. HMF was determined as:

HMF/100 g of honey = (Abs_{284} _ Abs_{336}) × 14, 97 × (5/g of sample)

Reducing sugars and apparent sucrose

Reducing sugars and apparent sucrose were determined by potentiometric titration using the Fehling's test (Lane-Eynone modified method) (Gomes et al., 2010).

Total phenolic content

The total phenolic content procedure was adapted from Zalibera et al. (2008) with some modifications. To the sample of honey (50 µL) was added 125 µL of Folin-Ciocalteu reagent. The mixture was sonicated for 5 min, then 625 µL of sodium carbonate was added and the absorbance was determined after 2 h at 760 nm. Results were expressed as milligram of gallic acid equivalents per kilogram of honey (mg GA/kg). All physicochemical analysis were performed in triplicate.

Antimicrobial activity

Disc diffusion method

Antibacterial activity was evaluated using the method originally described by Bauer et al. (1996). Sterile paper discs (6 mm Φ) were impregnated with honey at different doses and placed on the inoculated Mueller Hinton agar surface. The plates were then incubated at 37°C for 24 h. Distillated water served as negative control, while standard discs of Gentamycin (10 IU) served as positive control.

Determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

From the 50% (v/v) honey solution prepared in sterile water, 12 serial (1:1) dilutions were made, resulting in final concentrations of 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, 0.4, 0.2, 0.1, 0.04 and 0.02%. The MICs were determined using the two-fold serial broth microdilution assay (NCCLS, 2000). Briefly, 50 µl of 10^6 cfu/ml of bacteria logarithmic phase cultures were incubated with 50 µl of honey serial dilution in wells of a microtitration plate. H_2O and formaldehyde (0.7%) were used as negative and positive controls, respectively. The microbial growth was monitored after overnight incubation at 37°C by measuring absorbance at 630 nm using a microplate spectrophotometer. MICs were expressed as the lowest concentration of honey that inhibited bacterial growth completely and as the average value from three independent experiments. For MBC testing, aliquots (20 µl) of broth from tubes containing no growth were plated onto solid medium and again incubated overnight at 37°C. The highest dilution in which there were no survivors was recorded as the MBC. All MICs and MBCs were confirmed by triplicate assays.

Bacterial motility

Twitching (type IV pili) assays were performed with nutrient broth (Pronadisa, Hispanlab, Madrid, Spain: 5 g polypepton and 3 g meat extract per liter of distilled water, pH 7) solidified with 1.5% agar and honey. Twitch plates were briefly dried and sterile paper discs (6 mm Φ) were impregnated with strain and inoculated to the bottom of the Petri dish from an overnight-grown, then, after incubation at 37°C for 24 h, the zone of motility at the agar/Petri dish interface was determined. Swarming (flagella and type IV pili) medium used for assay is a 0.6% agar nutrient broth. Swarm plates were briefly dried sterile paper discs (6 mm Φ) impregnated with strain, and inoculated to the bottom of the Petri dish from an overnight-grown, then, after incubation at 37°C for 24 h, the zone of motility at the agar/Petri dish interface was measured (O'Toole and Kolter, 1998; Macé et al., 2008). In all cases, the experiments are carried out in triplicate and the data obtained was analyzed using statistica.

RESULTS AND DISCUSSION

Physicochemical analysis

Honeys physicochemical parameters were represented in Table 2. All samples were found to meet honeys quality European Legislation (Ec Directive 2001/110).

Honey moisture and density

Honey moisture content depends on the environmental conditions and the manipulation from apiarists at the harvest period, and was highly variable from year to year (Acquarone et al., 2007). Results in Table 2 show that the moisture content of honey samples were ranged from 15.9 to 18.9% which were well below to the imposed limit of ≤ 20% (EU, 2001), and were similar to the findings of other researchers (Rodriguez et al., 2004; Kahraman et al., 2010). These results were indicative of good storage ability of these honeys, since high moisture content could lead to fermentation during storage and decrease in durability of honey. Further, high moisture content could accelerate crystallisation in certain types of honey and increased its water activity to values where certain yeasts could grow. These results correlated with density of samples ranged from 1.334±0.02 to 1.431±0.1. According to Jean-Prost (1987), the density of honey at 20°C is between 1.39 and 1.44. The highest value is shown for sample 12 and this confirmed the moisture values. There were no significant differences, using the turkey test ($p<0.05$), between moisture or density values obtained for the 12 honey samples, these results were indicative of good honey storage.

