Physicochemical, Antibacterial and Antioxidant Property of Honey Samples Harvested From OuedZiz Region, Morocco.

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Abstract
Honey is a naturally sweet substance produced by honey bees, from the nectars of plant flowers and honeydew. The present study aimed to evaluate physicochemical characteristics, antioxidant and antibacterial activity of honey samples from OuedZiz region in Morocco. The results of this study showed that all the tested parameters are still within the standard limit and vary between a minimum and a maximum value as follows: Density 1.4382 and 1.4743; pH 3.52±0.03 and 5.02±0.07; free acidity 18±0.11 and 38.5±0.16 meq/kg; lactic acid 4.3±0.2 and 9.25±0.1 meq/kg; total acidity 22.3±0.22 and 45.25±0.19 meq/kg; moisture 13.53% and 20.8%; Electrical conductivity (EC) 0.329±0.005 and 0.701±0.002 mS/cm; Ash 0.32±0.02 and 0.83±0.01; DPPH scavenging activity (IC50) 12.5 and 130 mg/mL. Moreover, the antibacterial activity showed that the tested honey samples were more effective with gram positive bacteria than with gram negative ones. The current findings will provide the good quality of the tested honey samples and suggest that these honeys could be used as a functional or nutraceutical substance.

Keywords
Honey; Physicochemical Properties; Antibacterial Activity; Antioxidant Capacity; Morocco

Introduction
Honey is a precious substance offered by nature, is known and used by humans since the earliest times. This noble product is one of the most appreciated foods thanks to its nutritive and therapeutic properties. Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature [1]. This natural complex food stuff is produced in almost every

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country and largely used as a food source. Moreover, in the long human tradition, honey has been used not only as a nutrient but also as a medicinal product due to its therapeutic [2], antioxidant [3,4], antimicrobial [3], antitumoral [5,6], inflammatory [7], antiviral [8] and antiulcer [9] activities thanks to it several benefits in Modern medicine [10].

Honey contains about 200 substances, mainly carbohydrates (of which fructose and glucose are the main components) and water [11-13]. Also, it contains minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids and other compounds [12, 14]. Moreover, honey presents a very variable sensory and physicochemical characteristic due to its geographical floral origin, environmental factors, season and treatment of beekeepers [15-20].

Honey can be divided into 2 categories: Monofloral honey made from nectar and/or honeydew coming from a single plant species and this requires the beehives should be installed near the desired plant (Acacia, Orange, Thymus, Ziziphus, Rosemary, Lavender…) [16, 21, 22], and multifloral honey made from nectar and/or honeydew from several plant species [16].

In Morocco, honey is widely used in traditional medicine, unfortunately, there are just a few investigations regarding its quality and characterization. The present study aims to evaluate the physicochemical properties such as density, moisture, ash, pH, free acidity and electrical conductivity of honey samples harvested from OuedZiz region in Morocco. The antibacterial and antioxidant activities were also studied.

**Material and Methods**

**Samples Collections**

Ten honey samples of different botanical sources in South-East Region of Morocco were collected following their harvested seasons (Table1). All the samples were stored in a dry and dark place at 20°C till further analysis to avoid the effect of laboratory conditions on the chemical composition and physical properties of honey samples. The analyses were carried out in triplicates and the data were presented as means ± standard deviations.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Harvested date</th>
<th>Color</th>
<th>Botanic origin</th>
<th>Type of beehive</th>
<th>Beehive: stable/ mobile</th>
</tr>
</thead>
<tbody>
<tr>
<td>H 1</td>
<td>09/2016</td>
<td>Yellow</td>
<td>Ziziphus</td>
<td>Modern</td>
<td>Stable</td>
</tr>
<tr>
<td>H 2</td>
<td>08/2012</td>
<td>Brown</td>
<td>Multifloral</td>
<td>Traditional</td>
<td>Stable</td>
</tr>
<tr>
<td>H 3</td>
<td>08/2015</td>
<td>Orange</td>
<td>Multifloral</td>
<td>Modern</td>
<td>Stable</td>
</tr>
<tr>
<td>H 4</td>
<td>10/2016</td>
<td>White</td>
<td>Rosmarinus</td>
<td>Modern</td>
<td>Mobile</td>
</tr>
<tr>
<td>H 5</td>
<td>09/2016</td>
<td>Yellow</td>
<td>Multifloral</td>
<td>Modern</td>
<td>Stable</td>
</tr>
<tr>
<td>H 6</td>
<td>07/2016</td>
<td>Yellow</td>
<td>Multifloral</td>
<td>Modern</td>
<td>Mobile</td>
</tr>
<tr>
<td>H 7</td>
<td>08/2016</td>
<td>Orange</td>
<td>Thymus</td>
<td>Modern</td>
<td>Stable</td>
</tr>
<tr>
<td>H 8</td>
<td>09/2016</td>
<td>Brown</td>
<td>Cytinus</td>
<td>Modern</td>
<td>Stable</td>
</tr>
<tr>
<td>H 9</td>
<td>09/2016</td>
<td>Orange</td>
<td>Rosmarinus</td>
<td>Modern</td>
<td>Stable</td>
</tr>
<tr>
<td>H 10</td>
<td>09/2016</td>
<td>White</td>
<td>Thymus</td>
<td>Modern</td>
<td>Stable</td>
</tr>
</tbody>
</table>

