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Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics

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ABSTRACT

The aim of the present study was to characterise and classify Greek pine honeys according to geographical origin, based on the determination of volatile compounds and physicochemical parameters using MANOVA and Linear Discriminant Analysis. Thirty-nine pine honey samples were collected during the harvesting period 2011 from 4 different regions in Greece known to produce good quality pine honey. The analysis of volatile compounds was performed by Headspace Solid Phase Microextraction–Gas Chromatography/Mass Spectroscopy. Fifty-five volatile compounds were tentatively identified and semi quantified. Physicochemical parameter analysis included the determination of pH, free, lactonic and total acidity, electrical conductivity, moisture, ash, lactonic/free acidity ratio and colour parameters L^*, a^*, b^*. Using 8 selected volatile compounds and 11 physicochemical parameters, the honey samples were satisfactorily classified according to geographical origin using volatile compounds (84.6% correct prediction), physicochemical parameters (79.5% correct prediction) and the combination of both (74.4% correct prediction).

1. Introduction

The EU has recognised and supported the potential of differentiating quality products on a regional basis by introducing the following geographical indications to a food product: protected designation of origin (PDO), protected geographical indication (PGI) and traditional specialties guaranteed (TSG) (Council Directive 2001/110/EC relating to honey, 2001). Such products with unique composition and/or processing characteristics enjoy higher prices on domestic and international markets.

The composition and sensory attributes of honey vary considerably depending on its botanical and geographical origin. Consequently, the determination of botanical and geographical origin of honey is of increasing interest worldwide. European Legislation (Council Directive 2001/110/EC relating to honey, 2001) defines types and quality criteria for honey. With regard to origin, honeys are categorised as blossom or nectar honeys, obtained from the nectar of plants and honeydew honeys, obtained mainly from excretions of plant sucking insects (*Hemiptera*) in the living parts of plants or secretions of living parts of plants. Typical examples of blossom honeys are thyme, citrus and heather honeys while those of honeydew honeys are pine and fir honeys.

Greece produces an estimated 12,000–13,000 tons of honey annually, 60–65% of which is pine honey, 10% is thyme, 10% is citrus and 5–10% is fir honey. Chestnut, heather, oak, and cotton honeys are produced in smaller amounts. Presently the only honey classified as PDO in Greece is that of Menalon vanilla fir honey from Arcadia in southern Greece.

Initially, honeydew honeys were regarded as insect excrements by consumers. However, studies have shown that pine honey has high nutritional value due to its high mineral content (Tananaki, Thrasyvoulou, Giraudel, & Montury, 2007). Thus, pine honey became gradually accepted as honey of good quality. Nowadays, pine honey is considered as one of the most popular monofloral honeys on the Greek market with a price higher than that of blossom honey.

Techniques used to differentiate botanical and geographical origin of foodstuffs include High Performance Liquid chromatography (HPLC), Gas Chromatography (GC), Gas Chromatography/Mass

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Spectroscopy(GC/MS), High Performance Liquid Chromatography/Mass Spectroscopy(LC/MS), Inductively Coupled Plasma Mass Spectroscopy (ICP/MS), NMR Spectroscopy, Isotope Ratio Mass Spectroscopy (IRMS), SNIF-NMR Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Fluorescence Spectroscopy, Capillary Electrophoresis etc. (Luykx & van Ruth, 2008).

The determination of volatile compounds or physicochemical parameters has been used to differentiate honeys according to botanical origin (Escuredo, Fernandez-Gonzalez, & Carmen, 2012; Tananaki et al., 2007) and geographical origin (Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2007; Senyuva et al., 2009).

Volatile compounds have been determined using solvent extraction (SE) (D'Arcy, Rintoul, Rowland, & Blackman, 1997), simultaneous steam distillation extraction (SDE) (Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2005), Solid phase extraction (SPE) (Castro-Vazquez, Pırez-Coello, & Cabezudo, 2003), static head space (Rowland, Blackman, D_Arcy, & Rintoul, 1995), solid phase dynamic extraction (SPDE) (Ampuero, Bogdanov, & Bosset, 2004), purge and trap (Soria et al., 2008; Tananaki et al., 2007), solid phase microextraction (Alissandrakis et al., 2007; Senyuva et al., 2009) and a micro-scale simultaneous distillation/extraction technique (Castro-Vazquez, Diaz-Maroto, & Perez-Coello, 2007, 2009) followed by GC/MS analysis.

To the best of our knowledge there is no information in the literature on the classification of Greek pine honeys according to geographical origin.

Thus, the aim of the present study was to characterise and classify Greek pine honeys based on (a) volatile compounds (b) physicochemical parameter values and (c) their combination using chemometrics.

