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Original article

Antioxidant activities of some monofloral honey types produced across Turkey

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A B S T R A C T

This study was conducted with the aim of determining the chemical, biochemical properties, and antimicrobial capabilities of some of the monofloral honeys produced in Turkey. In this study, 23 different monofloral honey samples were obtained from diverse geographical regions of Turkey. Floral origin of the honey samples was determined by melissopalinological analyses. Additionally, antioxidant properties were determined. To determine the antioxidant properties of honey samples, four test methods of total phenolic content, DPPH, iron reduction power and β-carotene linoleic acid emulsion method were used. As a result of the antioxidant activity analysis among the honey samples, rhododendron and parsley honey showed most prominent results in terms of the amount of phenolic compounds and antioxidant activity. On the other hand, acacia and citrus honey samples showed least antioxidant activity. A positive correlation was determined between four methods. Differences between antioxidant activities of honey samples were significantly found ($P < 0.01$).

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1. Introduction

Honey is a natural dietary antioxidant whose components are responsible for the redox properties are likely to be flavonoids, phenolic acids, enzymes, vitamins and minerals such as copper and iron (Chua et al., 2013). Honey can originate from single or multiple plant species, and its biochemical composition is affected by the floral source (Elbanna et al., 2014). 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 (Can et al., 2015).

The melissoplaynological study is an effective method to determine the pollen inside the honey sample. Taxa of the pollen are usually used to indicate the floral nectar sources utilized by bees to produce honey (Louveaux et al., 1978; Moar, 1985). The relative pollen frequency is usually used to verify a honey sample as to the major and minor nectar sources.

A free radical is an atom, molecule or compound that is highly unstable because of its atomic or molecular structure. Free radicals are very reactive as they attempt to pair up with other molecules, atoms, or even individual electrons to create a stable compound (Wu and Cederbaum, 2003). Therefore reactive oxygen species (ROS) occur and free radicals cause molecular transformations and gene mutations in many types of organisms. This is called oxidative stress and is well known to cause many diseases (Küçük et al., 2007). Honey has been an important food for humans since the beginning. The relation between bees and humans started as early as the stone age. It has been used in alternative medicine since that time, and its role was to treat burns, gastrointestinal disorders, asthma, infections and some chronic wounds. Honey maintains an important place in terms of nutrients as it is known to be rich in antioxidants, including glucose oxidase, catalase, ascorbic acid, phenolic compounds, carotenoids, organic acids, amino acids and proteins. The botanical origin of honey is one of its main quality parameters, and it has been reported that the composition and antioxidant capacity of honey depend on the floral source used to collect nectar, seasonal and environmental factors, as well as processing(Kıvrak and Kıvrak, 2017). These factors may also have an effect on the honey composition and antioxidant activity. Price depends on the quality and is also related to the

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floral origin. Over the past two decades, intensive studies have been conducted on the effects of free oxygen radicals, known as experimental and clinical cues, reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Halliwell and Gutteridge, 2015). Many studies have shown that oxidative stress causes many diseases such as cancer formation, inflammation, aging (Di Mascio et al., 1991), the pathogenesis and progression of diabetes (Lau et al., 2013), cardiovascular diseases, weakening of the immune system, degenerative diseases of the nervous system (Diplock et al., 1998; Koca and Karadeniz, 2003), heart and lung diseases, cataracts (Aras, 2006).

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 (Can et al., 2015). Consuming nutrients rich in natural antioxidants, may be effective in the prevention and treatment of chronic diseases that have increased in recent years. These antioxidant compounds can function as an endogenous cellular antioxidant defense against free radicals (Bozdogan Konuskan and Mungan, 2016; Cardozo et al., 2013; Kang et al., 2008; Kelsey et al., 2010; Lau et al., 2013). Honey with rich antioxidant content is one of the characteristics according to the studies (Alves et al., 2013; Da Silva et al., 2013; Isla et al., 2009; Wilczyńska, 2014). Honey presents several biological activities and its use as a medicine has been known since ancient times. Honey has antimicrobial, antiviral, antiparasitic, antiinflammatory, antimutagenic, anticancer, and immuno-suppressive activities (Bogdanov et al., 2008).

Therapeutic techniques utilizing and integrating 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 (Can et al., 2015). Honey is a natural product and consists of a highly concentrated complex solution of sugars. Honey contains other minor constituents such as minerals, proteins, amino acids, enzymes, vitamins, organic acids, phenolic compounds, volatile compounds, and carotenoids and the composition depends mainly on its botanical and geographical origin and, to a lesser extent, on its processing, handling, and storage (Manyi-Loh et al., 2011).

