



## Review

# An overview of physicochemical characteristics and health-promoting properties of honeydew honey

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## ABSTRACT

Honeydew honey has differentiated chemical and physicochemical characteristics besides potential functional properties such as antimicrobial, anti-inflammatory and antioxidant. In this sense, the interest and consumption of this honey as a functional product by the food industry and consumers have increased. Honeydew honeys usually present dark color, a lower content of monosaccharides and higher values of pH, acidity, electric conductivity, proteins, minerals, phenolic compounds, and oligosaccharides compared to blossom honeys, which contribute to its outstanding biological activities. Consequently, contaminations and adulterations of this honey can occur and compromise the quality, safety and authenticity of honeydew honey. Thus, detailed knowledge of the composition and properties of honeydew honeys is of great importance, especially considering that honeydew honeys are still few studied and therefore underestimated. Therefore, in this review, the physicochemical characteristics, chemical and bioactive composition, functional and health-promoting properties of honeydew honey as well as contamination, adulteration and authenticity of this honey are summarized.

## 1. Introduction

Honey is a natural product traditionally used as a sweetener and for therapeutic applications. It is constituted mainly of the monosaccharides fructose and glucose, besides water and other components in minor concentrations such as proteins, enzymes, amino acids, phenolic compounds, minerals, vitamins, organic acids, important contributors to the quality and health-promoting properties of honey (Alvarez-Suarez et al., 2012; Alvarez-Suarez, Tulipani, Romandini, Bertoli, & Battino, 2010; Can et al., 2015; Escuredo, Míguez, Fernández-González, & Seijo, 2013).

The interest in an unconventional type of honey, known as honeydew honey, has increased due to the differentiated nutritional, sensorial and possible therapeutic characteristics of this honey (Castro-Vázquez, Díaz-Maroto, & Pérez-Coello, 2006; Flores, Escuredo, & Seijo, 2015; Pérez, Iglesias, Pueyo, Gonzalez, & de Lorenzo, 2007). Honeydew honey is produced by bees (*Apis mellifera*) from secretions of living parts of plants or excretions of plant-sucking insects, while blossom honey, commonly known, is produced from the nectar of flowers (European Commission, 2002a), resulting in honeys with very different characteristics. Honeydew honey usually presents higher values of pH,

electrical conductivity, net absorbance, ashes percentage, higher content of disaccharides, trisaccharides, and lower level of monosaccharides, besides darker color and peculiar sensory features compared to blossom honeys (Bogdanov, Ruoff, & Persano-Oddo, 2004; Escuredo et al., 2013; Manzanares, García, Galdón, Rodríguez, & Romero, 2011; Mateo & Bosch-Reig, 1998; Pita-Calvo & Vázquez, 2017).

Actually, honey has been investigated through *in vivo* and *in vitro* studies as a promise agent in the treatment of different diseases, including wound healing (Oryan, Alemzadeh, & Moshiri, 2016), non-healing leg ulcers (Mayer, Slezak, Takac, Olejnik, & Majtan, 2014), eye wounds (Cernak, Majtanova, Cernak, & Majtan, 2012; Majtanova et al., 2015), disorders of the skin (McLoone, Warnock, & Fyfe, 2016) and the gastrointestinal system (Nasuti, Gabbianelli, Falcioni, & Cantalamessa, 2006). Honeydew honey usually presents higher content of bioactive compounds such as phenolics, proteins, and amino acids compared to blossom honeys. As a consequence, they present higher antimicrobial and antioxidant activity (Bertoncelj, Dobersek, Jamnik, & Golob, 2007; Bogdanov et al., 2004; Can et al., 2015; Escuredo et al., 2013; Mateo & Bosch-Reig, 1998; Osés et al., 2016; Seraglio et al., 2017; Silva, Gonzaga, Fett, & Costa, 2018), highlighting this honey as a potential

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health-promoting food.

However, even though the papers have highlighted honeydew honeys in relation to blossom honeys as to their potential to exert beneficial effects on human health, there are still few studies on honeydew honeys. The few papers available in the literature may be due to the increase in demand for this product, which also makes necessary to ensure the quality and safety as well as the authenticity of the honeydew honeys (Can et al., 2015; Flores et al., 2015; Manzanares et al., 2011; Seraglio et al., 2016; Tananaki, Thrasyvoulou, Giraudel, & Montury, 2007). Thus, the knowledge and characterization of the honeydew honeys may contribute with its authenticity, through the identification of origin markers (Pita-Calvo & Vázquez, 2018). These markers could link the product to its botanical and geographical origin, and in addition to avoiding fraud, enhance its production chain (Azevedo et al., 2017; Azevedo et al., 2017; Madejczyk & Baralkiewicz, 2008; Oroian, Ropciuc, & Paduret, 2018; Pita-Calvo & Vázquez, 2018; Ruiz-Matute, Soria, Martínez-Castro, & Sanz, 2007; Simova, Atanassov, Shishiniova, & Bankova, 2012).

Therefore, considering the relevance of honeydew honey in the international scenario, this review aims to provide a detailed document on this product, summarizing important information on the physicochemical characteristics, chemical and bioactive composition, functional and health-promoting properties, possible contaminants, adulteration, and authenticity of honeydew honey.

## 2. Composition and physicochemical characteristics

### 2.1. Carbohydrates

Honeydew honey is a concentrated aqueous solution of sugars (Can et al., 2015; Karabagias et al., 2018). As shown in Table 1, the content of reducing monosaccharides (fructose + glucose) in honeydew honeys ranging from 59.9 to 79.7 g 100 g<sup>-1</sup>. Therefore, honeydew honeys are in accordance with the permissible limit, since it is established that, for honeydew honey and mixtures thereof, the concentration of these reducing sugars should not be < 45 g 100 g<sup>-1</sup> (Codex Alimentarius Commission, 2001; European Commission, 2002a). Also, fructose and glucose were the major monosaccharides in all honeydew honeys evaluated, with content ranging between 28.2 and 48.3 g 100 g<sup>-1</sup> and 21.7 to 37.7 g 100 g<sup>-1</sup>, respectively (Table 1).

The reducing sugars (fructose and glucose) and also sucrose can be used as indicative of maturity in honeydew honey (Dominguez, Jacksén, Emmer, & Centurión, 2016; Schlabit, da Silva, & de Souza, 2010). The sucrose content should not exceed 5 g 100 g<sup>-1</sup> (Codex Alimentarius Commission, 2001; European Commission, 2002a), since high sucrose content may indicate a premature honey harvesting, in which sucrose was not completely converted into glucose and fructose or reveal possible adulterations of the product with commercial sugar syrup during its production (Boussaid et al., 2018; Schlabit et al., 2010). According to Table 1, honeydew honeys did not exceed 5 g 100 g<sup>-1</sup> of sucrose, suggesting adequate maturity and absence of adulteration.

Besides sucrose, disaccharides such as maltose, turanose, isomaltose, gentiobiose, melibiose, and trehalose, besides the presence of trisaccharides such as erlose, maltotriose, isomaltotriose, panose, raffinose, melezitose and other oligosaccharides have been reported in honeydew honeys, but normally at low concentrations (Can et al., 2015; Pascual-Maté et al., 2018; Rybak-Chmielewska, Szczęśna, Waś, Jaśkiewicz, & Teper, 2013). As shown in Table 1, maltose was the disaccharide in higher concentration, presenting 5.5 g 100 g<sup>-1</sup> in honeydew honeys (origin unknown) of Spain (Manzanares et al., 2011). Honeydew honeys of different botanical origins are usually characterized by a higher concentration of oligosaccharides, especially the trisaccharides raffinose and melezitose, which are commonly not found or in very low concentrations in blossom honeys (Kaškonienė, Venskutonis, & Čeksterytė, 2010; Victorita et al., 2008; Weston,

Brocklebank, & Lu, 2000). Melezitose was quantified in honeydew honeys from Spain, Turkey, Poland, and Romania with concentration ranging from 0.1 to 3.2 g 100 g<sup>-1</sup> (Table 1). The content of melezitose has been considered characteristic of honeydew honeys and normally the presence of this sugar may represent that a considerable part of honey was made from honeydew (Rybak-Chmielewska et al., 2013).

The content of carbohydrates can also affect the physical characteristics of the honeys (Dominguez et al., 2016). In general, the crystallization of the honeys is slow when the fructose/glucose (F/G) ratio is higher than 1.3 and is fast when this ratio is < 1.0 (Dobre, Georgescu, Alexe, Escuredo, & Seijo, 2012). In honeydew honeys, the crystallization process does not usually occur (Dominguez et al., 2016), since high F/G ratio is obtained, as demonstrated in Table 1.

#### 2.1.1. 5-Hydroxymethylfurfural

The 5-hydroxymethylfurfural (5-HMF) is formed slowly and naturally during honey storage (Fechner, Moresi, Ruiz Díaz, Pellerano, & Vazquez, 2016). However, long storage period and heating during the processing and storage of the honey can increase the 5-HMF content. Therefore, this compound has been considered a good indicator of honey freshness (Fallico, Arena, & Zappala, 2008; Tornuk et al., 2013; Yücel & Sultanoglu, 2013).

In honeydew honeys, 5-HMF content lower than 10.0 mg kg<sup>-1</sup> is frequently reported (Table 2), indicating acceptable freshness and proper handling of these honeys, since it is established a maximum value of 40 mg kg<sup>-1</sup> of 5-HMF for honeys in general and a maximum value of 80 mg kg<sup>-1</sup> is accepted for honeys of locations with tropical climate and blends of these honeys (Codex Alimentarius Commission, 2001; European Commission, 2002a). In contrast, 42.0 mg kg<sup>-1</sup> of 5-HMF was reported in one commercial honeydew honey (unknown origin) from European Union. Since 5-HMF content and diastase activity are factors closely related, it was possible to assume that the processing and/or storage conditions of this sample were not adequate, resulting in high 5-HMF content and low diastase activity (4.7 Göthe units) (Halouzka, Tarkowski, & Zeljković, 2016).