Electrical conductivity and free acidity

Results showed that electrical conductivity values were ranged from 0.19±0.01 to 0.7±0.02 Ms/cm. Free acidity values were ranged from 14±2 and 25±1 meq /kg. Electrical conductivity and free acidity values were also within the limit (lower than 0.8 Ms/cm and 50, respect-tively). None of the samples exceeded the limit allowed, which may be taken as indicative of freshness of all honeys

Table 2. Physicochemical parameters of honey samples.

Parameter	S1	S2	S3	S 4	S 5	S6	S7	S8	S9	S10	S11	S12
Moisture (%)	16±0.1	17.01±0.4	17.01±0.4	17.04±0.2	15.9±0.1	15.8±0.1	16.03±0.2	17.02±0.03	17.01±0.01	16.4±0.3	16.9±0.2	18.9±0.01
Density	1.389±0.01	1.423±0.01	1.402±0.01	1.421±0.01	1.334±0.02	1.324±0.1	1.34±0.01	1.351±0.02	1.391±0.01	1.347±0.2	1.421±0.1	1.431±0.1
Conductivity (mS/cm)	0.4±0.01	0.3±0.01	0.19±0.01	0.38±0.01	0.26±0.02	0.4±0.01	0.7±0.02	0.33±0.03	0.21±0.02	0.4±0.01	0.31±0.01	0.29±0.01
Free acidity (meq/Kg)	18±2	18±1	17±4	14±2	15±1	18±2	17±2	15±2	20±1	23±0.5	23±1	25±1
Ashes (%)	0.26±0.01	0.3±0.01	0.5±0.01	0.3±0.02	0.5±0.01	0.3±0.02	0.52±0.01	0.4±0.01	0.42±0.01	0.5±0.03	0.1±0.01	0.09±0.01
pH	4.33±00	4.3±00	3.21±00	3.97±00	3 .05±00	3.67±00	3.44±00	3.22±00	4.1±00	3.62±00	3.3±00	3.54±00
HMF	27.84±0.5	47.2±1	50.3±1	30.5±3	27.45±2	55.4±2	42.24±2	26.19±2	57.4 ±1	32.96±0.5	55.87±0.3	79.33±2
Apparent sucrose	7.4±0.2	4.1±0.2	4.6±0.1	4.1±0.5	3.1±0.2	6.2±0.2	6.7±0.3	6.3±0.1	4.7±0.3	8.7±0.1	4.4±0.1	4.5±0.1
Reducing sugars	78.28±2	69.12±1	70.72±3	69.42±2	69.64±3	67.64±3	71 .29±5	72.64±3	69.29±3	78.9±2	71.64±2	70.66±2
Total phenolic (mg GA/Kg)	901±20	441±4	595±3	430±3	301±1	711.5±4	411±6	820±29	292±22	997±25	320±23	345±45

(Average standard deviation, n = 3).

samples. Further, none of the analyzed honey samples showed electrical conductivity values superior to 0.8 mS/cm suggesting that all samples were from nectar honey. The free acidity of honey may be explained by taking into account the presence of organic acids in equilibrium with their corresponding lactone, or internal esters, and some inorganic ions, such as phosphate. High acidity can be indicative of fermentation of sugars to organic acids (Gomes et al., 2010).

pH

All of the analyzed samples of honeys have an acid pH ranged from 3.21±1 to 4.33±0.1 and they meet the values reported by other authors (Razzaqh et al., 2012; Gomes et al., 2010; Ouchemouk et al., 2007; Azeredo et al., 2003; Terrab et al., 2002; Al-Khalifa and Al-Arify 1999; Andrade et al., 1999). In general, regardless of the geographical origin of honey, it has naturally acidic pH (Saxena et al., 2010). This parameter has a great importance during the extraction and storage of honey, it influence the texture, stability and shelf life of honey (Terrab et al., 2002). In fact, low pH of honey inhibits the presence and growth of microorganisms, improving the stability and durability of honey.