The goal of this study was to determine the antioxidant capacity of some monofloral honey types produced from the diverse regions of Turkey. To determine the origin of honey, melissoplaynological analyses were done before determining the antioxidant capacity of samples.

2. Materials and methods

2.1. Chemicals and instruments

Unless otherwise noted, all reagents and chemicals used were analytical grade from Sigma Chemical Company (St. Louis, MO, USA). A Shimadzu UV-1208 model UV–VIS spectrophotometer (UV-1280 Multipurpose UV-Visible Spectrophotometer, Shimadzu) was used for absorbance measurements.

2.2. Honey samples

Honey samples were directly obtained from the beekeepers during the period of 2015–2016, living across different locations throughout Turkey and registered to the Turkish Beekeeping Associations. A total of 23 monofloral honey samples, not subjected to any heating process, were collected for analyses. Honey samples of different floral sources were obtained from the local beekeepers living in the different regions of Turkey as apparent in Table 1 and Fig. 1. All honey samples were kept at room temperature (24 ± 2) throughout the process of analysis.

Table 1
Honey samples, location, pollen frequency (%).

No	Honey types	Location	Pollen frequency (%)
H1	Anise (*Pimpinella* sp.)	Antalya	0.45
H2	Chestnut (*Castanea* sp.)	Ordu	0.75
H3	Astragalus (*Astragalus* sp.)	Konya	0.52
H4	Sainfoin (*Onobrychis* sp.)	Van	0.47
H5	Wild Mint (*Mentha* sp.)	Istanbul	0.51
H6	Cesme thyme (*C. Capitatus*)	Izmir	0.63
H7	Acacia (*R. pseudoacacia*)	Ordu	0.54
H8	Cedrus (*C. libani*)	Antalya	0.58
H9	Cotton (*Gossypium* sp.)	Diyarbakır	0.53
H10	Thyme (*Thymus* sp.)	Batman	0.48
H11	Euphorbia (*Euphorbia* sp.)	Mardin	0.60
H12	Linden (*Tilia* sp.)	Ordu	0.66
H13	Eucalyptus (*Eucalyptus* sp.)	Adana	0.73
H14	Ferula (*Ferula* sp.)	Mersin	0.47
H15	Yellowstar-thistle (*C. solstitialis*)	Diyarbakır	0.46
H16	Parsley (*Petroselinum* sp.)	Hatay	0.79
H17	Chasteberry (*V. agnus-castus*)	Izmir	0.86
H18	Sunflower (*H. Annuus*)	Adana	0.86
H19	Citrus (*Citrus* sp.)	Adana	0.57
H20	Rhododendron (*Rhododendron* sp.)	Ordu	0.56
H21	Strawberry tree (*Arbutus* sp.)	Mersin	0.61
H22	Carob bean (*C. siliqua*)	Mersin	0.46
H23	Pine honey (*Marchalina hellenica*)	Muğla	–

2.3. Melissopalynological analysis of honey samples

Floral nectar sources of honey samples were determined by melissapalinological analyses according to Louveaux et al. (1978) and Sorkun (2008). Accordingly, 10 g of each honey sample were dissolved in 20 mL of distilled water in a test tube. Each diluted sample was then shaken by a stirrer for 10 min. The solution was then centrifuged at 3500 rpm for 15 min and the supernatant fraction was poured off. The decanted sediment was washed with 10 ml of distilled water and re-centrifuged. Following the second centrifugation, the precipitate remaining at the bottom of the tube was infused with an added quantity of basic-fucose glycerin-gelatin taken from the needle tip, and this material was then transferred onto the slide. Then, a lamella was placed on top for examination through a microscope. When one pollen type represented >45% of the total number of pollen grains, the sample was identified as a monofloral honey (Sorkun, 2008). Moar (1985) points out that since 45% of a single pollen type is the "universal minimal limit" needed for a honey to be identified as monofloral (Moar, 1985). Honey samples which had 45% or more dominant pollen, were selected and analyzed for antioxidant capacity.

2.4. Determination of antioxidant activity

2.4.1. Determination of total phenolic content (TPC)

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) (Singleton et al., 1999; Yorulmaz and Konuskan, 2017) with some modifications. Briefly, each honey sample (1 g) was dissolved in methanol (5ml) and filtered through Whatman No: 1. This solution was used (40 µl) and mixed with 2.4 ml of distilled water and 200 µl non-diluted Folin–Ciocalteu reagents for 3 min and then 0.6 ml of sodium carbonate was added (20%, Na_2CO_3). After incubation in the dark at 25 °C for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank using a UV-VIS Spectrophotometer (Hitachi U-1900, Japan). All measurements were made in triplicate. Gallic acid (0–1000 mg/L) was used as a standard to derive the calibration curve. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per kg of honey.

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