#### 2.2. Moisture and water activity ( $a_w$ )

Water is the second largest constituent of honeys, and its content is also related to the maturity of this product (Gallina, Stocco, & Mutinelli, 2010). The moisture content of honeydew honeys is shown in Table 2. For pine honeydew honeys from Greece, moisture content ranged from 10.5 to 20.5 g 100 g<sup>-1</sup> while for honeydew honeys (unknown origin) from Spain little variation in moisture content (16.4 to 16.5 g 100 g<sup>-1</sup>) was observed. Moisture content of honeydew honeys can be influenced by botanical and geographical origin, climatic conditions, season of the year, processing and storage conditions, besides the degree of maturity of the honey reached in the hive (Bergamo, Seraglio, Gonzaga, Fett, & Costa, 2018b; Escuredo, Dobre, Fernández-González, & Seijo, 2014; Frink & Armstrong, 2016; Gallina et al., 2010; Kadri, Zaluski, & Orsi, 2017; Zamora & Chirife, 2006). Despite the variability of the moisture content (Table 2), it is possible to assume that the honeydew honeys has adequate maturity, since moisture values were in general below 20 g 100 g<sup>-1</sup>, maximum value establishes for moisture content in honeys (Codex Alimentarius Commission, 2001; European Commission, 2002a).

Besides the F/G ratio, the glucose/moisture (G/M) ratio also affect the crystallization of honeys. The crystallization can be considered slow or null when this ratio (G/M) is < 1.7 and fast when the ratio is higher than 2.0 (Dobre et al., 2012). According to Table 1, honeydew honeys present G/M ratio in general lower than 2.0, suggesting considerable slow crystallization.

A critical factor that determines the enzymatic activity, the survival and limitation of the growth of microorganisms and the product deterioration by fermentation, is the water activity ( $a_w$ ) (Abramovic, Jamnik, Burkan, & Kac, 2008). The  $a_w$  of honey depends mainly on the

**Table 1**  
Mean concentration of carbohydrate in honeydew honeys.

|                                       | Geographical origin / botanical origin |   |                                 |                        |                                      |  |  |                                       |  |   |                            |
|---------------------------------------|--|---|---------------------------------|------------------------|--------------------------------------|--|--|---------------------------------------|--|---|----------------------------|
|                                       | Spain / nr<br>(n = 24)                 | Romania /<br><i>Brassica napus</i><br>(n = 3) | Poland / nr<br>(n = 27)         | Spain / nr<br>(n = 13) | Spain and<br>Romania /<br>nr (n = 1) | Turkey /<br><i>Quercus robur</i> L.<br>(n = 3) | Turkey /<br><i>Pinus</i> L.<br>(n = 4) | India /<br>Pine<br>(n = 8)            | Brazil /<br><i>Mimosa scabrella</i><br>Benth<br>(n = 16) | Greece / Pine<br>and Fir<br>(n = 70) <sup>a</sup> | Spain / nr<br>(n = 18)     |
| Carbohydrate (g 100 g <sup>-1</sup> ) |  |   |                                 |                        |                                      |  |  |                                       |  |   |                            |
| Fructose                              | 39.6                                   | 28.2  | 34.2                            | 35.6                   | 32.9                                 | 43.3   | 39.8                                   | 32.0                                  | 36.5   | 41.2–48.3   | 36.2                       |
| Glucose                               | 31.2                                   | 37.7  | 27.8                            | 26.5                   | 23.2                                 | 21.7   | 23.7                                   | 32.8                                  | 23.3   | 27.1–36.7   | 26.9                       |
| Sucrose                               | 0.5                                    | 1.8   | nd                              | 0.2                    | 0.5                                  | nd   | nd                                     | 1.1                                   | < 0.1  | 0.9–3.5   | 0.4                        |
| Maltose                               | 5.5                                    | 1.5   | 3.2                             | 2.2                    | 1.4                                  | 0.2  | 0.5                                    | 1.0                                   | 2.7  | 0.4–4.0   | 4.3                        |
| Trehalose                             | 1.9                                    | 0.3   | 2.7                             | 0.2                    | –                                    | 0.4  | 0.2                                    | –                                     | –  | –   | 0.1                        |
| Gentiobiose                           | –                                      | –   | –                               | –                      | –                                    | –  | –                                      | –                                     | –  | –   | 0.2                        |
| Isomaltose                            | 1.1                                    | –   | nd                              | –                      | –                                    | –  | –                                      | –                                     | –  | –   | 1.7                        |
| Raffinose                             | –                                      | –   | –                               | –                      | –                                    | –  | –                                      | –                                     | nd   | 0.6–2.5   | 0.1                        |
| Erllose                               | –                                      | –   | –                               | –                      | –                                    | –  | –                                      | –                                     | –  | –   | 0.8                        |
| Melezitose                            | 0.7                                    | 0.6   | 3.2                             | 0.2                    | 0.1                                  | 0.9  | 0.6                                    | –                                     | nd   | –   | 0.5                        |
| Maltotriose                           | –                                      | –   | –                               | –                      | –                                    | –  | –                                      | –                                     | –  | –   | 0.1                        |
| Panose                                | –                                      | –   | –                               | –                      | –                                    | –  | –                                      | –                                     | –  | –   | 0.2                        |
| Isomaltotriose                        | –                                      | –   | –                               | –                      | –                                    | –  | –                                      | –                                     | –  | –   | < 0.1                      |
| Maltotetraose                         | –                                      | –   | –                               | –                      | –                                    | –  | –                                      | –                                     | –  | –   | < 0.1                      |
| Melibiose                             | –                                      | –   | –                               | –                      | –                                    | nd   | 0.3                                    | –                                     | –  | –   | –                          |
| Turanose                              | 1.3                                    | –   | 1.8                             | –                      | –                                    | –  | –                                      | –                                     | –  | 0.5–1.7   | –                          |
| F + G (g 100 g <sup>-1</sup> )        | 70.8                                   | 65.9  | 62.0                            | –                      | –                                    | 65.0   | 63.5                                   | –                                     | 59.9   | 70.4–79.7   | 63.1                       |
| F / G ratio                           | 1.3                                    | 1.4   | 1.2                             | –                      | –                                    | 2.0  | 1.7                                    | 1.1                                   | 1.6  | 1.1–2.8   | 1.3                        |
| G / M ratio                           | 1.8                                    | 1.6   | –                               | –                      | –                                    | –  | –                                      | 1.8                                   | 1.5  | 1.5–2.7   | 1.7                        |
| Analytical technique                  | HPLC-RID                               | HPAEC-PAD                                     | HPLC                            | ICS-PAD                | HPAEC-PAD                            | HPLC-RID                                       | HPLC-RID                               | HPLC-RID                              | CZE-DAD  | NMR and HPLC-RID                                  | GC-FID                     |
| Reference                             | Manzanares et al. (2011)               | Dobre et al. (2012)                           | Rybak-Chmielewska et al. (2013) | Escuredo et al. (2013) | Escuredo et al. (2014)               | Can et al. (2015)                              | Can et al. (2015)                      | Nayik, Suhag, Majid, and Nanda (2018) | Bergamo et al. (2018b)                                   | Karabagias, Vlasiou, et al. (2018)                | Pascual-Maté et al. (2018) |

CZE – Capillary Zone Electrophoresis; DAD – Diode Array Detector; FID – Flame Ionization Detection; GC – Gas Chromatography; HPAC – High Performance Anion-Exchange Chromatography; HPLC – High Performance Liquid Chromatography; ICS – Ion Chromatography System; MS – Mass Spectrometry; NMR – Nuclear Magnetic Resonance; PAD – Pulsed Amperometric Detection; RID – Refractive Index Detector; F + G – Fructose + Glucose; nd – not detected; (–) – not investigated; nr – not reported.

<sup>a</sup> Data presented as range of values, not as mean.

glucose content, since its sugar influence the crystallization of honey (Gleiter, Horn, & Isengard, 2006). The influence of crystallization process in the  $a_w$  of honeydew honeys was observed by Gleiter et al. (2006) and Abramovic et al. (2008), where the  $a_w$  was higher in the crystallized honey (0.59 and 0.55, respectively) than in uncrystallized or liquid honey (0.57 and 0.52, respectively). In liquid honey, glucose is bound to five molecules of water, while in crystallized honey glucose is bound to only one of these molecules, and the others released, contributing to the increase of  $a_w$  (Gleiter et al., 2006).

The  $a_w$  in honeydew honey (unknown origin) from Slovenia presented variation from 0.48 to 0.59 (Abramovic et al., 2008) and in honeydew honey (unknown origin) from Germany ranged from 0.53 to 0.61 (Gleiter et al., 2006), suggesting that these honeydew honeys are possible safe in relation to fermentation, since  $a_w$  below 0.60 is considered sufficient to inhibit the growth of osmophilic yeasts (Zamora & Chirife, 2006).

### 2.3. Organic acids, acidity, and pH

Honeydew honeys contain a small amount of low molecular mass aliphatic organic acids (LMMAOA) that can be used as an indicator of quality, freshness, and authenticity of honeys (Navarrete et al., 2005; Nozal, Bernal, Diego, Gómez, & Higes, 2003; Tezcan, Kolayli, Ulusoy, & Erim, 2011), besides contributing to the sensorial properties such as aroma and flavor and to the physicochemical properties of honeydew honeys such as color, acidity, pH, and electrical conductivity (Mato, Huidobro, Simal-Lozano, & Sancho, 2003, 2006; Tezcan et al., 2011).

A wide range of LMMAOA has been identified in honeydew honeys, as shown in Table 3. The origin of LMMAOA in honeydew honeys is considered unknown, although many of them are natural intermediates of the metabolism of microorganisms, Krebs cycle (citric, succinic, glutaric, fumaric, and oxaloacetic) or enzymatic reactions (Mato et al., 2003).

Gluconic acid, in equilibrium with gluconolactone, is usually the major LMMAOA found in honeydew honeys (Tezcan et al., 2011). As shown in Table 3, with the exception of *Quercus* honeydew honeys (Sanz, Gonzalez, de Lorenzo, Sanz, & Martínez-Castro, 2005), the other studies reported gluconic acid as the major LMMAOA, with concentration ranging from 350.0 to 370.5 mg 100 g<sup>-1</sup>. This acid can be originated from the conversion of the monosaccharide D-glucose by the action of the enzyme D-glucose oxidase, which comes from the hypopharyngeal gland of the bees (Cherchi, Spanedda, Tuberoso, & Cabras, 1994; Mato et al., 1997).

