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Effect of late harvest and floral origin on honey antibacterial properties and quality parameters

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ABSTRACT

Honey is a food of high importance due to its nutritional value. The effect of late harvest and pollen composition on honey quality and antimicrobial activity was studied. Different physicochemical parameters were determined as quality indicators. The HMF content, diastase activity, sugar content, antimicrobial activity, and hydrogen peroxide content were selected in current work as the crucial parameters for evaluation. The results proved that the late harvested honey sample showed a vast number of pollens from different blossom periods. The hydrogen peroxide content and antimicrobial activity of late harvested honey was significantly increased (proved by *t*-test), whereas the honey seems to be affected significantly from weather conditions since the HMF content was also increased. The diastase activity was proved to be lower than the respective in fresh honey, whereas no difference was observed on the sugar content. Finally, the effect of origin and processing in late harvested honey was discussed.

1. Introduction

During the last few years, honey consumption has increased since it is a natural product with high nutritional value. As part of a balanced diet honey may contribute to health promotion due to its various bioactive compounds. It has an important antioxidant activity and is usually used as a food additive in many beverages and foodstuffs. The chemical composition of honey is perfectly described by Bogdanov, Jurendic, Sieber, and Gallman (2008) and Silva, Gauche, Gonzaga, Costa, and Fett (2016) in their reviews (Bogdanov et al., 2008; Silva et al., 2016).

The rapid growth in honey production has made the sector important to the economy of many developing countries, whereas there is an increased concern for public health since honey undergoes many changes in its composition during storage and processing (Barra, Ponce-Díaz, & Venegas-Gallegos, 2010; Tornuk et al., 2013). Furthermore, the inappropriate agricultural practice and the small amounts of honey production have provided a heightened interest in its adulteration (Wang, Juliani, Simon, & Ho, 2009). The detection of the adulteration of honey is very difficult and modern analytical techniques are required, such as liquid chromatography coupled to isotope ratio mass spectrometry, elemental analyzer-isotope ratio mass spectrometry and gas chromatography coupled to mass spectrometry (Cabañero, Recio, & Rupérez, 2006; Luo et al., 2016; Padovan, De Jong, Rodrigues, & Marchini, 2003).

Numerous studies have been carried out on chemical composition of different pollen type honeys and from different regions (Silva et al., 2016). To the best of our knowledge, there is no similar study concerning the effect of late harvesting on the honey quality and pollen content. Parameters such as melissopalynological characterization Hydroxymethylfurfural (HMF) content, diastase activity, hydrogen peroxide content, iron content antimicrobial activity and sugars profile were investigated, since are the most important parameters for the quality control of different honey samples. The results were compared with other obtained by different multifloral honeys, Manuka honey, and a sample form the same region harvested under usual conditions by the same beekeeper.

2. Materials and methods

2.1. Honey samples

Honey samples were harvested after a whole year remaining in the honeycomb (coded as "Argos late"). The honeycomb was left closed

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without any external intervention and the sample "Argos late" was taken one year later. The honey was produced under good agricultural practice. No pesticides, antibiotics or smoke were used. In order to have a more representative estimation of the harvest effect four different samples were collected from the same honeycomb of Attica region, Greece. Furthermore, a sample was also collected with the normal harvest, coded as "Argos early" from the same region, and from the same beekeeper composed mainly with *Eucalyptus* pollens, and other different samples were also purchased from the local market (Manuka honey and multifloral honeys) and analyzed for comparison reasons. All multifloral honeys were marked as Greek honeys from different producers and under different brand names. The production dates were in 2016 and the expiration date was in 2017. The procedure of harvesting was taken place during 8 o'clock in the morning for fresh and non fresh honey. The date of harvesting was July of 2016. The temperature was around 30 °C. All samples were immediately transferred to the laboratory in glass vessels.

Samples were stored at room temperature in a dark place until analysis. The storage time was less than a month. All honey samples were characterized on the basis of melissopalynological characterization according to their specific botanical variety (Louveaux, Maurizio, & Vorwohl, 1978).

2.2. HMF determination

The HMF content determination was based on the official AOAC method (AOAC 980.23, 1983). Five grams of honey were dissolved in 25 mL of water, transferred quantitatively into a 50 mL volumetric flask, added by 0.5 mL of Carrez solution I and 0.5 mL of Carrez II and make up to 50 mL with water. The solution was filtered through paper rejecting the first 10 mL of the filtrate. Aliquots of 5 mL were put in two test tubes; 5 mL of distilled water were added to one tube (sample solution); 5 mL of sodium bisulphite solution 0.2% were added to the second tube (reference solution). The absorbance of the solutions at 284 and 336 nm was determined using a HACH LANGE DR 5000 UV–visible spectrometer. The HMF content was calculated by the Eq. (1):

$$\text{HMF (mg/kg)} = (A_284)-(A_336) \times 149.7, \quad (1)$$

where: A_{284}: the absorbance at 284 nm

A_{336}: the absorbance at 336 nm

149.7: a factor calculated by the molecular weight of HMF and the mass of the sample.