**Density and Moisture content**

Moisture content was determined from the refractive index (RI) of the honey samples using a standard model Abbe type refractometer at 20°C regularly calibrated with distilled water [23]. Water content (%) was then obtained from the Chataway table. Then, the density was measured at 20°C according to the method previously described by Mehryar et al. [24].

**Electrical Conductivity (EC)**

Electrical conductivity (EC) was measured at 20°C in solutions of honey samples in distilled water (20%, w/v) with specific electrical conductivity 1S/cm using a conductivity meter (WTW 1970i) [25].
pH

pH measurements were performed potentiometrically at 20°C using a pH-meter (Adwa, AD1000) in honey samples diluted with distilled water, according to the method described by Silva et al. [26].

Acidity (free, lactone, and total)

Free, lactone and total acidity were determined using the titrimetric method [23]: Briefly, 10 g honey samples were dissolved in 75 ml in a 250 ml beaker. The electrode of the pH meter (Adwa, AD1000) was immersed in the solution, stirred with a magnetic stirrer and titrated with 0.05 N NaOH to pH 8.5 (free acidity). Then the addition was stopped; immediately 10 ml of 0.05 N NaOH were added and without delay back-titrated with 0.05 N HCl to pH 8.30 (lactone acidity). Total acidity resulted from adding free plus lactone acidities. The results were expressed as milliequivalents/kg (meq/kg).

Ash Content

5 g of honey was placed in combustion pots, which required preheating to darkness with a gas flame to prevent honey foaming. Then, the samples were incinerated at high temperature (550°C) in a burning muffle for 5 h. After cooling at room temperature, the obtained ash was weighed [27].

Antibacterial Assay

Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) were performed according to the microplates method [28, 29]. This method consists in inoculating a decreasing concentration of honey samples by a bacterial inoculum. After incubation, the range of bacterial growth indicates the MIC and MBC. The MIC is defined by the lowest concentration of essential oil capable of inhibiting the growth of 90% of the bacterial population. However, the MBC is the lowest concentration of essential oil capable of killing more than 99.9% of initial microbial inoculum.

Antioxidant Assay

1 mL of a 0.2mM solution of DPPH radical in methanol was added to 2 mL of honey samples at different concentrations. The absorbance (A) of the resulting solution was measured after 30 min in dark at 517nm with a spectrophotometer [30]. The percentage inhibition of activity was calculated as:

\[ \% \text{Inhibition} = \frac{(A_{\text{blank}} - A_{\text{samble}})}{A_{\text{blank}}} \times 100 \]

The concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage plotted against the honey samples concentration.

Results and Discussion

The Density of Honey

The density of honey samples was measured using the method previously described. The results of this study showed that the density of honey samples harvested from OuedZiz in Morocco ranged between 1.4382 and 1.4743 (Table 2). These results were similar to those previously reported in Iran (1.462 and 1.499) [24], but higher than that reported in Saud Arabia whose the density of the studied honeys varies between 1.3498 and 1.4429 [31].

pH Determination

The pH is correlated with honey storage and with microorganism growth that could change the texture and honey stability [32, 33]. In this study, the pH values of the ten honey samples were measured and the obtained results were presented in table 2. These results showed that all tested samples were acidic and have a pH value between 3.52±0.03 and 5.02±0.07 within the standard limit that ensures honey samples’ freshness (pH 3.40–6.10) [1]. This pH values are in agreement to those reported previously in Morocco (3.52–5.13) [16] and Algeria (4.02 and 5.7) [34]. Boussaid and his group reported that the pH values of Tunisian honeys vary between 3.67±0.01 and 4.11±0.02 [22]. However, the pH values of honey samples harvested from the arid regions varies between 3.99±0.02 and 6.33±0.02 [35].