Citric acid can be used to discriminate honeys according to their botanical and/or geographical origin and had already been cited as an important compound for differentiation between blossom honey (lower amount of citric acid) and honeydew honey (higher amount of citric acid) (Mato et al., 1998; Sanz et al., 2005). In honeydew honeys, its content ranged from 12.4 to 142.3 mg 100 g<sup>-1</sup> (Table 3). Other organic acids can be obtained from the hydration of the 5-HMF with two molecules of water, forming a molecule of formic acid and also a molecule of levulinic acid (Cavia, Fernández-Muiño, Alonso-Torre, Huidobro, & Sancho, 2007). Lactic acid can come from a natural fermentative process that occurs in the digestive system of bees. Olofsson and Vásquez

**Table 2**  
Mean content of moisture, free acidity, pH, diastase, 5-hydroxymethylfurfural (5-HMF), and electrical conductivity in honeydew honeys.

| Geographical origin / botanical origin                                 | Moisture (g<br>100 g <sup>-1</sup> ) | Free acidity (meq kg <sup>-1</sup> ) | pH      | Diastase (units) | 5-HMF (mg kg <sup>-1</sup> ) | Electrical conductivity (mS<br>cm <sup>-1</sup> ) | Reference   |
|--|--------------------------------------|--------------------------------------|---------|------------------|------------------------------|---|---|
| Croatia / <i>Salix</i> spp. (n = 2)                                    | 16.1                                 | -                                    | -       | 30.5 (S)         | 3.0                          | 3.1   | Tuberoso et al. (2011)  |
| Spain / Suspected honeydew honeys (n = 24)                             | 17.0                                 | 34.6                                 | 4.6     | 16.7 (G)         | 10.8                         | 1.2   | Manzanares et al. (2011)  |
| Poland / Mainly <i>Abies alba</i> (n = 27)                             | 16.8                                 | 27.6                                 | 4.6     | 28.4 (S)         | nr                           | 1.1   | Rybak-Chmielewska et al. (2013)                                   |
| Spain / Forest (n = 8)   | 15.3                                 | -                                    | -       | 20.5 (D)         | 3.9                          | 1.0   | Escríche et al. (2014)  |
| Greece / Pine (n = 39) <sup>a</sup>                                    | 10.5–20.5                            | 18.1–41.5                            | 4.4–5.3 | -                | -                            | 0.8–1.8   | Karabagias et al. (2014)  |
| Spain / nr (n = 2)   | 15.4                                 | 31.6                                 | 5.2     | 53.2 (D)         | 4.6                          | 1.0   | Visquert et al. (2014)  |
| Turkey / <i>Pinus</i> L. (n = 4)                                       | 17.2                                 | -                                    | -       | 11.6 (D)         | 3.6                          | 1.0   | Can et al. (2015)   |
| Turkey / <i>Quercus robur</i> L. (n = 3)                               | 17.1                                 | -                                    | -       | 10.5 (D)         | 0.6                          | 1.1   | Can et al. (2015)   |
| India / Pine (n = 10)  | 18.2                                 | -                                    | 3.8     | 26.0 (D)         | 6.8                          | 0.8   | Nayik and Nanda (2015)  |
| Spain / <i>Quercus pyrenaica</i> (n = 32) <sup>a</sup>                 | 17.0–18.1                            | -                                    | 4.3–4.5 | 21.9–26.4 (D)    | 0.0–3.0                      | 0.9–1.0   | Flores et al. (2015)  |
| Bulgaria / nr (n = 30)   | 17.3                                 | -                                    | 4.3     | -                | -                            | 1.1   | Atanasova et al. (2016)   |
| Morocco / nr (n = 2)   | 14.6                                 | 21.4                                 | 4.9     | 19.1 (G)         | 1.9                          | 1.1   | Chakir, Romane, Marcazzan, and Ferrazzi (2016)                    |
| Czech Republic, Italy, and European Union / nr<br>(n = 5) <sup>a</sup> | 15.0–18.4                            | 15.1–35.0                            | 3.9–5.1 | 4.7–24.9 (G)     | 2.2–42.0                     | 0.4–1.6   | Halouzka et al. (2016)  |
| Lebanon / nr (n = 16)  | 15.2                                 | -                                    | 5.3     | -                | 4.3                          | 1.3   | Jaafar et al. (2017)  |
| Spain / nr (n = 3) <sup>a</sup>  | 16.4–16.5                            | -                                    | -       | -                | -                            | 0.8   | Juan-Borrás, Soto, Gil-Sánchez, Pascual-Maté, and Escríche (2017) |
| Romania / nr (n = 9)   | 16.3                                 | 16.1                                 | 4.9     | -                | -                            | 1.0   | Oroian and Ropciuc (2017)   |
| Romania / nr (n = 9) <sup>a</sup>                                      | 14.4–17.2                            | 11.8–20.0                            | 4.2–5.2 | -                | -                            | 0.8–1.3   | Oroian, Ropciuc, Paduret, and Sanduleac (2017)                    |
| Brazil / <i>Mimosa scabrella</i> Benthham (n = 16)                     | 16.6                                 | 49.4                                 | 4.8     | 13.7 (S)         | nd                           | 1.4   | Bergamo et al. (2018b)  |
| Serbia / nr (n = 8) <sup>a</sup>                                       | 14.0–19.2                            | 19.8–43.7                            | -       | 31.8–60.0 (S)    | 0.4–13.1                     | 0.9–1.3   | Matović et al. (2018)   |
| Romania / nr (n = 10)  | -                                    | 16.7                                 | 4.5     | -                | -                            | 1.0   | Oroian, Paduret, and Ropciuc (2018)                               |
| Greece / Pine (n = 8)  | -                                    | 34.3                                 | 4.3     | -                | -                            | 1.0   | Karabagias, Karabagias, and Gatzias (2018)                        |
| Greece / Fir (n = 8)   | -                                    | 33.7                                 | 4.6     | -                | -                            | 1.3   | Karabagias, Karabagias, et al. (2018)                             |

nd – not detected; nr – not reported; (-) – not investigated; S – Schade units; D – Diastase units; G – Göthe units.

<sup>a</sup> Data presented as range of values, not as mean.

**Table 3**  
Mean concentration of low molecular mass aliphatic organic acids (LMMAOA) in honeydew honeys.

|                                  | Geographical origin / botanical origin |                                     |                                     |                            |   |  |  |
|----------------------------------|--|-------------------------------------|-------------------------------------|----------------------------|---|--|--|
|                                  | Spain / <i>Quercus robur</i> (n = 2)   | Spain / <i>Quercus ilex</i> (n = 2) | Spain / <i>Quercus</i> sp. (n = 17) | Turkey / <i>Pinus</i> (nr) | Turkey / <i>Quercus</i> sp. (n = 1)                   | Turkey / <i>Pinus</i> sp. (n = 7) <sup>a</sup> | Germany / nr (n = 2) <sup>a</sup>                        |
| LMMAOA (mg 100 g <sup>-1</sup> ) |  |                                     |                                     |                            |   |  |  |
| Maleic                           | –                                      | –                                   | –                                   | –                          | nd  | nd - 1.4                                       | –  |
| Malonic                          | –                                      | –                                   | –                                   | –                          | –   | –  | –  |
| Fumaric                          | –                                      | –                                   | –                                   | –                          | 3.1   | 0.6–5.7  | 2.0–4.0  |
| Tartaric                         | –                                      | –                                   | –                                   | –                          | nd  | nd - 20.9                                      | 58.0–66.0  |
| Formic                           | nd                                     | nd                                  | –                                   | 8.9                        | –   | –  | 8.0–11.0   |
| Citric                           | 27.7                                   | 21.9                                | –                                   | 12.4                       | 142.3   | 78.2–139.4                                     | 25.0–28.0  |
| Succinic                         | –                                      | –                                   | –                                   | nd                         | nd  | nd - 132.9                                     | 17.0–28.0  |
| Gluconic                         | 554.7                                  | 370.5                               | 12.0                                | 350                        | –   | –  | –  |
| Malic                            | –                                      | –                                   | –                                   | 25.7                       | 113.4   | 3.8–34.4                                       | nd   |
| Acetic                           | –                                      | –                                   | –                                   | –                          | –   | –  | –  |
| Oxalic                           | 5.4                                    | 6.3                                 | –                                   | nd                         | –   | –  | –  |
| Pyruvic                          | –                                      | –                                   | –                                   | –                          | –   | –  | nd   |
| Glutaric                         | nd                                     | 17.0                                | –                                   | –                          | –   | –  | –  |
| Propionic                        | 33.7                                   | 65.7                                | –                                   | –                          | –   | –  | –  |
| D-glucuronic                     | 1.5                                    | nd                                  | –                                   | –                          | –   | –  | –  |
| Analytical technique             | HPLC-DAD                               | HPLC-DAD                            | GC-MS                               | CZE-DAD                    | HPLC-DAD  | HPLC-DAD                                       | NMR  |
| Reference                        | Nozal et al. (2003)                    | Nozal et al. (2003)                 | Sanz et al. (2005)                  | Tezcan et al. (2011)       | Haroun, Konar, Poyrazoglu, Hospolar, and Artik (2012) | Haroun, Konar, et al. (2012)                   | Ohmenhaeuser, Monakhova, Kuballa, and Lachenmeier (2013) |

CZE – Capillary Zone Electrophoresis; DAD – Diode Array Detector; GC – Gas Chromatography; HPLC – High Performance Liquid Chromatography; MS – Mass Spectrometry; NMR – Nuclear Magnetic Resonance; nd – not detected; (–) – not investigated; nr – not reported.

<sup>a</sup> Data presented as range of values, not as mean.

(2008) reported a new bacterial flora composed of lactic acid bacteria (*Lactobacillus* and *Bifidobacterium*) in the stomach of *Apis mellifera*. Acetic acid is the main LMMAOA that can come from the fermentation process, however, none of the studies reported in Table 3 evaluated this compound.

The acidity of honey can be evaluated as free, lactonic, and total (free + lactonic) acidity (Navarrete et al., 2005). Free acidity is a parameter that can assist in assessing the deterioration level of honey, being its limit established as 50 meq kg<sup>-1</sup> (Codex Alimentarius Commission, 2001; European Commission, 2002a). The free acidity of the honey is influenced by the presence of LMMAOA in equilibrium with the respective lactones, esters and some inorganic ions, such as phosphate (de Almeida-Muradian et al., 2013; Navarrete et al., 2005), as well as the botanical source, amount of minerals, harvest time (Kadri et al., 2017), which can result in a considerable variability of free acidity values. As shown in Table 2, all the honeydew honeys analyzed presented free acidity values between 15.1 and 49.4 meq kg<sup>-1</sup>, indicating acceptable quality and low or null deterioration level.