Ash

As other physicochemical parameters, ash content of honey is affected by geographical and climatic conditions of the production site. The ash content of honey is generally low and mainly dependent upon the nectar ingredients of the plants used for honey production (Al-Khalifa and Al-Arify, 1999). Our results showed that all analyzed samples of honey have a content of total ashes inferior to 0.6%. These results confirmed values of electrical conductivity and were completely consistent with the ash contents of honey samples measured in other studies by Gomes et al. (2010), Razzaqh et al. (2012) and Mendes et al. (1998). In addition, the amount of ash in honey samples in various areas showed no statistically significant differences ($P \leq 0.05$).

HMF

The HMF content is widely recognized as a parameter of honey samples freshness, because it is absent in fresh honeys and tends to increase during processing and/ or aging of the product. In the present study, all samples of honey have a value of HMF ≤ 80 mg/kg and are ranged from 27.84 ± 0.5 to 79.33 ± 2 mg/kg. Several factors influence the levels of HMF, such as temperature and time of heating, storage conditions, pH and floral source, thus it provides an indication of overheating and storage in poor conditions (Fallico et al., 2006). The highest value of HMF was obtained for sample 12 which was indicative of temperature abuse during processing and or bad storage practices.

Reducing sugars

In respect to reducing sugars (fructose and glucose), EC Directive 2001/110 imposes reducing sugars ≥ 60 /100 g. These samples of honey do not only meet the standards but also

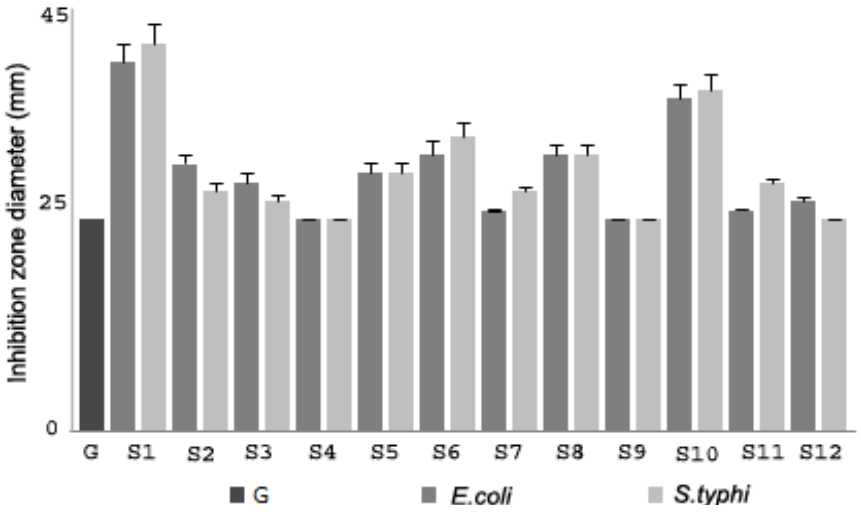

Figure 1. Inhibition effect of honey on bacterial growth: antimicrobial effect of honey samples by disc diffusion method. Inhibition zone diameter was measured (mm). Gentamycin served as positive control. G: gentamycin, S: sample of honey. Bacterial strains used are *E. coli* and *S. typhi.*

correspond to the levels observed in the other studies (Gomes et al., 2010; Rodriguez et al., 2004; Andrade et al., 1999; Kucuk et al., 2007). No significant differences were observed between reducing sugars values obtained for the 12 analyzed honey samples. Glucose and fructose represent 85 to 95% of honey sugars (Peter et al., 2007). The content of glucose is an important indicator of quality of honey because it tells us about the trend of crystallization of honey; in fact, more honey is rich in glucose more rapid is crystallisation (Gomes et al., 2010). Non reducing sugars (apparent sucrose) were set to be ≤ 5/100 g for the majority of honeys, expect for citrus and eucalyptus honeys, which had higher limits (≤ 10/100 g (EC Directive, 2001/110). Results in Table 2 showed that honey samples S1, S6, S8 and S10, originated from Eucaluptus flower, presented the highest content of apparent sucrose but it meet the standard (≤ 10/100g).

Also, all other samples were ranged from 3.1 and 4.7, these samples meet the standards expect sample 7 (6.7 ± 0.3) this value can be explained by its multifloral origin. Higher sucrose contents could be the result of an early harvest of honeys, that is, the sucrose has not been converted to fructose and glucose (Azeredo et al., 2003).