Moisture Content

Moisture content is an important parameter of honey quality and defines the amount of water present in honey; it is a limiting factor in determination of its quality, stability and spoilage resistance against yeast fermentation by the influencing of physical properties of honey such as viscosity and crystallization, as well as other parameters: colour, flavour, taste, specific gravity, solubility and conservation [36]. However, the low moisture content helps to promote longer shelf life during storage [37]. In
Table 2: Physicochemical parameters of analyzed honey samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Density (g/cm³)</th>
<th>Moisture (%)</th>
<th>EC (mS/cm)</th>
<th>Ash (%)</th>
<th>pH</th>
<th>Free acidity (meq/Kg)</th>
<th>Lactic acid (meq/Kg)</th>
<th>Total acidity (meq/Kg)</th>
<th>DPPH (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>1.4743</td>
<td>13.5±0.43</td>
<td>0.701±0.002</td>
<td>0.83±0.01</td>
<td>5.02±0.07</td>
<td>18±0.11</td>
<td>4.3±0.2</td>
<td>22.3±0.22</td>
<td>81.5±0.65</td>
</tr>
<tr>
<td>H2</td>
<td>1.4655</td>
<td>14.3±0.55</td>
<td>0.336±0.005</td>
<td>0.32±0.02</td>
<td>3.74±0.09</td>
<td>30.5±0.15</td>
<td>7.5±0.18</td>
<td>38±0.19</td>
<td>40.75±0.38</td>
</tr>
<tr>
<td>H3</td>
<td>1.4593</td>
<td>14.1±0.49</td>
<td>0.460±0.005</td>
<td>0.5±0.02</td>
<td>3.95±0.04</td>
<td>38±0.21</td>
<td>6.25±0.12</td>
<td>44.25±0.17</td>
<td>50±0.49</td>
</tr>
<tr>
<td>H4</td>
<td>1.4667</td>
<td>14.5±0.37</td>
<td>0.510±0.004</td>
<td>0.61±0.03</td>
<td>3.73±0.07</td>
<td>35.5±0.12</td>
<td>6.5±0.17</td>
<td>42±0.18</td>
<td>90±0.32</td>
</tr>
<tr>
<td>H5</td>
<td>1.4480</td>
<td>18.6±0.71</td>
<td>0.594±0.005</td>
<td>0.49±0.01</td>
<td>3.85±0.04</td>
<td>25.5±0.23</td>
<td>9.25±0.1</td>
<td>34.75±0.11</td>
<td>31.25±0.15</td>
</tr>
<tr>
<td>H6</td>
<td>1.4660</td>
<td>19.1±0.33</td>
<td>0.476±0.003</td>
<td>0.54±0.01</td>
<td>3.84±0.08</td>
<td>35.5±0.19</td>
<td>7.25±0.14</td>
<td>42.75±0.15</td>
<td>43.75±0.26</td>
</tr>
<tr>
<td>H7</td>
<td>1.4677</td>
<td>19.3±0.23</td>
<td>0.621±0.004</td>
<td>0.52±0.02</td>
<td>4.19±0.07</td>
<td>30.5±0.18</td>
<td>6.5±0.17</td>
<td>37±0.16</td>
<td>12.5±0.23</td>
</tr>
<tr>
<td>H8</td>
<td>1.4382</td>
<td>19.8±0.41</td>
<td>0.402±0.006</td>
<td>0.40±0.02</td>
<td>3.55±0.04</td>
<td>28±0.24</td>
<td>8.25±0.18</td>
<td>36.25±0.18</td>
<td>130±0.5</td>
</tr>
<tr>
<td>H9</td>
<td>1.4520</td>
<td>20.3±0.39</td>
<td>0.329±0.005</td>
<td>0.34±0.01</td>
<td>3.52±0.03</td>
<td>35.8±0.16</td>
<td>6.75±0.14</td>
<td>45.25±0.19</td>
<td>37±0.39</td>
</tr>
<tr>
<td>H10</td>
<td>1.4655</td>
<td>20.8±0.29</td>
<td>0.335±0.008</td>
<td>0.38±0.03</td>
<td>3.59±0.05</td>
<td>25.5±0.15</td>
<td>8.5±0.14</td>
<td>34±0.14</td>
<td>31.25±0.28</td>
</tr>
</tbody>
</table>

In the present study, the percentage of moisture content was between 13.53% and 20.8% (Table 2), which is within the allowed parameters [1]. These results were similar to those previously reported in honey samples from arid regions (13.63% to 20.60%) [35]. In Malaysia, the moisture content of the studied honeys varies between 14.86 and 17.53% [38]. However, this parameter varies between 13% and 25.5% in Algerian honeys [34].