The pH can limit and inhibit the growth of microorganisms, contributing to the stability of honey since a decrease in pH values may indicate honey fermentation (Cavia et al., 2007; Ouchemoukh, Louaileche, & Schweitzer, 2007; Terrab, Recamales, Hernanz, & Heredia, 2004). The pH of honeys depends on organic acids, inorganic ions, such as phosphate and chloride, minerals ionized and its botanical source (nectar and honeydew) (Pita-Calvo & Vázquez, 2017). For this reason, the pH values of honeydew honeys can vary, as shown in Table 2, presenting values normally between 3.8 and 5.3. Although pH is an important parameter in quality control of honeys, there is no legislation that establishes acceptable limits. Nayik and Nanda (2015) reported a positive correlation between pH and electrical conductivity, been both parameters dependent on the amount of ions in honey. This relationship was also observed in the studies presented in Table 2, since honeydew honeys with higher pH values also presented high electrical conductivity.

#### 2.4. Ash, electrical conductivity, and minerals

In honeys, the ash content is composed mainly of minerals such as K, Ca, Na, Mg. The high ash content may indicate an excess of inorganic

material from external contaminants (such as handling and equipment) as well as from environmental pollution (Anklam, 1998). For these reasons, it is considered an important quality parameter in honeys (Saxena, Gautam, & Sharma, 2010). Honeydew honeys naturally present high ash values, but generally lower than 1.2 g 100 g<sup>-1</sup> (w/w) (Ouchemoukh et al., 2007; Popek, 2002; Terrab, González, Díez, & Heredia, 2003b).

Currently, the determination of ash content has been replaced by the measurement of electrical conductivity, mainly because it is more sensitive to small changes in the mineral levels than the ash content (Alqarni, Owayss, Mahmoud, & Hannan, 2014; Saxena et al., 2010). Although it is strongly associated with ash and mineral contents, the electrical conductivity can also be influenced by the presence of ions, organic acids, and proteins (Fechner, Moresi, Díaz, Pellerano, & Vazquez, 2016). Honeydew honeys present higher electrical conductivity than blossom honeys, being a good parameter to differentiate both types of honeys (Can et al., 2015; Mateo & Bosch-Reig, 1998; Soria, González, de Lorenzo, Martínez-Castro, & Sanz, 2005). The minimum value of 0.8 mS cm<sup>-1</sup> for electrical conductivity is established for honeydew honeys (Codex Alimentarius Commission, 2001; European Commission, 2002a), where lower values may indicate frauds and mixtures with blossom honeys. As shown in Table 2, generally honeydew honeys present electrical conductivity above 0.8 mS cm<sup>-1</sup>. However, lower values were reported in commercial honeydew honeys and one sample obtained directly from beekeepers (Halouzka et al., 2016), indicating possible mixing with blossom honeys.

The minerals play several roles in the biological activities of the human body, such as in the regulation of metabolic functions (Vaquero, 2002) and as structural components of tissues (Özcan, 2004). Additionally, the minerals of honeydew honeys are highly bioaccessible (Pohl, Stecka, Greda, & Jamroz, 2012; Seraglio et al., 2017). The minerals present in honey directly represent the profile and amount of these elements present in the soil and plants where the bees collect the nectar, honeydew or pollen (Madejczyk & Baralkiewicz, 2008; Nayik & Nanda, 2015). Additionally, the botanical and geographical origin of honeydew honey specifically affects the content of each mineral. These facts may be associated with the great variability of these elements in the honeydew honeys presented in Table 4.

**Table 4**  
Mean concentration of minerals in honeydew honeys.

| Geographical origin / botanical origin  | Minerals (mg kg <sup>-1</sup> ) |       |         |     |            |      |      |    |   |    |    |    |           |         |        |
|---|---------------------------------|-------|---------|-----|------------|------|------|----|---|----|----|----|-----------|---------|--------|
|   | Al                              | As    | B       | Ba  | Ca         | Cd   | Co   | Cr | K | Mg | Mn | Mo | Na        | Ni      | P      |
| Italy / nr (n = 2)  | -                               | -     | -       | -   | 397.9      | -    | -    | -  | - | -  | -  | -  | -         | -       | -      |
| Czech Republic / nr (n = 9)   | 14.3                            | -     | 30.4    | -   | 34.9       | -    | -    | -  | - | -  | -  | -  | 62.4      | -       | -      |
| Poland / nr (n = 19) <sup>a</sup>   | 5.1–36.3                        | -     | 1.0–6.8 | -   | 3.3–48.2   | -    | -    | -  | - | -  | -  | -  | -         | 0.8     | -      |
| Italy / nr (n = 4)  | -                               | 0.01  | -       | 1.0 | 356.0      | 0.02 | 0.04 | -  | - | -  | -  | -  | 10.3–88.4 | 0.3–1.3 | -      |
| Poland / nr (n = 21)  | 25.7                            | -     | 4.1     | 0.1 | 21.4       | 0.04 | -    | -  | - | -  | -  | -  | -         | -       | -      |
| New Zealand / a (n = 1)   | 5.4                             | 0.07  | 2.2     | -   | 7.2        | 0.3  | -    | -  | - | -  | -  | -  | -         | -       | nd     |
| Poland (commercial samples) / Coniferous and deciduous trees (n = 4) <sup>a</sup> | -                               | -     | -       | -   | 32.7–100.8 | -    | -    | -  | - | -  | -  | -  | -         | -       | -      |
| Spain/ nr (n = 13)  | -                               | -     | -       | -   | 14.7       | -    | -    | -  | - | -  | -  | -  | -         | -       | -      |
| Indian / Pine (n = 10)  | -                               | -     | -       | -   | -          | -    | -    | -  | - | -  | -  | -  | 0.2       | -       | -      |
| Bulgaria / nr (n = 30)  | 1.3                             | < 0.3 | -       | -   | 103.0      | -    | -    | -  | - | -  | -  | -  | nd        | nd      | < 0.04 |
| Indian / Pine (n = 8)   | -                               | -     | -       | -   | 122.9      | -    | -    | -  | - | -  | -  | -  | -         | -       | -      |
| Brazil / <i>Mimosa scabrella</i> Benthham (n = 13)                                | -                               | -     | -       | -   | 43.3       | -    | -    | -  | - | -  | -  | -  | -         | -       | -      |

  

| Geographical origin / botanical origin  | Minerals (mg kg <sup>-1</sup> ) |          |                 |            |          |    |       |       |       |    |    |   |    |   |    |
|---|---------------------------------|----------|-----------------|------------|----------|----|-------|-------|-------|----|----|---|----|---|----|
|   | Cu                              | Fe       | K               | Mg         | Mn       | Mo | Na    | Ni    | P     | Sr | Sb | S | Th | U | Zn |
| Italy / nr (n = 2)  | 1.9                             | 8.6      | 2569.0          | 64.2       | 0.9      | -  | -     | -     | -     | -  | -  | - | -  | - | -  |
| Czech Republic / nr (n = 9)   | 0.4                             | -        | -               | 70.1       | 7.0      | -  | -     | -     | -     | -  | -  | - | -  | - | -  |
| Poland / nr (n = 19) <sup>a</sup>   | nd – 1.8                        | 3.8–16.1 | 1743.–9–365–9.3 | 1.4–6.6    | 1.7–7.3  | -  | -     | -     | -     | -  | -  | - | -  | - | -  |
| Italy / nr (n = 4)  | 4.4                             | 10.1     | 3440.0          | 139.0      | 1.7      | -  | 148.0 | 0.5   | -     | -  | -  | - | -  | - | -  |
| Poland / nr (n = 21)  | 1.1                             | -        | 2641.9          | 26.1       | 4.1      | -  | 28.4  | 1.0   | -     | -  | -  | - | -  | - | -  |
| New Zealand / a (n = 1)   | 0.1                             | 3.3      | 3640.0          | 86.3       | 0.4      | nd | 8.5   | 0.6   | 255.0 | -  | -  | - | -  | - | -  |
| Poland (commercial samples) / Coniferous and deciduous trees (n = 4) <sup>a</sup> | 0.6–1.4                         | 2.1–8.9  | -               | 42.1–138.5 | 2.1–13.8 | -  | -     | -     | -     | -  | -  | - | -  | - | -  |
| Spain/ nr (n = 13)  | 0.1                             | 0.5      | 239.7           | 21.3       | -        | -  | 3.9   | -     | 16.3  | -  | -  | - | -  | - | -  |
| Indian / Pine (n = 10)  | 0.1                             | 2.1      | -               | -          | 1.0      | -  | -     | -     | -     | -  | -  | - | -  | - | -  |
| Bulgaria / nr (n = 30)  | 0.5                             | 3.0      | 1331.0          | 83.0       | 12.0     | -  | 17.0  | < 0.9 | 123.0 | -  | -  | - | -  | - | -  |
| Indian / Pine (n = 8)   | -                               | -        | 752.2           | -          | -        | -  | 93.7  | -     | 69.2  | -  | -  | - | -  | - | -  |
| Brazil / <i>Mimosa scabrella</i> Benthham (n = 13)                                | -                               | -        | 5416.4          | 65.0       | nd       | -  | 7.2   | -     | -     | -  | -  | - | -  | - | -  |

| Geographical origin / botanical origin  | Minerals (mg kg <sup>-1</sup> ) |      |       |     |       |      |         |   |   |   |   |          |   |   | Analytical technique | Reference           |   |
|---|---------------------------------|------|-------|-----|-------|------|---------|---|---|---|---|----------|---|---|----------------------|---------------------|---|
|   | Pb                              | S    | Sb    | Sr  | Th    | U    | Zn      |   |   |   |   |          |   |   |                      |                     |   |
| Italy / nr (n = 2)  | -                               | -    | -     | -   | -     | -    | -       | - | - | - | - | -        | - | - | -                    | AAS and GF-AAS      | Conti, Stripekakis, Campanella, Cucina, and Tudino (2007) |
| Czech Republic / nr (n = 9)   | -                               | -    | -     | -   | -     | -    | -       | - | - | - | - | 2.5      | - | - | -                    | FAAS and ICP-OES    | Lachman et al. (2007)                                     |
| Poland / nr (n = 19) <sup>a</sup>   | -                               | -    | -     | -   | -     | -    | -       | - | - | - | - | nd – 9.9 | - | - | -                    | FAAS-AES and ICP-MS | Madejczyk and Baralkiewicz (2008)                         |
| Italy / nr (n = 4)  | 0.09                            | -    | 0.007 | 1.7 | 0.002 | 0.01 | 1.8     | - | - | - | - | 1.8      | - | - | -                    | ICP-OES             | Pisani et al. (2008)                                      |
| Poland / nr (n = 21)  | 0.2                             | -    | -     | -   | -     | -    | 5.6     | - | - | - | - | 5.6      | - | - | -                    | ICP-MS              | Chudzinska and Baralkiewicz (2010)                        |
| New Zealand / a (n = 1)   | nd                              | 46.1 | -     | -   | -     | -    | 2.4     | - | - | - | - | 2.4      | - | - | -                    | ICP-OES             | Vanhanen et al. (2011)                                    |
| Poland (commercial samples) / Coniferous and deciduous trees (n = 4) <sup>a</sup> | -                               | -    | -     | -   | -     | -    | 1.3–4.9 | - | - | - | - | 1.3–4.9  | - | - | -                    | FAAS                | Pohl et al. (2012)  |