For quality assurance the Lamia Laboratory took a z-score equal to −1.5 in an interlaboratory proficiency test for HMF content, organized by the General Chemical State Laboratory of Greece (Schema 70 06), which is a well performed value (z-score among −2 and +2).

2.3. Diastase activity and conductivity measurement

Diastase activity was determined using 10 g of honey weighted into a 50 mL beaker and 5 mL of acetate buffer were added, together with 20 mL of water. When the sample was completely dissolved 3 mL of sodium chloride 0.5 M were added and the solution was diluted to 50 mL with water. Moreover, a starch solution was standardized using an iodine solution. Both solutions were warmed at 40 °C. 5 mL of starch solution were added into 10 mL of honey solution and start stop-watch. An aliquot was taken every 5 min and was added to 10 mL of iodine solution. The absorbance was recorded and a calibration curve was obtained. According to the official AOAC method the number 300 was divided by the time needed to reach the absorbance value of 0.235 and expressed as DN (Shade units) or diastase number (AOAC official method 958.09-1977, 2010). For quality assurance the Lamia Laboratory took a z-score equal to −1.2 in an interlaboratory proficiency test for diastase activity, organized by the General Chemical State Laboratory of Greece (Schema 70 06), a well performed value (z-score among −2 and +2). The conductivity was measured in a 20% (w/v) honey solution diluted with ultra-pure water.

2.4. Sugar content

Honey samples were analyzed for their sugar profile by GC-FID. Samples were diluted with ultrapure water (18.2 mΩ cm) and 1 mL was transferred in a vial and were dried at 100 °C. The dried samples were derivatised by the addition of 150 μL of trimethylsilyl imidazole (98%, Fluka, Saint Quentin Fallavier, France) and 1 mL of pyridine (Fluka, Saint Quentin Fallavier, France). The vial was capped and the solution heated at 80 °C 1 h; then 1 μL of mixture was injected into the GC system. All experiments were performed with a Shimadzu GC 2010 PLUS, GC-FID system, using an Agilent DB-1 30 m × 0.32 mm × 1 μm. The optimized conditions were: injection volume 1 μL in split mode 1:50, injector temperature 260 °C, carrier gas He at a constant flow 1.0 mL/min, detector temperature 320 °C. The initial oven temperature was 105 °C and then programmed at 4 °C/min to 240 °C, where was held for two min, then programmed at 30 °C/min to 300 °C where was held for 10 min. The estimation of the accuracy was also calculated by the the certified reference material FAPAS T2830QC and the participation of Lamia Laboratory in an interlaboratory proficiency test for sugar content, organized by the General Chemical State Laboratory of Greece (Schema 70 06) where it achieved a z-score among −2 and +2 for all analytes determined.

2.5. Hydrogen peroxide determination

Samples were diluted to a final concentration 20% (v/v) with ultrapure water (18.2 mΩ cm), and 5 mL of the diluted sample was mixed with 1 mL H_2SO_4 0.5 M and 5 mL of V_2O_5 (0.2% w/v in 0.5 M H_2SO_4). The absorbance of the solutions at 454 nm was determined using a HACH LANGE DR 5000 UV–visible spectrometer Standard addition method was used for the quantification of hydrogen peroxide in honey samples.

2.6. Antimicrobial analysis

An exact amount of each honey (0.5 g) was swabbed and transferred to ready to use 10 mL tubes containing Buffered Peptone Water (Bioprepare S.A, Athens, Greece). The balance (Gibertini balance, CRYSTAL 500 CAL) had three significant digits. After weighing, tubes were vortexed for 20 s and water bathed for two hours in 35 °C. 50 μL from the material of the tubes was then transferred to petri dishes 60 mm diameter with a ready to use substrate Chromagar *S. aureus* MRSA (Chromagar S.A.).

Samples were spiked using BD Microtrol discs S. Aureus MRSA. Each disc was diluted in the ready to use 10 mL tubes containing Buffered Peptone Water. The tubes were vortexed for 20 s and water bathed for two hours in 35 °C. Then a sequential dilution took place and the last dilution gave us the preferred concentration (log = 1). The material was then swabbed to petri dishes of 60 mm diameter with a ready to use substrate Chromagar *S. Aureus* MRSA bought from Chromagar S.A. The spiking was repeated three times for each sample and the means of the results are presented below. A blank sample was also prepared in triplicate to set the minimum percentage (0%) of inhibition. The percentage of inhibition as calculated based on the Eq. (2), as proposed by Ker-Woon, Ghafar, Hui, Yusof, and Ngah (2015).

$$X = (AB) \times 100/A \quad (2)$$

Where

X = Percentage of inhibition.

A = Initial columns of S. aureus after seeding

B = Columns that were measured at day 2 after seeding