Total phenolic contents

Since phenolics substances have been shown to be responsible for the antioxidant activity of honey, the total phenol contents of honey samples were investigated (Table 2). The values showed a range of 292 ± 22 to 997 ± 25 mg GA/kg. For the monofloral samples, the eucalyptus flower (S1, S8, S6 and S10) had the highest total phenolic contents values, while in sample 9, a multifloral honey had the lowest value. Similar phenolic honey contents were reported for Mexican (Rodrıguez et al., 2012) and Croatian honeys (Piljac et al., 2009).

Antimicrobial effect of honeys

Honeys were tested against *E. coli* and *S. typhimurium* by the disc diffusion method. The values of diameter zone of inhibition were represented as histograms in Figure 1. Results showed that all honeys samples inhibited the growth of *E. coli* and *S. typhi*. The most important inhibition was shown in samples S1, S6, S8 and S10 which were from different areas but had the same floral origin as the Eucalyptus. We have also determined the MIC and MIB of all honeys samples (Table 3). Results have demonstrated that the lowest value of CMI was observed for S1, S10, S6 and S8. The antimicrobial activity of honey has been documented previously; it has been attributed to its high sugar concentration, low water activity and to the presence of hydrogen peroxide generated by glucose oxidase and nonperoxide compounds such as phenolic compounds (Mundo et al., 2004). Reports describing the inhibition of growth of numerous bacteria of clinical significance such as *E. coli* and *S. typhi* have been published (Shamala et al., 2002; Miorin et al., 2003; Vorlova et al., 2005). However, there is no information published regarding the effect of the honey of the North of Tunisia on these bacteria. In this study, we showed the importance of the floral origin in the antibacterial effect of honey. Indeed, Eucalyptus is a medicinal plant and its antibacterial activity against bacteria causing respiratory tract disorders has been reported (Salari et al., 2006).

Table 3. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of honey.

Honey	*E. coli*		*S. typhi*	
	MIC % v/v	MBC % v/v	MIC % v/v	MBC % v/v
S1	3.1	3.1	3.1	3.1
S2	6.2	6.2	12.5	12.5
S3	12.5	25	12.5	12.5
S4	12.5	12.5	12.5	12.5
S5	6.2	6.2	25	25
S6	6.2	6.2	6.2	6.2
S7	12.5	12.5	12.5	12.5
S8	6.2	6.2	6.2	6.2
S9	12 .5	12.5	12 .5	12.5
S10	3.1	6.2	3.1	3.1
S11	12.5	25	12.5	12.5
S12	12.5	25	25	25

S1: samples, MIC: minimal inhibitory concentration, MBC: minimal bactericidal concentration. Bacterial strains used are *E. coli* and *S. typhi.*

Table 4. Effect of honey on the bacterial motility: swarming essay.

Bacteria	T	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
E. coli Ø (cm)	2.25±0.2	0.8±0.1	1.6±0.1	1.1±0.2	1.5±0.2	1.3±0.01	0.8±0.07	1.3±0.1	0.9±0.04	1.8±0.1	0.8±0.01	1.6±0.1	1.3±0.3
S. typhi Ø (cm)	-	1 ±0.09	2±0.1	1.4±0.1	1.6±0.08	1.52±0.2	0.8±0.05	1.31±0.1	1.20±0.09	1.5±0.2	0.9±0.09	1.6±0.1	1.5±0.1

T: Control within honey, S: samples of honeys, Ø (cm): diameter in centimeter of zone of motility of bacteria. (Average ± standard deviation). Bacterial strains used are *E. coli* and *S. typhi.*

Oil of eucalyptus has been used traditionally as an antiseptic and in the treatment of respiratory tract infections. However, scientific and toxicological data regarding its bacterial actions are lacking, and its current applications are focused on topical use as an antiseptic (Erdoorul, 2002; Kumar, 1988).