(continued on next page)

Table 4 (continued)

| Geographical origin / botanical origin             | Minerals (mg kg <sup>-1</sup> ) |      |    |     |    |   |    |   |   |   | Analytical technique | Reference |                              |
|--|---------------------------------|------|----|-----|----|---|----|---|---|---|----------------------|-----------|------------------------------|
|  | Pb                              | S    | Sb | Sr  | Th | U | Zn |   |   |   |                      |           |                              |
| Spain/ nr (n = 13)                                 | -                               | -    | -  | -   | -  | - | -  | - | - | - | 0.2                  | AAS       | Escuredo et al. (2013)       |
| Indian / Pine (n = 10)                             | 0.2                             | -    | -  | -   | -  | - | -  | - | - | - | 1.0                  | AAS       | Nayik, Dar, and Nanda (2015) |
| Bulgaria / nr (n = 30)                             | < 0.3                           | 53.0 | -  | 0.3 | -  | - | -  | - | - | - | 1.2                  | ICP-AES   | Atanassova et al. (2016)     |
| Indian / Pine (n = 8)                              | -                               | -    | -  | -   | -  | - | -  | - | - | - | -                    | AAS       | Nayik and Nanda (2016)       |
| Brazil / <i>Mimosa scabrella</i> Benthham (n = 13) | -                               | -    | -  | -   | -  | - | -  | - | - | - | -                    | CE-DAD    | Bergamo et al. (2018a)       |

Al – Aluminium; As – Arsenic; B – Boron; Ba – Barium; Ca – Calcium; Cd – Cadmium; Co – Cobalt; Cr – Chromium; Cu – Copper; Fe – Iron; K – Potassium; Mg – Magnesium; Mn – Manganese; Mo – Molybdenum; Na – Sodium; Ni – Nickel; P – Phosphorus; Pb – Lead; S – Sulfur; Sb – Antimony; Sr – Strontium; Th – Thorium; U – Uranium; Zn – Zinc; AAS – Atomic Absorption Spectrometer; AES – Atomic Emission Spectrometry; CE – Capillary Electrophoresis; DAD – Diode Array Detector; FAAS – Flame Atomic Absorption Spectrometry; GF – Graphite furnace; ICP – Inductively Coupled Plasma; MS – Mass Spectrometry; OES – Optical Emission Spectroscopy; a – Produced by scale insects *Ultracoelostoma assimile* and *U. Brittoni* inhabiting *Nothofagus solandri* and *N. fusca* beech trees in New Zealand native forests; nd – not detected; (–) – not investigated; nr – not reported.

<sup>a</sup> Data presented as range of values, not as mean.

As shown in Table 4, K can be highlighted as the most abundant mineral, usually corresponding to > 70% of the minerals found in honeydew honeys. Other minerals often reported in honeydew honey are Ca, Mg, Na, and P, normally in concentrations higher than 1%. For the Ca reported in Italian honeydew honeys (unknown origin), Pisani, Protano, and Riccobono (2008) attribute the high concentration found to the presence of carbonate rocks in the sample collection area. This fact collaborates to emphasize that the environmental and soil conditions are factors that directly influence the mineral composition of the honeydew honeys.

Cu, Mn, Fe, and Zn were generally reported at concentrations below 1% of the total minerals investigated (Table 4). For the Cu, Madejczyk and Baralkiewicz (2008) found in their study that dark honeys tend to accumulate this mineral, being the content found in honeydew honeys generally superior to that found in blossom honeys. For Zn content, Madejczyk and Baralkiewicz (2008) related the highest value found in their study in honeydew honeys with the presence of this mineral in plants and soil in Poland, where the samples were collected.

The presence of other minerals as Al, As, B, Ba, Cd, Co, Cr, Mo, Ni, Pb, S, Sb, Sr, Th, and U has also been reported in honeydew honeys (Table 4). The presence of heavy metals (Pb and Cd) and toxic elements (Cr and As) in honeydew honey has been reported in some studies as originating from environmental contamination, mainly from soil, pharmacological treatment, and incorrect procedures in the processing and conservation of honey (Atanassova, Lazarova, & Yurukova, 2016; Vanhanen, Emmertz, & Savage, 2011). Also, Th and U were reported by Pisani et al. (2008) and their low concentrations in honeydew honeys were justified due to low availability in nature.

## 2.5. Color

The color of honey is a factor that defines your identity and has a great impact on the acceptance of a particular type of honey (González-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia, 2005; Terrab, Escudero, González-Miret, & Heredia, 2004). In honeys, the color can range from almost colorless to brown-dark (Codex Alimentarius Commission, 2001; European Commission, 2002a) and according to Belay, Solomon, Bultossa, Adgaba, and Melaku (2015), the color of honey is related to its flavor: honeys that have light colors have a mild flavor while that dark honeys have a more pronounced flavor and this feature may influence consumer choice and product price.

Dark tones have been related to larger amounts of total phenolic compounds and higher antioxidant activity (Bertoncelj et al., 2007; Kuš & Van Ruth, 2015; Özcan & Ölmez, 2014) and mineral levels (Bath & Singh, 1999; Bogdanov et al., 2004; González-Miret et al., 2005; McComb & Frew, 2013). Also, during the heating or storage for a prolonged period, honey undergoes transformations resulting from non-enzymatic browning reactions, such as the Maillard reaction (Oroian & Ropciuc, 2017). These reactions form compounds such as furfural and 5-HMF, implicating in the honey browning (Silva, Gauche, Gonzaga, Costa, & Fett, 2016).

Generally, honeydew honey is darker than blossom honey mainly due to mineral and phenolic contents and other color compounds (Can et al., 2015; Oroian & Ropciuc, 2017; Tezcan et al., 2011). Additionally, some authors have reported that color associated with other physico-chemical parameters and chemometric analysis, can be used for honey (honeydew or blossom) differentiation (Bertoncelj et al., 2007; González-Miret et al., 2005; Manzanares et al., 2011; Terrab, González, Díez, & Heredia, 2003a).

## 2.6. Proteins and amino acids

### 2.6.1. Proteins

In honeys, a large part of the proteins is from the bees, been especially enzymes and Major Royal Jelly Protein (MRJP) (Girolamo, D'Amato, & Righetti, 2012; Sakač & Sak-Bosnar, 2012). In pine

honeydew honeys from Turkey and Spanish honeydew honeys (unknown origin), the protein content was similar, presenting mean values of 1.16 and 1.00 g 100 g<sup>-1</sup>, respectively (Escuredo et al., 2013; Nisbet, Guler, Ciftci, & Yarim, 2009). In *Acacia mangium*, *Hevea brasiliensis*, *Elaeis oleifera*, and forest honeydew honeys from Malaysia, the protein content ranged from 0.43 to 1.02 g 100 g<sup>-1</sup> (Chua & Adnan, 2014). Low protein content was reported in bracinga (*Mimosa scabrella* Benth) honeydew honeys from Brazil and *Abies alba* honeydew honeys from Slovakia (mean values of 0.04 g 100 g<sup>-1</sup>) (Azevedo, Valentim-Neto, et al., 2017; Horniackova, Bucekova, Valachova, & Majtan, 2017), besides Lebanon honeydew honeys (unknown origin) (0.08 to 0.14 g 100 g<sup>-1</sup>) (Jaafar et al., 2017) and Croatian honeydew honeys (unknown origin) (0.03 to 0.10 g 100 g<sup>-1</sup>) (Flanjak, Strelec, Kenjeric, & Primorac, 2016).

The low concentration of proteins in honeys and the difficulty of its extraction and characterization by conventional methods contribute to that few studies are found in the literature, especially related to honeydew honeys (Azevedo, Valentim-Neto, et al., 2017; Chua, Lee, & Chan, 2013). The presence of seven peaks comprising proteins with a molecular weight between 13.1 and 94.0 kDa was observed in honeydew honeys (unknown origin) and blossom honeys from Spain using fast protein liquid chromatography. According to the discriminant analysis of the area of four peaks, the differentiation between both types of honey (honeydew and blossom) was observed (Iglesias, Martín-Álvarez, Polo, de Lorenzo, & Pueyo, 2006). In pine honeydew honey, different blossom honeys and adulterated honey (saccharose syrup) from Turkey, three protein bands (94 kDa, 87 kDa and 84 kDa) were determined in all honeys. However, the intensity of the protein bands and the total protein content were lower in the adulterated honey, being able to assist in the discrimination of pure honeys and adulterated honeys (Nisbet et al., 2009). The use of proteins was also explored to differentiate filtered and unfiltered honeys. Two dominant protein bands of 40 and 65 kDa from sucrose enzyme fraction were found in unfiltered forest honeydew honeys and blossom honeys from different geographical origins, whereas in filtered honeys the protein band of 65 kDa was almost vanished (Beckmann, Beckh, Luellmann, & Speer, 2011). The MRJP-1 was indicated as the most abundant protein in Revamil honey, manuka honey and Slovakia honeydew honey (unknown origin). However, honey proteins with a molecular weight above 10 kDa, including MRJP-1, were indicated as not responsible for the antimicrobial activity of honey (Bucekova & Majtan, 2016). The effect of gamma radiation was investigated in fir (*Abies alba*) honeydew honey from Slovakia. The antimicrobial and antibiofilm activity and defensin-1 of the honey were not affected by the dose of 10 kGy. However, doses above 10 kGy significantly reduced the concentration of the peptide defensin-1 (Horniackova et al., 2017). Two-dimensional electrophoresis and principal component analysis were used to discriminate blossom and honeydew honeys from bracinga (*Mimosa scabrella* Benth) botanical species from Brazil. In bracinga honeydew honey, total soluble protein was higher (0.042 g 100 g<sup>-1</sup>) than in bracinga blossom honey (0.016 g 100 g<sup>-1</sup>). Besides that, were detected 160 protein spots in the honeydew honey and 84 spots in the blossom honey, suggesting that the proteome profile is useful for discrimination between these kinds of honeys (Azevedo, Valentim-Neto, et al., 2017). Considering the studies mentioned above, it is observed that advances must be made about the evaluation of the protein profile and, mainly, to the identification of the main proteins of honeydew honeys.

