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ORIGINAL ARTICLE

Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia

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Abstract The present study was undertaken to determine the physicochemical, biochemical, and antioxidant activities of Tunisian honey samples. All the extracted honey samples appeared to conform to the European Legislation (EC Directive 2001/110) for all parameters. Mint honey, for instance, possesses significant pH value ($p < 0.05$), invertase activity, water, and protein contents. In addition, this study demonstrates that the color of the Tunisian honeys is highly variable and ranges from pale yellow to dark brown. The total phenolic, flavonoid and carotenoid contents significantly vary ($p < 0.05$). The highest values were found in mint honey, which has a very dark color. Correlations between the analyzed parameters are statistically significant ($p < 0.05$). The DPPH radical scavenging activity of rosemary honey was determined as lower ($p < 0.05$) than the other analyzed honey samples. Yet, the highest activity was detected in mint honey. The results suggest that Tunisian honeys could be beneficially used as a functional or nutraceutical substance as they prevent or moderate oxidative stress-related diseases.

1. Introduction

Honey is defined as "the sweet substance produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honey combs" (Codex Alimentarius Commission, 2001). Naturally, honey has been traditionally recognized as a valuable source of energy. It has also been recognized for its antimicrobial and antioxidant characteristics (Alvarez-Suarez et al., 2010;

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Basualdo et al., 2007). It is a concentrated aqueous solution of invert sugar that contains a mixture of other carbohydrates, amino and organic acids, minerals, aromatic substances, pigments, waxes and pollen grains to make it complex (Alvarez-Suarez et al., 2010; Ajlouni and Sujirapinyokul, 2010; Manzanares et al., 2011; Rashed and Soltan, 2004). Many studies have demonstrated that honey serves as a source of natural antioxidants, which are effective in reducing the risk of heart disease, cancer, immune system deficiency, cataracts, different inflammatory processes, etc. (National Honey Board, 2002). In recent years, the antibiotic and wound healing properties of honey have been scientifically proven (Molan and Betts, 2004).

In the past three decades, the large number of published studies concerning the physicochemical characteristics of honeys of different botanical and geographical origins illustrates the importance of determining honey's quality. Very few studies, however, have analyzed honey's physicochemical properties, and none of them has determined the physicochemical characteristics and antioxidant activities of any Tunisian honey variety. It is known that the physicochemical parameters of natural honeys, such as pH, moisture, sugar composition and hydroxymethylfurfural (HMF) contents, color, acidity and specific conductivity, are strictly defined and represent the quality indicators that characterize each individual honey variety. It is important to note that glucose and fructose are the major honey sugars while sucrose remains very scarce (Ajlouni and Sujirapinyokul, 2010; Fallico et al., 2004; Tosi et al., 2008). HMF is practically not present in fresh food, but it is naturally generated in sugar-containing food during heat-treatments like drying or cooking. HMF can be used as an indicator for excess heat-treatment. For instance, fresh honey only has low amounts of HMF less than 15 mg/kg, and the European Union (EU, 2001) requires an HMF limit in honey of 40 mg/kg and 80 mg/kg for honey coming from countries or regions with tropical temperatures, 15 mg/kg for honey with low enzymatic level (8–3 Schade Units). This standard guarantees that the honey has not undergone heating during processing (Codex Alimentarius, 2000). The geographical origin of honey has previously been studied by many researchers around Europe, especially in Slovenia, Romania, Spain, Denmark and Portugal (Bertoncelj et al. 2011; De la Fuente et al. 2011; Stolzenbach et al., 2011; Feás et al., 2010), in Africa mainly in Morocco, Burkina Fasan and Algeria (Terrab et al., 2002; Meda et al., 2005; Ouchemoukh et al., 2007), in South America mainly in Argentina, Cuba and Brazil (Alvarez-Suarez et al., 2010; Chirifie et al., 2006; Moreira et al., 2010), and in Australia and New Zealand (Ajlouni and Sujirapinyokul, 2010; Vanhanen et al., 2011). The authors have determined the physicochemical parameters including water content, pH, conductivity and sugar composition. They found that the geographical area influences and distinguishes the physicochemical properties of honey to a large extent.

In Tunisia, nevertheless, honey has always had a valued place in traditional medicine. It has been principally employed for wound healing and diseases of the gut. Unfortunately, there are no ample investigations regarding its quality and/or its biochemical characteristics. Besides, there are few Tunisian varieties that have never been analyzed before, i.e. mint, orange, eucalyptus, thyme, rosemary and horehound.

The present study aims at identifying natural honey varieties harvested in Northwest Tunisia with respect to their floral origin, physicochemical properties such as moisture, ash, pH, free acidity, electrical conductivity, HMF content, reducing sugars and invertase activity. Note that the total carotenoids, total flavonoids and antioxidant activities were also identified.

2. Materials and methods

2.1. Chemicals and reagents

Glucose, fructose, sucrose, maltose, Folin–Ciocalteu reagent, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl, (+)-catechin, L-proline and methanol were purchased from Sigma Chemical Co. (St. Louis, USA). Ultrapure water was made using a Milli-Q water purification system (Millipore, Bedforde, MA, USA). All the other used chemicals were high purity analytical grade reagents.

2.2. Samples

There are six honey samples coming from different botanical origins and produced in Tunisia. They were collected throughout a whole year in different regions in Tunisia (see Fig. 1). Table 1 shows different origins, and type of the examined honeys. Obviously, all honey samples were directly obtained from beekeepers. The beekeepers directly extracted the samples into 250 mL sterilized glass sample bottles with glass caps. The samples were then stored in a dry and dark place at a temperature of 20 °C. Subsequently, the honey samples were classified into six categories as they belong to the following diverse floral origin: eucalyptus, orange, thyme, mint, rosemary, and horehound.

2.3. Proximate composition analyses

Water content was measured by a Carl Zeiss 16531 refractometer at 20 °C and the corresponding water content was calculated using the Association of Official Analytical Chemists method (AOAC, 1990). While the total nitrogen (TN) was determined using the Kjeldahl method from which the crude protein could be calculated as % $N \times 6.25$. The ash content was determined by burning the samples in a muffle furnace at 500 °C for 6 h. The proline content was determined according to the Bogdanov method (Bogdanov et al., 1997). The pH, however, was measured using a Mettler Toledo pH meter (California, USA). The pH probe was immersed in a 250 mL beaker that contained a solution of 4 grams of honey dissolved in 30 mL of ultrapure water. Free acidity was determined according to the AOAC method (AOAC, 2000), by the titrimetric method. To make things clear, the electrode of the pH meter was immersed in the solution, stirred with a magnetic stirrer and titrated to pH 8.5 by adding a 0.05 N of NaOH solution. The Electrical conductivity (EC) of a honey solution, at 20% (w/v) (dry matter basis) in ultrapure water, was measured at 20 °C using a Consort conductometer (Consort C830, Belgium). The results were expressed as milli Siemens per centimeter (mS/cm). The water activity (a_w) was determined at 25 ± 0.02 °C using an AquaLab water activity meter (AquaLab CX2T, Decagon Devices, USA). Viscosity was determined by an Ostwald viscometer at 25 °C and at a shear rate of 5 rpm.

Note that all the tests were performed in triplicate and were expressed as mean ± standard deviation.