Inhibition of bacterial motility

In order to well understand the effect of honey on bacteria, we investigated the effect of honey on the motility of *E. coli* and *S. typhimurium* by twitching and swarming essay. For the first essay, the swarming (a flagella-driven movement) was altered. As shown, in Table 4, for all samples of honey, the motility of bacteria is altered. Remarkably, this alteration is most important for bacteria treated with honey originated from eucalyptus flower. The zone of motility for bacteria without honey is 2.25 cm, while for treated bacteria by honey of eucalyptus flower value are ranged from

0.8 ± 0.01 to 0.9 ± 0.04 and 0.8 ± 0.05 to 1.20 ± 0.09 for *E. coli* and *S. typhi*, respectively. Whereas, honey had no effect on twitching a type IV pili-dependent motility. This result demonstrates that inhibition of motility was due to the inhibition of activity of flagella but not pilli IV. For a lot of pathogens, virulence and motility are often intimately linked by complex regulatory networks (Josenhans and Suerbaum, 2002). The flagellar hook, a constituent of the bacterial motile flagellum, is a short connection between the flagellar motor and the long filament acting as a helical propeller. It is made of about 120 copies of a single protein, FlgE, and its function is essential for dynamic and efficient bacterial motility and taxis (Samatey et al., 2004). This bacterial motility decrease was due to the up-regulation of the STY1416 protein, which was shown to exhibit a negative effect on bacterial motility (Nachin et al., 2005; Snoussi et al., 2012). This result suggests the probable effect of honey on these proteins.

Conclusion

Honey of the North of Tunisia is of good quality because all physicochemical parameters are in standards. Also, almost all samples of honey, especially those originated from eucalyptus flower, inhibited growth of *S. typhi* and *E. coli* and altered the flagella driven motility of this bacteria strains. These results suggest the effect of honey on proteins controlling the bacterial motile flagellum.

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REFERENCES

Acquarone C, Buera P, Elizalde B (2007). Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. Food. Chem. 101:695-703.

Al-Khalifa AS, Al-Arify IA (1999). Physicochemical characteristics and pollen spectrum of some Saudi honeys. Food. Chem. 67:21-25.

Andrade PB, Amaral MT, Isabel P, Carvalho JCMF, Seabra RM, Cunha AP (1999). Physicochemical attributes and pollen spectrum of Portuguese heather honeys. Food. Chem. 66:503-510.

Association of Official Analytical Chemists (AOAC) (1990). Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Inc., Arlington.

Azeredo LC, Azeredo MAA, Souza SR, Dutra VML (2003). Protein contents and physicochemical properties in honey samples of *Apis mellifera* of different floral origins. Food. Chem. 80:249-254.

Bauer SW, Kirby WM, Sherris JC, Thurck M (1996). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Path. 45:493-496.

Erdoorul OT (2002). Antibacterial activities of some plant extracts used in folk medicine. Pharm. Biol. 40: 269-273.

EU (2001). Council Directive 2001/110 relating to honey. Official Journal of the European Communities.

Fallico B, Arena E, Verzera A, Zappala M (2006). The European Food Legislation and its impact on honey sector. Accredit. Qual. Assur. 11:49-54.

Gomes S, Dias GL, Moreira LL, Rodrigues P, Estevinho L (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food. Chem. Toxicol. 48:544-548.

Ibrahim AS (1985). Antibacterial action of honey. Bull. Islam. Med. 1:363-365.

Jean-Prost P (1987). L'apiculture. Connaître l'abeille .conduire le rucher. 6éme édition Lavoisier. 597 p.

Josenhans C, Suerbaum S (2002). The role of motility as virulence factor in bacteria. Int. J. Med. Microbiol. 291(8):605-614.

Kahraman T, Buyukunal SK, Vural A, Altunatmaz SS (2010). Physicochemical properties in honey from different regions of Turkey. Food. Chem. 123:41-44.

Kucuk M, Kolaylı S, Karaoglu S, Ulusoy E, Baltacı C, Candan F (2007). Biological activities and chemical composition of three honeys of different types from Anatolia. Food. Chem. 100:526-534.

Kumar A (1988). Antibacterial properties of some eucalyptus oils. Fitoterapia. 59:141-144.

Macé C, Seyer D, Chemani C, Cosette P, Di-Martino P, Guery B, Filloux A, Fontaine M, Molle V, Junter GA, Jouenne T (2008). Identification of Biofilm-Associated Cluster (bac) in *Pseudomonas aeruginosa* involved in biofilm formation and virulence. PLoS ONE. 3 (12): 3897.

Mendes E, Brojo PE, Ferreira IM, Ferreira MA (1998). Quality evaluation of Portuguese honey. Carbohydrate. 37:219-223.

Miorin PL, Levy-Junior NC, Custodio AR, Bretz WA, Marcucci MC (2003). Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca angustula* against *Staphylococcus aureus*. J. Appl. Microbiol. 95:913-20.

