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An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles

Zehra Can [a], Oktay Yildiz [b], Huseyin Sahin [c,*], Emine Akyuz Turumtay [d], Sibel Silici [e], Sevgi Kolayli [f]

[a] Giresun University, Şebinkarahisar Technical Sciences Vocational School, Giresun, Turkey
[b] Maçka Vocational School, Karadeniz Technical University, Maçka 61750, Trabzon, Turkey
[c] Giresun University, Espiye Vocational School, Espiye 28600, Giresun, Turkey
[d] Department of Chemistry, Faculty of Science and Art, R.T.E. University, 53100 Rize, Turkey
[e] Erciyes University, Agriculture Faculty, Department of Agricultural Biotechnology, Kayseri, Turkey
[f] Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey

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ABSTRACT

This study investigated some physico-chemical and biochemical characteristics of different honey types belonging to Turkish flora. Sixty-two honey samples were examined on the basis of pollen analyses, including 11 unifloral honeys (chestnut, heather, chaste tree, rhododendron, common eryngo, lavender, Jerusalem tea, astragalus, clover and acacia), two different honeydew honeys (lime and oak), and 7 different multifloral honeys. Electrical conductivity, moisture, Hunter color values, HMF, proline, diastase number, and sugar analyses of the honey samples were assessed for chemical characterization. Some phenolic components were analyzed by reverse phase high performance liquid chromatography (RP-HPLC) to determine honeys' phenolic profiles. Total phenolic compounds, total flavonoids, ferric reducing antioxidant capacity (FRAP) and 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity were measured as antioxidant determinants. The study results confirm that physico-chemical and biological characteristics of honeys are closely related to their floral sources, and that dark-colored honeys such as oak, chestnut and heather, have a high therapeutic potential.

1. Introduction

The adverse effects on human health of synthetic drugs and chemicals in the age of technology have encouraged the use of more traditional and natural methods. Therapeutic techniques using bee products that protect and strengthen the immune system are known as apitherapy. Honey, a rediscovered natural product, has also begun being used for numerous purposes.

Although the great majority of the dry weight of honey (95–98%) consists of carbohydrates, 2–5% is made up of various secondary metabolite agents and minerals. The main sugars in the structure are fructose and glucose, although it also contains small quantities of mono-, di- and trisaccharides and oligosaccharides. Some of these are criteria for honey purity recognized in international food codices (IHC, CEU, TSE etc.) (Codex, Standard 12-1981), but these do not indicate honey's bioactive potential and apitherapic functions. The true quality of honey is associated with the presence, variety and amounts of its bioactive compounds, and this again depends on the geographic and floral structure of the region in which it is produced. Studies show that the great majority of the bioactive compounds in honey consist of molecules with phenolic structures, such as phenolic acids, flavonoids, procyanidins and anthocyanins (Küçük et al., 2007; Sahin, Aliyazıcıoğlu, Yıldız, Kolayli, & Supuran, 2011; Tezcan, Kolayli, Sahin, Ulusoy, & Erim, 2011).

Both animal studies and clinical trials in different parts of the world are providing highly promising results regarding the healing potential of honey (Kassım et al., 2012; Yıldız et al., 2013). For example, with its high antimicrobial activity, New Zealand Manuka honey is used in the treatment of wounds and burns. Manuka is a honey classified on the basis of the amount of methyl syringate it contains and is known for its dark color and high phenolic content (Jonathan et al., 2010). Similar to Manuka honey, other honeys such as Tualang and Gelam are also known to possess high levels of biological activity and to have potential for use in apitherapy (Kassım et al., 2012; Küçük et al., 2007). Many studies have suggested that the antioxidant and antimicrobial activities of honey samples correlate with the total phenolic contents and the color pigments within samples (Kassım et al., 2012; Tezcan et al.,

* Corresponding author. Tel.: +90 454 611 6007/22; fax: +90 454 611 6008.
E-mail address: huseyin.sahin@giresun.edu.tr (H. Sahin).

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2011). Over the last two decades, research into the role of apitherapic products in the prevention and treatment of human diseases has intensified, and their antioxidant, antibacterial, antitumoral, and anti-inflammatory potentials have been revealed (Nasuti, Gabbianelli, Falcioni, & Cantalamessa, 2006).

Due to features such as its geographical position, climatic conditions and three seasons of the year being suited to honey production, Turkey is one of the richest regions of the world in terms of honey production and variety. It is home to a wide variety of nectar and honeydew honey types, both unifloral and multifloral. The purpose of this study was to reveal the physical and chemical characterizations, and antioxidant and antimicrobial activities of honeys from different flora produced in Turkey and to identify honeys with a high apitherapy potential for future studies.

2. Materials and methods

2.1. Chemicals

2,4,6-Tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 5-hydroxymethlfurfural (HMF) were purchased from Sigma Chemical Co. (St Louis, MO, USA).