2. Materials and methods

2.1. Honey samples

A total of 39 pine honey (*Pinus* spp.) samples were collected from selected beekeepers during the harvesting period 2011 from four different geographical regions: Halkidiki, northern Greece (10 samples), Evia, central Greece (12 samples), Thassos, north-eastern Greece (10 samples) and Samos, eastern Greece (7 samples) known to produce good quality pine honey (Table 1). In order to ensure that the honeys were unifloral the melissopalynological procedure was applied to all honey samples (Louveaux, Maurizio, & Vorwohl, 1978). Samples were stored in glass containers, shipped to the laboratory and maintained at 4 °C until analysis.

2.2. Melissopalynological analysis

Melissopalynological analysis was performed according to Louveaux et al. (1978) Ten grams of each honey sample were diluted in 20 mL of distilled water and centrifuged at 3000 rpm for 10 min. The sediment was dried at 40 °C and mounted with Entellan Rapid (Merck, 1.07961.0500). The honeydew elements and pollen grains were counted and identified in 20 optical areas at 200× magnification using an OLYMPUS BX 40 light microscope.

2.3. Determination of volatile compounds

2.3.1. HS-SPME-GC/MS analysis

A divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber 50/30 μm (Supelco, Bellefonte, PA, USA) was used to extract headspace volatiles from honey. Prior to use, the fiber was conditioned following the manufacturer's recommendations. The samples (2 g honey in 2 mL of distilled water, plus 0.20 g NaCl (Merck, Darmstadt, Germany) plus 20 μL of an internal standard (benzophenone,100 μg/mL, Sigma Aldrich), were placed in 15 mL screw-cap vials equipped with PTFE/silicone septa. The vials were maintained at 45 °C in a water bath under stirring at 600 rpm during the entire extraction procedure. A cross shaped PTFE-coated magnetic stirrer (diameter 10 mm) (Semadeni, Ostermundigen – Bern, Switzerland) was placed inside the vials. Screening of the parameters affecting the extraction (sampling time (10/15/20/25/30 min), extraction temperature (40/50/60 °C), vial size (10/15/25 mL), sample volume (1:1, 1:2, 1:4 g honey/mL distilled H_2O), fibre coating film thickness (50/30 μm or 70/30 μm), salt addition (NaCl) or not) revealed the optimum conditions to be: 15 min equilibration time, 30 min sampling time, 4 mL sample volume and 45 °C water bath temperature. Test samples were prepared daily prior to HS-SPME-GC/MS analysis. Each sample was run in duplicate.

2.3.2. GC/MS instrumentation and conditions

An Agilent 7890 A GC unit coupled to an Agilent 5975 MS detector was used to analyse the honey solutions. A DB-5MS (cross linked 5% PH ME siloxane) capillary column (60 m × 320 μm i.d., × 1 μm film thickness) was used, with helium as the carrier gas (purity 99.999%), at 1.5 mL/min flow rate. The injector and MS-transfer line were maintained at 250 °C and 270 °C, respectively. For the SPME analysis, oven temperature was held at 40 °C for 3 min, increased to 260 °C at 8 °C/min (6 min hold). Electron impact mass spectra were recorded at 50–550 mass range. An electron ionisation system was used with ionisation energy of 70 eV.

2.3.3. Mass spectral data processing

The identification of compounds was achieved using the Wiley 7, NIST 2005 (National Institute of Standards and Technology) mass spectral library (J. Wiley & Sons Ltd., West Sussex, England). For the determination of retention indices a mixture of n-alkanes (C_8–C_{20}) dissolved in n-hexane, was employed. The mixture was supplied by Supelco (Bellefonte, PA, USA). The calculation was carried out for components eluting between n-octane and n-eicosane. Data were expressed as ratios of the abundance of each m/z fragment against the abundance of the fragment $m/z = 182$, which is the molecular ion of the internal standard (benzophenone) used. Extraction of the ion chromatogram for this fragment ($m/z = 182$) revealed that benzophenone does not naturally occur in any of the isolated compounds.

2.4. Determination of physicochemical parameters

The following physicochemical parameters (pH, moisture content, electrical conductivity, free acidity, lactonic acidity, total acidity, ash content, lactonic/free acidity ratio and colour parameters L^*, a^*, b^*) were determined according to the Harmonized Methods of the International Honey Commission (IHC), 1997 and AOAC, 1990 Official methods of Analysis with some modifications.

2.4.1. pH

The pH was measured using a Delta OHM, model HD 3456.2, pH-meter (Padova, Italy) with a precision of ± 0.002 pH units in a solution of 10 g honey in 75 mL of CO_2 free distilled water. All measurements were performed in duplicate.

2.4.2. Moisture content

Moisture was determined using a KERN, model MLB50-3, moisture analyzer (Balingen, Germany) by weighing 10 g of honey in a metal dish and drying at a temperature of 100 °C for 10–12 min. If the weighing result of 3 successive measurements remained constant then drying was terminated. Results were expressed as moisture (g/100) = weight loss/initial weight × 100. All measurements were performed in duplicate.