Molan PC (1992). The antibacterial activity of honey. 1. The nature of the antibacterial activity. Bee. World. 73(1): 5-28.

Mundo MA, Padilla-Zakour OI, Worobo RW (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. Int. J. Food. Microbiol. 97: 1-8.

Nachin L, Nannmark U, Nystrom T (2005). Differential roles of the universal stress proteins of *Escherichia coli* in oxidative stress resistance, adhesion, and motility. J. Bacteriol. 187(18): 6265-6272.

O'Toole GA, Kolter R (1998). Flagellar and twitching motility are necessary for *P. aeruginosa* biofilm development. Mol. Microbiol. 30(2):295-304.

Ouchemouk S, Louaileche H, Schweitzer P (2007). Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food. Cont. 18: 52-58.

Peter BO, Olufemi EA, Iyabo OO (2007). Honey: a reservoir for microorganisms and an inhibitory agent for microbes. Afr. Health. Sci. 7:3.

Piljac ZL, Stipcevic T, Belscak A (2009). Antioxidant properties and phenolic content of different floral origin honeys. J. Api. Prod. Api. Med. Sci. 1:43-50.

Radwan S, El- Essawy A, Sarhan MM (1984). Experimental evidence for the occurrence in honey of specific substances active against Microorganisms. Cent. Microbiol. 139:249-255.

Razzaqh M, Payman Z, Hossein T, Sina S, Farzad N (2012). Biochemical properties and microbial analysis of honey from North-Western regions of Iran: Seasonal effects on physicochemical properties of honey. Afr. J. Biotechnol. 11 (44):10227-10231.

Rodrıguez BA, Mendoza S, Iturriga MH, Castano-Tostado E (2012). Quality Parameters and Antioxidant and Antibacterial Properties of Some Mexican Honeys. J. Food. Sci. 71:1.

Rodriguez GO, Ferrer BS, Ferrer A, Rodriguez B (2004). Characterization of honey produced in Venezuela. Food. Chem. 84:499-502.

Salari MH, Amine G, Shirazi MH, Hafezi R, Mohammadypour M (2006). Antibacterial effects of Eucalyptus globules leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. Clin. Microbiol. Infect.12(2):194-196.

Samatey FA, Matsunami H, Imada K, Nagashima S, SHaikh T R, Thomas DR, Chen JZ, Derosier DJ, Kitao A, Namba K (2004). Structure of the bacterial flagellar hook and implication for the molecular universal joint mechanism. Nature 431 (7012):1062-1068.

Sancho MT, Muniategui S, Huidobro JF, Simal J (1992). Evaluating

soluble and insoluble ash, alkalinity of soluble and insoluble ash and total alkalinity of ash in honey using electrical conductivity measurements at 20 °C. Apidologie 23:291-297.
Saxena S, Gautam S, Sharma A (2010). Physical, biochemical and antioxidant properties of some Indian honeys. Food. Chem. 118: 391-397.
Shamala TR, Jyothi YPS, Saibaba P (2002). Antibacterial effect of honey on the *in vitro* and *in vivo* growth of *Escherichia coli*. World. J. Microbiol. Biotechnol. 18:863-865.
Snoussi S, May AE, Coquet L, Chan P, Jouenne T, Landoulsi A, Dé E (2012). Adaptation of Salmonella enterica Hadar under static magnetic field: effects on outer membrane protein pattern. Proteome Sci. 3(10):1-6.
Subramanyam M (1991). Tropical application of honey in treatment of burns. Br. J. Surg. 78:497-498.
Terrab A, Diez MJ, Heredia FJ (2002). Characterization of Moroccan unifloral honeys by their physicochemical characteristics. Food Chem. 79:373-379.
Vorlova L, Karpiskova R, Chabiniokova I, Kalabova K, Brazdova A (2005). The antimicrobial activity of honeys produced in the Czech Republic. Czech. J. Anim. Sci 50:376-384.
White JW (1975). Composition of honey. In: Crane, E. (Ed.), Honey, A Comprehensive Survey, vol. 5. Heinemann, London, UK, pp. 157-206
Zalibera M, Stasko A, Slebodava A, Jancovicova V, Cermakova T, Brezova V (2008). Antioxidant and radical-scavenging activities of Slovak honeys: an electron paramagnetic resonance study. Food Chem. 110:512-21.