### 2.6.2. Diastase

Diastase is a group of enzymes, that include  $\alpha$ - and  $\beta$ -amylase, naturally present in honeys. Diastase activity can be expressed in Schade, Göthe or diastase units and it is a parameter commonly explored as indicator of honey freshness (Fechner et al., 2016; Flores et al., 2015; Yücel & Sultanoglu, 2013). Honeys in general should present diastase activity of at least 8 Schade units, minimum value accepted by regulatory organizations (Codex Alimentarius Commission,

2001; European Commission, 2002a). Therefore, honeys with diastase activity below to the permissible limits, suggest that long storage periods and/or heating during its processing or storage may have occurred (Fechner et al., 2016; Flores et al., 2015; Yücel & Sultanoglu, 2013).

In honeydew honeys, a wide range of values of diastase activity is reported (Table 2). Honeydew honeys rarely present values lower than 8 Schade units, indicating acceptable freshness. In one commercial honeydew honey (unknown origin) from European Union, Halouzka et al. (2016) reported diastase activity of 4.7 Göthe units. This low value combined with the high 5-HMF content (42.0 mg kg<sup>-1</sup>), suggest that this honey was possibly storage for long periods or heating during its processing or storage, which affected its quality. Values of diastase activity ranging from 9.0 to 30.0 diastase units are frequently reported for honeydew honeys, but values higher than 30.0 diastase units were also found, such as in honeydew honeys (unknown origin) from Spain (Visquert, Vargas, & Escriche, 2014) and Serbia (Matović et al., 2018) (Table 2). The wide range of diastase values suggests that different factors such as botanic origin, processing and storage conditions, secretion of bees and plant-sucking insects, and honey composition can influence the diastase activity of honeydew honeys (Escuredo, Seijo, & Fernández-González, 2011; Nalda, Yagüe, Calva, & Gómez, 2005; Oddo, Piazza, & Pulcini, 1999; Vorlová & Piidal, 2002). However, there is still a lack of information that clarifies more accurately what factors and how much these affect the diastase activity of honeydew honeys.

### 2.6.3. Amino acids

It is estimated that around 40 to 65% of the total nitrogen in honeys belongs to proteins and the remainder to free amino acids (Girolamo et al., 2012). In Turkish honeydew honey (unknown origin), 6454.5 mg kg<sup>-1</sup> of total free amino acids was reported (Kivrak, 2015). Intermediate concentrations were found in Italian honeydew honeys (unknown origin), ranging from 810.0 to 1243.0 mg kg<sup>-1</sup> of total free amino acids (Carratù, Ciarrocchi, Mosca, & Sanzini, 2011) and in Polish and Slovakian honeydew honeys from forest origin (773.9 and 749.2 mg kg<sup>-1</sup> of total free amino acids, respectively) (Kowalski, Kopuncová, Ciesarová, & Kukurová, 2017). Low content of total free amino acids was reported in Polish honeydew honey (unknown origin), with content of 398.4 mg kg<sup>-1</sup> (Janiszewska, Aniolowska, & Nowakowski, 2012) and in *Acacia mangium*, *Hevea brasiliensis*, *Elaeis oleifera*, and forest honeydew honeys from Malaysia, ranging from 5.9 to 23.3 mg kg<sup>-1</sup> (Chua & Adnan, 2014).

Proline is usually the major amino acid present in honeydew honeys corresponding to up to 90% of the total free amino acid content (Table 5). Proline is originated mainly from the bee (Azevedo, Seraglio, et al., 2017; Crailsheim & Leonhard, 1997; Von Der Ohe, 1994) and has been suggested as a ripeness indicator of honey and, in some cases, sugar adulteration. It is proposed that pure honeys must have a minimum value of 180 mg kg<sup>-1</sup> of proline, however, considerable variation in proline content occurs according to the type of honey (Bogdanov et al., 1999) and low values can be found even in ripeness and non-adulterated honeys (Carratù et al., 2011). According to Table 5, mean concentration of proline in honeydew honeys ranged from not detected to 9600.0 mg kg<sup>-1</sup>. Only in *Acacia mangium*, *Hevea brasiliensis*, *Elaeis oleifera*, and forest honeydew honeys from Malaysia were reported mean content of proline (Chua & Adnan, 2014) lower than 180 mg kg<sup>-1</sup>. Even than low proline content can be found in ripeness or non-adulterated honeys, the low diastase activity (< 2 diastase units) of these honeydew honeys reinforce the possibility that their quality has been affected. Therefore, the proline content can contribute to the assessment of ripeness and purity of honeydew honeys.

Although proline is usually the major amino acid found in honeydew honeys, some studies have found other amino acids in highest concentration (Table 5). In Turkish honeydew honeys (unknown origin), phenylalanine was the amino acid found in highest concentration (Kivrak, 2015), while in *Mimosa scabrella* Benth honeydew honeys from Brazil, glutamic acid was the main amino acid



(Azevedo, Seraglio, et al., 2017). Also, in *Hevea brasiliensis* honeydew honey from Malaysia, proline was not detected (Chua & Adnan, 2014). These results suggest the influence especially of the botanical and/or geographical origin, as well as processing and manipulation conditions in the profile and amount of free amino acids in the honeydew honeys.

As shown in Table 5, besides proline, at least 29 amino acids have been reported in honeydew honeys: aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine,  $\alpha$  and  $\beta$ -alanine,  $\alpha$  and  $\beta$ -aminobutyric acid, tyrosine, valine, tryptophan, phenylalanine, isoleucine, leucine, ornithine, lysine, cysteine, methionine, hydroxyproline,  $\alpha$ -aminoadipic acid,  $\gamma$ -aminobutyric acid, ethylamine, homoserine, and taurine. The profile and amount of free amino acids vary intensely according to the geographical and botanical origin of the honeydew honey. However, can be highlighted as the main amino acids present in honeydew honeys, besides proline, the amino acids: aspartic acid, glutamic acid, asparagine, glutamine, phenylalanine,  $\alpha$ -alanine, arginine, tyrosine, leucine, and  $\gamma$ -aminobutyric acid, usually ranging from not detected at values close to 200.0 mg kg<sup>-1</sup> (Table 5).

Besides the characterization of the amino acids and possible indication of adulteration or immaturity of honeys, the profile and amount of free amino acids has been a promising indicator to the differentiation between honeydew honeys and blossom honeys (Chua & Adnan, 2014; Iglesias, Martín-Álvarez, Polo, de Lorenzo, González, et al., 2006; Iglesias, de Lorenzo, Polo, Martín-Álvarez, & Pueyo, 2004; Janiszewska et al., 2012; Kivrak, 2015; Kowalski et al., 2017; Silici & Karaman, 2014), as well as between honeydew honeys from different geographical origins (Azevedo, Seraglio, et al., 2017). However, due to their great variability, it has not yet been possible to determine any amino acid or a certain amount that can be considered as a common characteristic of honeydew honeys.

## 2.7. Vitamins

In honeydew honeys, the vitamins generally found are the vitamins of B group and the vitamin C. León-Ruiz, Vera, González-Porto, and Andrés (2011) determined vitamin C in blossom honeys and honeydew honeys (unknown origin) from Spain. Honeydew honeys presented a mean value of 7.70 mg kg<sup>-1</sup> of vitamin C, very low value compared to blossom honey of thyme, which reached a mean value of 571.5 mg kg<sup>-1</sup>. Some factors that may contribute to the loss of vitamin C in honeys are the commercial filtration process and its oxidation by the hydrogen peroxide, which is naturally present in honeys (Ciulu et al., 2011).

The simultaneous determination of the water-soluble vitamins in blossom honeys and honeydew honeys (unknown origin) was performed by León-Ruiz, Vera, González-Porto, and Andrés (2013). In honeydew honeys, the vitamins B1, B2, B3N, B3H, B5, B6, and C were determined. The vitamin B1 was quantified in high concentration in honeydew honeys (mean value of 4.00 mg kg<sup>-1</sup>), while the vitamin B2 presented the highest concentration in this honey type (mean value of 0.16 mg kg<sup>-1</sup>) compared to the blossom honeys.

Kaygusuz et al. (2016) evaluated vitamin B2 in honeydew honeys of *Pinus L.* and *Quercus robur L.* from Turkey. The concentration of vitamin B2 in honeydew honey of *Pinus L.* ranged from 0.12 to 0.27 mg kg<sup>-1</sup> and in honeydew honey of *Quercus robur L.* ranged from 0 to 0.21 mg kg<sup>-1</sup>. These concentrations were lower than those found by Tuberoso et al. (2012) in honeydew honey of *Castanea sativa Mill.* from Italy and Croatia, which the mean value was 6.10 mg kg<sup>-1</sup>.

## 2.8. Phenolic compounds

Phenolic compounds in different types of honey has been evaluated both to study biological activities (Can et al., 2015; Escriche, Kadar, Juan-Borrás, & Domenech, 2014) and to use this profile as a marker of origin or authenticity (Campono et al., 2014; Seraglio et al., 2016).

Table 6 presents the studies regarding phenolic compounds identification in honeydew honeys. Honeydew honeys have been reported by some authors as sources of higher amounts of phenolic compounds than blossom honeys, ranging from approximately 5 to 1500 mg gallic acid equivalent kg<sup>-1</sup> (Can et al., 2015; Halouzka et al., 2016; Pichichero, Canuti, & Canini, 2009; Socha et al., 2011; Tuberoso, Jerković, Bifulco, & Marijanović, 2011).