Electrical Conductivity (EC)

Electrical conductivity EC is an important factor in the determination of the physical characteristics of honey [39]. This parameter is closely related to the concentration of mineral salts, organic acids, proteins... and shows a great variability according to the floral origin. In this study, the EC values of the ten analysis honey samples were varied from 0.329±0.005 to 0.701±0.002 mS/cm (Table 2), and are below the maximum limit indicated by the international regulations of quality (0.8 mS/cm) [1]. These values are similar to those previously reported in Malaysia (0.35-0.76 mS/cm) [38]. However, a study carried out in the arid regions showed that the EC varies between 154.67±0.58 and 690.67±0.58 µS/cm [35].

Acidity Measurement

Honey acidity is due to the presence of organic acids, mainly gluconic acid, in equilibrium with their corresponding lactones or internal esters, and to inorganic ions, such as sulfate, phosphate and chloride [37]. Free, lactone and total acidity were measured and presented in table 2. The obtained results illustrate that the total acidity observed in the current study for different honey samples were acceptable (below 50 meq/kg) [1], indicating the absence of undesirable fermentation. These results are in agreement to those reported previously from other geographical locations [35, 37].

Ash Content

In the present study, the ash content of honeys samples ranged from 0.32±0.02 to 0.83±0.01 (Table 2). These results are higher than that found in arid regions whose the ash content varies between 0.066% and 0.316% [35]. In Tunisia, Boussaid and his group reported that the ash content of the analyzed honeys varies between 0.08% and 0.69% [22]. However, the ash content of the Algerian honeys varies between 0.0002 and 0.83% [34].

Antibacterial Assay

The antibacterial activity of honey samples against 6 pathogenic bacteria was determined and presented in Table 3. The results showed that all the tested honeys have an antibacterial activity against Escherichia coli, Salmonella enterica, Staphylococcus aureus, Klebsiella pneumonia and Streptococcus faecalis but without effect on Pseudomonas aeruginosa. This activity was more effective with gram positive bacteria than with gram negative ones. In a study carried out in Brazil, the authors reported that the tested honey samples have a greater inhibitory activity against Staphylococcus aureus and Bacillus cereus [3]. The antibacterial activity may be due to the complex
composition, osmolarity, acidity and the ability to generate hydrogen peroxide in honeys samples and is influenced by the time of storage, composition, and source of nectar on which the reared bees were fed [40-43].

Antioxidant Assay
Antioxidant compounds play an important role as a health protecting factor and can reduce the risk for chronic diseases. DPPH is an unwavering nitrogen-centered radical that has been extensively used to test the free radical scavenging ability of various samples. During the reaction, the color changes from purple to yellow. In this study, the DPPH scavenging activity of honey samples showed that the IC50 vary between 12.5 and 130 mg/mL (Table 2). These results are similar to those found in China, who the IC50 varies between 35 and 122 mg/mL [44]. Moreover, the DPPH scavenging activity of Tunisian honeys showed that the IC50 varies between 11.08±0.32 and 93.26±0.37 [22].

Table 3: Antibacterial activity of the studied honey samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>E. coli</th>
<th>Sal</th>
<th>SA</th>
<th>PS</th>
<th>KL</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMI</td>
<td>CMB</td>
<td>CMI</td>
<td>CMB</td>
<td>CMI</td>
<td>CMB</td>
</tr>
<tr>
<td>H1</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>H2</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>12.5</td>
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</tr>
<tr>
<td>H3</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
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<td>25</td>
<td>25</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>H5</td>
<td>12.5</td>
<td>12.5</td>
<td>50</td>
<td>50</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>H6</td>
<td>12.5</td>
<td>12.5</td>
<td>25</td>
<td>50</td>
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<td>6.25</td>
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<td>12.5</td>
<td>25</td>
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<tr>
<td>H8</td>
<td>12.5</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>H9</td>
<td>12.5</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
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<tr>
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<td>12.5</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
<td>6.25</td>
<td>6.25</td>
</tr>
</tbody>
</table>

-: no effect; E. coli: Escherichia coli; Sal: Salmonella enterica; SA: Staphylococcus aureus; PS: Pseudomonas aeruginosa; KL: Klebsiella pneumonia; SF: Streptococcus faecalis.

these honeys could be used as a functional or nutraceutical substance.

References