All chemical standards were HPLC-grade pure, and the common phenolic compounds and sugars were obtained from Sigma–Aldrich (Munich, Germany). Of the HPLC grade organic reagents, acetonitrile was supplied by Sigma–Aldrich Co. (St. Louis, MO, USA) and methanol by Merck KGaA, (Darmstandt, Germany). HPLC syringe filters (RC-membrane, 0.45 μm) were purchased from Sartorius Minisart RC 15, Sartorius (Germany).

2.2. Honey samples

Thirteen varieties of unifloral honey, chestnut (*Castanea sativa* Mill.), heather (*Calluna vulgaris* L.), chaste tree (*Vitex agnus-castus* L.), rhododendron (*Rhododendron ponticum* L.), lime (*Tilia platyphyllos*), clover (*Trifolium* spp. L.), oak (*Quercus robur* L.), pine (*Pinus brutia* L.), lavender (*Lavandula stoechas* L.), Jerusalem tea (*Phlomis armeniaca* Willd.), astragalus (*Astragalus microcephalus*), black locust (*Robinia pseudoacacia*) and common eryngo (*Eryngium campestre* L.), and seven different multifloral honeys from Turkey obtained by experienced beekeepers in the 2011–2012 harvest season were included in the study.

2.3. Melissoplaynological analysis of honey samples

Honey samples were classified on the basis of melissopalynological characterization according to their specific botanical variety. The preparation of honey samples followed the standardized method described by Louveaux, Maurizio, and Vorwohl (1978).

The pollen types present in the honey samples were identified, counted, and classified, according to their percentages, as dominant pollen (45% or more of the total pollen grains counted) and, secondary pollen (16–44%), important minor pollen (3–15%), and minor pollen (less than 3%). Counts were expressed as percentages after counting a minimum 1000 pollen grains on four slides from sample.

2.4. Analytical examinations of honey samples

Some physicochemical characteristics of the honeys were in agreement with European Union (Bogdanov et al., 1997). The color value of the honeys was determined using a Hunter spectrometer (CR-400, Minolta, Osaka, Japan). Moisture was measured using a refractometer (Atago, Tokyo, Japan), electrical conductivities with a conductometer (WTW inoLab Cond/720, Germany), and optical activity or rotation with a polarimeter (Beta PPP7, England).

Sugar analysis of samples was performed using a refractive detector (RID) with HPLC (Elite LaChrom, Hitachi, Japan) and a reverse phase–amide column (200/4.6 Nucleosil 100-5 NH_2). Quantitative and qualitative sugar analyses were performed using the method described by Ozturk, Tuncel, and Tuncel (2007). The calibration curves of all analyzed sugars were between 0.994 and 1.000.

Hydroxymethylfurfural (HMF) was measured with HPLC-UV (Elite Lachrom Hitachi, Japan) using a C_{18} column of LiChroCART® 250-4 RP (10 μm) (Jeuring & Kupper, 1980). Proline content was measured using spectrometric assay (Ough, 1960). Diastase activity was determined with the spectrophotometric method using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 °C (Bogdanov et al., 1997).

2.5. Honey extraction for antioxidant activity and phenolics analysis

Methanolic extracts of the honeys were used for antioxidant analyses. Approximately 15 g honey was placed in a falcon tube (50 mL) and 50 mL 99% methanol was added. The mixture was continuously stirred with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h. Particles were removed with filter paper. The final volume of the solution was adjusted with methanol. The methanolic extract was divided into two parts, the first being used for antioxidant tests and the second for phenolic analysis. Liquid–liquid extraction procedure was applied to this second part for phenolic determining (Akyuz, Sahin, Islamoglu, Kolayli, & Sandra, 2014).

2.6. Analysis of phenolic compounds by HPLC

Eighteen standards of phenolic compounds were analyzed using HPLC (Thermo Finnigan Surveyor), in a UV–Vis detector supplying a double wavelength simultaneously. Phenolic profile was determined according to Akyuz et al. (2014). For quantitative determining, each phenolic component calibration curves were between 0.998 and 1.000.

2.7. Determination of total phenolic content (TPC) and total flavonoids

TPCs of the methanolic extracts were determined following the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Folin assay was also based on all phenolic contents including phenolic acids, flavonoids, and anthocyanins in the aquatic solution. This gives a blue-color complex whose maximum absorbance can be read at 760 nm.

The amount of total flavonoid was measured with a spectrophotometric method at 415 nm as reported previously (Fukumoto & Mazza, 2000) using quercetin as standard.

2.8. Determination of total antioxidant capacity and free radial scavenging activity

The reducing ability of ferric tripyridyltriazine (Fe-III-TPTZ) complex was used for total antioxidant capacity assay (Benzie & Strain, 1996). And also, trolox was used as positive control to construct a reference curve (62.5–1000 μM).

The scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was used to determine the radical scavenging activity of the methanolic honey samples. The colorimetric test was assayed using the Molyneux method (2004). DPPH radical has a purple color which decays in the presence of antioxidant agents, thus the change of the absorbance is monitored at 517 nm.