As shown in Table 6, ferulic, caffeic, *p*-coumaric, syringic, and gallic acids commonly show up in honeydew honeys from different sources. Some phenolic compounds were reported only in one honeydew honey, like myricetin and genistein in *Thymus vulgare* (Pichichero et al., 2009), kynurenic acid in *Salix* spp. (Tuberoso et al., 2011), syringaldehyde, coniferaldehyde, pinobanksin, hesperidin in *Mimosa scabrella* Benth (Seraglio et al., 2016). Other compounds seems to be specific to a region, like gentisic and isoferulic acids and taxifolin from the Czech Republic (Halouzka et al., 2016), ellagic acid, acacetin, catechin and epicatechin from Turkey (Can et al., 2015; Haroun, Poyrazoglu, Konar, & Arti, 2012; Silici, Sarioglu, & Karaman, 2013).

The main focus of studies on phenolic compounds in honeydew honey has been the differentiation of these from blossom honeys and these studies have reported larger amounts of phenolic compounds in honeydew honeys, which have been important for the stimulation to studies in biological systems of these honeys (Jaromír Lachman et al., 2010).

## 2.9. Volatile compounds

In Table 7, the main volatile compounds reported in honeydew honeys are summarized. The compounds 3-methyl-3-buten-1-ol, ethanol, 2-methyl-1-butanol, 3-hydroxy-2-butanone, and acetic acid were reported in relative high proportions in Spanish honeydew honeys from forest origin (Escriche, Visquert, Juan-Borrás, & Fito, 2009), while especially the compounds 2,3-butanediol, 1-hydroxy-2-propanone, and 3-hydroxy-2-butanone were abundant in Spanish honeydew honeys from unknown origin (Soria et al., 2005; Soria, González, de Lorenzo, Martínez-Castro, & Sanz, 2004). Besides the 2,3-butanediol, other 19 compounds were considerate exclusive to Spanish honeydew honeys from forest origin, including 2-methyl-propanoic acid, 3-hexen-1-ol, and 3-methyl-2-butanol (Escriche et al., 2009). High levels of acetic acid were also found in honeydew honeys (unknown origin) from Spain (Soria et al., 2005) and from Brazil, which was proposed as indicative of the presence of honeydew in honeys (Campos, Nappi, Raslan, & Augusti, 2000). In honeydew honeys (unknown origin) from Slovakia, the presence of 2,3-butanediol, 3-hydroxy-2-butanone, and acetic acid was observed and the compounds methyl ester of 2,6-dihydroxybenzoic acid, 4-oxapentanoic acid, 2-oxooctanoic acid, and allyl ester of acetic acid were considered markers of Slovakian honeydew honeys (Jánošková, Vyviurska, & Špánik, 2014). In honeydew honeys (forest origin) from different geographical locations, a characteristic pattern was observed, with an abundance of terpenes, such as *cis*-linalool oxide and limonene. Also, the compounds 3-hydroxy-2-butanone, *trans*-2-pentenal, and 3-methylbutanol were proposed as characteristics of honeydew honeys (Gerhardt, Birkenmeier, Schwolow, Rohn, & Weller, 2018). Therefore, the compounds 2,3-butanediol, 3-hydroxy-2-butanone, and acetic acid appear to be commonly found in higher concentrations in honeydew honeys.

Among the Croatian honeys evaluated by Lušić, Koprivnjak, Čurić, Sabatini, and Conte (2007), only in fir (*Albies alba*) honeydew honeys were found the compounds acetonitrile, methyl-2-buten-1-ol, *n*-hexanol, 3-hexanol, 1-propyne, 2-furanmethanol, 5-methyl-2 (5H)-furanone, 4-methylphenol, hexadecanoic acid, and methylheptanoate, featured as characteristics of *Albies alba* honeydew honeys. To the best of our knowledge, acetonitrile was reported for the first time in honeydew honeys in this study, not being considered a natural product. In Polish fir (*Albies alba*) honeydew honeys, the compounds coniferyl alcohol isomers, methyl syringate, benzaldehyde, 4-hydroxy-3,5,6-

**Table 5**  
Mean concentration of free amino acids in honeydew honeys.

| Amino acid | Geographical origin / botanical origin                 |                                 |                                |   |                     |                       |                       |                           |                                   |                       | Analytical technique<br>Reference |
|------------|--|---------------------------------|--------------------------------|---|---------------------|-----------------------|-----------------------|---------------------------|-----------------------------------|-----------------------|-----------------------------------|
|            | Spain / nr (n = 5)                                     | Spain / Ilex (n = 10)           | Spain / Oak (n = 10)           | Spain / nr (n = 5)                                | Spain / nr (n = 5)  | Turkey / nr (n = 6)   | Italy / nr (n = 3)    | Poland / nr (n = 3)       | Malaysia / Acacia mangium (n = 1) |                       |                                   |
| Ala        | mg kg <sup>-1</sup> DM                                 | %*                              | %*                             | mg kg <sup>-1</sup> DM                            | mg kg <sup>-1</sup> | mg kg <sup>-1</sup>   | mg kg <sup>-1</sup>   | mg kg <sup>-1</sup>       | mg kg <sup>-1</sup>               | mg kg <sup>-1</sup>   |                                   |
| alle       | -  | 2.7                             | 2.3                            | -   | -                   | nr                    | 17.0                  | 9.2                       | nd                                | -                     |                                   |
| Arg        | 86.8   | 1.6                             | 2.5                            | 44.9  | 73.6                | nr                    | 33.3                  | 1.9                       | nd                                | -                     |                                   |
| Asn        | 239.4  | 9.4                             | 6.2                            | 137.8   | 216.3               | nr                    | 24.7                  | 3.2                       | -                                 | -                     |                                   |
| Asp        | 267.0  | 2.6                             | 4.1                            | 144.0   | 132.2               | nr                    | 68.7                  | 12.2                      | nd                                | -                     |                                   |
| C-C        | -  | -                               | -                              | -   | -                   | nr                    | -                     | -                         | -                                 | -                     |                                   |
| Cth        | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| Cys        | -  | -                               | -                              | -   | -                   | nr                    | -                     | -                         | 0.04                              | -                     |                                   |
| EA         | -  | -                               | -                              | -   | -                   | -                     | -                     | 2.1                       | -                                 | -                     |                                   |
| Gln        | 116.3  | 5.1                             | 3.4                            | 88.2  | 162.2               | nr                    | 38.7                  | 12.5                      | -                                 | -                     |                                   |
| Glu        | 389.7  | 4.1                             | 4.7                            | 215.0   | 276.0               | nr                    | 194.7                 | 11.4                      | 0.2                               | -                     |                                   |
| Gly        | 22.2   | 0.6                             | 0.3                            | 22.0  | 21.0                | nr                    | 7.0                   | 2.3                       | nd                                | -                     |                                   |
| Gpr        | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| His        | 12.5   | 0.8                             | 3.2                            | 3.4   | nd                  | nr                    | nd                    | 0.9                       | 4.1                               | -                     |                                   |
| Hly        | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| Hser       | -  | 0.3                             | 0.2                            | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| Hyp        | -  | -                               | -                              | -   | -                   | nr                    | -                     | -                         | -                                 | -                     |                                   |
| Ile        | 20.4   | 0.4                             | 0.8                            | 20.5  | 22.5                | nr                    | 7.0                   | 7.5                       | 0.3                               | -                     |                                   |
| Leu        | 16.2   | 0.2                             | 0.4                            | 19.4  | 19.9                | nr                    | 10.0                  | 9.3                       | 1.2                               | -                     |                                   |
| Leu/Ile    | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| Lys        | 23.4   | 0.9                             | 1.5                            | 26.3  | 31.1                | nr                    | 2.7                   | 2.9                       | nd                                | -                     |                                   |
| Met        | nd   | 0.8                             | 1.1                            | nd  | nd                  | nr                    | nd                    | 3.3                       | 0.3                               | -                     |                                   |
| Orn        | nd   | 0.2                             | 1.9                            | 24.4  | 26.2                | -                     | 2.7                   | 0.6                       | -                                 | -                     |                                   |
| Phe        | 112.2  | 2.7                             | 6.4                            | 66.5  | 84.2                | nr                    | 17.3                  | 5.8                       | nd                                | -                     |                                   |
| Php        | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| Pro        | 904.6  | 49.5                            | 37.6                           | 844.1   | 726.8               | nr                    | 449.7                 | 263.4                     | 16.3                              | -                     |                                   |
| Sar        | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| Ser        | 71.2   | 1.3                             | 1.4                            | 42.6  | 53.7                | nr                    | 15.7                  | 9.6                       | nd                                | -                     |                                   |
| Tau        | -  | 4.3                             | 3.1                            | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| Thr        | 34.8   | 0.5                             | 0.9                            | 27.5  | 34.5                | nr                    | 43.0                  | 4.0                       | 0.7                               | -                     |                                   |
| Tpr        | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| Trp        | 11.5   | 1.3                             | 4.4                            | 32.8  | 37.5                | nr                    | nd                    | -                         | -                                 | -                     |                                   |
| Tyr        | 55.0   | 1.6                             | 1.1                            | 46.6  | 58.2                | nr                    | 16.3                  | 9.4                       | 0.2                               | -                     |                                   |
| Val        | 34.2   | 0.5                             | 0.5                            | 23.9  | 26.9                | nr                    | 10.3                  | 9.4                       | nd                                | -                     |                                   |
| α-Aaa      | -  | 0.1                             | 0.2                            | -   | -                   | -                     | -                     | 1.0                       | -                                 | -                     |                                   |
| α-Aba      | nd   | -                               | -                              | -   | -                   | -                     | -                     | 6.5                       | -                                 | -                     |                                   |
| α-Ala      | 85.4   | -                               | -                              | 25.2  | 53.3                | -                     | -                     | -                         | -                                 | -                     |                                   |
| α-Apa      | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| β-Aba      | -  | -                               | -                              | -   | -                   | -                     | -                     | 2.6                       | -                                 | -                     |                                   |
| β-Aib      | -  | -                               | -                              | -   | -                   | -                     | -                     | -                         | -                                 | -                     |                                   |
| β-Ala      | 2.7  | -                               | -                              | 55.7  | 18.0                | -                     | -                     | 5.1                       | -                                 | -                     |                                   |
| γ-Gaba     | 56.1   | 2.2                             | 7.2                            | 65.7  | 88.6                | -                     | 19.7                  | 2.6                       | -                                 | -                     |                                   |
| LC-APCI-MS | Iglesias et al. (2004)                                 | HPLC-FLD; CM                    | HPLC-FLD; CM                   | HPLC-FLD; CM                                      | HPLC-FLD; CM        | LC-APCI-MS            | HPLC-FLD              | AAA                       | HPLC-DAD                          | HPLC-DAD              |                                   |
| Reference  | González-Paramás, García-Villanova, and Sánchez (2006) | González-Paramás, et al. (2006) | González-Paramás et al. (2006) | Álvarez, Polo, de Lorenzo, González et al. (2006) | Pérez et al. (2007) | Senyuva et al. (2009) | Carratù et al. (2011) | Janiszewska et al. (2012) | Chua and Adnan (2014)             | Chua and Adnan (2014) |                                   |

(continued on next page)

Table 5 (continued)

| Amino acid | Geographical origin / botanical origin       |                           |   |                                     |                             |   |                             |                             |   |  |
|------------|--|---------------------------|---|-------------------------------------|-----------------------------|---|-----------------------------|-----------------------------|---|--|
|            | Malaysia / <i>Hevea brasiliensis</i> (n = 1) | Malaysia / Forest (n = 1) | Malaysia / <i>Elaeis oleifera</i> (n = 1) | Turkey / <i>Pinus</i> spp. (n = 12) | Turkey / nr (n = 7)         | Brazil / <i>Mimosa scabrella</i> Benthiam (n = 21) <sup>a</sup> | Slovakia / Forest (n = 3)   | Poland / Forest (n = 3)     | Greece / Pine and fir (n = 70) <sup>a</sup> |  |
| Ala        | 0.2  | mg kg <sup>-1</sup><br>nd | mg kg <sup>-1</sup><br>nd                 | mg kg <sup>-1</sup><br>40.5         | mg kg <sup>-1</sup><br>26.2 | mg kg <sup>-1</sup><br>nd                                       | mg kg <sup>-1</sup><br>14.2 | mg kg <sup>-1</sup><br>11.6 | mg kg <sup>-1</sup><br>-                    |  |
| alle       | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| Arg        | nd   | nd                        | nd  | 101.3                               | 5.5                         | -   | 5.5                         | 1.9                         | -   |  |
| Asn        | -  | -                         | -   | -                                   | 4.9                         | 100.0–520.0   | 6.0                         | 4.7                         | -   |  |
| Asp        | nd   | nd                        | nd  | 39.8                                | nd                          | 130.0–380.0   | 15.3                        | 10.2                        | -   |  |
| C-C        | -  | -                         | -   | -                                   | nd                          | nd  | -                           | -                           | -   |  |
| Cth        | -  | -                         | -   | -                                   | nd                          | nd  | -                           | -                           | -   |  |
| Cys        | nd   | 0.1                       | 0.1                                       | 19.1                                | nd                          | -   | -                           | -                           | -   |  |
| EA         | -  | -                         | -   | -                                   | -                           | -   | -                           | -                           | -   |  |
| Gln        | -  | -                         | -   | 20.9                                | 24.7                        | nd  | 271.5                       | 271.9                       | -   |  |
| Glu        | 0.06   | nd                        | nd  | 42.4                                | 85.1                        | 200.0–1060.0  | 36.3                        | 24.1                        | -   |  |
| Gly        | nd   | nd                        | nd  | 17.5                                | nd                          | nd  | 3.5                         | 3.4                         | -   |  |
| Gpr        | -  | -                         | -   | -                                   | nd                          | nd  | 2.7                         | nd                          | -   |  |
| His        | 4.3  | nd                        | 0.1                                       | 48.8                                | nd                          | nd  | -                           | -                           | -   |  |
| Hly        | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| Hser       | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| Hyp        | -  | -                         | -   | 10.2                                | 8.9                         | nd  | 8.3                         | 7.7                         | -   |  |
| Ile        | 0.7  | 0.05                      | nd  | -                                   | 597.4                       | nd  | 5.9                         | 4.5                         | -   |  |
| Leu        | nd   | nd                        | nd  | -                                   | 877.8                       | nd  | 7.4                         | 4.9                         | -   |  |
| Leu/Ile    | -  | -                         | -   | 10.9                                | -                           | -   | -                           | -                           | -   |  |
| Lys        | 0.2  | nd                        | nd  | 166.8                               | 38.7                        | nd  | 9.9                         | 6.6                         | -   |  |
| Met        | 0.3  | nd                        | 0.1                                       | 18.3                                | 05.0                        | nd  | 0.27                        | 0.08                        | -   |  |
| Orn        | -  | -                         | -   | -                                   | -                           | nd  | 0.9                         | 0.26                        | -   |  |
| Phe        | nd   | nd                        | nd  | 9.3                                 | 3002.0                      | nd  | 15.2                        | 13.1                        | nd – 5800.0                                 |  |
| Php        | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| Pro        | nd   | 14.9                      | 12.2                                      | 207.3                               | 943.6                       | 422.0–920.0   | 316.8                       | 389.6                       | 400.0–9600.0                                |  |
| Sar        | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| Ser        | nd   | nd                        | nd  | 4.7                                 | nd                          | 80.0–270.0  | 8.3                         | 5.8                         | -   |  |
| Tau        | -  | -                         | -   | -                                   | -                           | -   | -                           | -                           | -   |  |
| Thr        | nd   | 1.5                       | 2.3                                       | 14.6                                | 3.1                         | nd  | 7.8                         | 4.7                         | -   |  |
| Tpr        | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| Trp        | -  | -                         | -   | nd                                  | 53.4                        | nd  | 1.3                         | 0.81                        | -   |  |
| Tyr        | 0.1  | nd                        | nd  | 1.5                                 | 664.2                       | nd  | -                           | -                           | -   |  |
| Val        | nd   | nd                        | nd  | 25.4                                | 114.1                       | nd  | 12.0                        | 7.1                         | -   |  |
| α-Aaa      | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| α-Aba      | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| α-Ala      | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| α-Apa      | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| β-Aba      | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| β-Alb      | -  | -                         | -   | -                                   | -                           | nd  | -                           | -                           | -   |  |
| β-Ala      | -  | -                         | -   | -                                   | -                           | -   | -                           | -                           | -   |  |
| γ-Gaba     | -  | -                         | -   | -                                   | -                           | -   | -                           | -                           | -   |  |

(continued on next page)

Table 5 (continued)

| Geographical origin / botanical origin                          |   | Analytical technique | Reference                          |
|---|---|----------------------|------------------------------------|
| Malaysia / Hevea <i>brasiliensis</i> (n = 1)                    | Malaysia / Forest (n = 1)                                       | HP/LC-DAD            | Chua and Adnan (2014)              |
| Malaysia / <i>Elaeis oleifera</i> (n = 1)                       | Malaysia / Forest (n = 1)                                       | HP/LC-DAD            | Chua and Adnan (2014)              |
| Turkey / <i>Pinus</i> spp. (n = 12)                             | Turkey / <i>Pinus</i> spp. (n = 12)                             | LC-APCI-MS           | Silici and Karaman (2014)          |
| Turkey / nr (n = 7)   | Turkey / nr (n = 7)   | UPLC-ESI-MS/MS       | Kivrak (2015)                      |
| Brazil / <i>Mimosa scabrella</i> Benthiam (n = 21) <sup>a</sup> | Brazil / <i>Mimosa scabrella</i> Benthiam (n = 21) <sup>a</sup> | GC-MS                | Azevedo, Seraglio, et al. (2017)   |
| Slovakia / Forest (n = 3)                                       | Slovakia / Forest (n = 3)                                       | LC-MS/MS             | Kowalski et al. (2017)             |
| Poland / Forest (n = 3)   | Poland / Forest (n = 3)   | LC-MS/MS             | Kowalski et al. (2017)             |
| Greece / Pine and fir (n = 70) <sup>a</sup>                     | Greece / Pine and fir (n = 70) <sup>a</sup>                     | NMR                  | Karabagias, Vlastou, et al. (2018) |

Ala – alanine; alle – allo-isoleucine; Arg – arginine; Asn – asparagine; Asp – aspartic acid; C-C – cystine; Cth – cystathionine; Cys – cysteine; EA – ethylamine; Glu – glutamine; Glu – glutamic acid; Gly – glycine; Gpr – glycy-l-proline; His – histidine; Hly – hydroxylysine; Hser – homoserine; Hyp – hydroxyproline; Ile – isoleucine; Leu/Ileu – leucine/isoleucine; Lys – lysine; Met – methionine; Orn – ornithine; Phe – phenylalanine; Phip – proline-hydroxyproline; Pro – proline; Sar – sarcosine; Ser – serine; Tau – taurine; Thr – threonine; Trp – tryptophan; Tyr – tyrosine; Val – valine; α-Aaa – α-amino adipic acid; α-Aba – α-aminobutyric acid; α-Ala – α-alanine; α-Apa – α-aminopimelic acid; β-Aba – β-aminobutyric acid; β-Ala – β-alanine; γ-Gaba – γ-aminobutyric acid; AAA – Automatic Amino Acid Analyzer; APCI – Atmospheric Pressure Chemical Ionization; CM – Colorimetric Method; DAD – Diode Array Detector; ESI – Electrospray Ionization; FLD – Fluorescence Detector; GC – Gas Chromatography; HPLC – High Performance Liquid Chromatography; LC – Liquid Chromatography; MS – Mass Spectrometry; MS/MS – Mass Spectrometry/Mass Spectrometry; NMR – Nuclear Magnetic Resonance; UPLC – Ultra Performance Liquid Chromatography; DM – dry matter; nd – not detected; (–) – not investigated; nr – not reported; %\* – percentage of the compound in relation to the total of free amino acids investigated.

<sup>a</sup> Data presented as range of values, not as mean.

trimethyl-4-(3-oxobut-1-enyl)cyclohex-2-en-1-one, linalool derivatives, and borneol were predominant, and the protocatechuic acid was suggested as a potential marker of this honey (Kuš, Jerković, Marijanović, & Tuberoso, 2017). *Albis alba* honeydew honeys mentioned above showed the distinct profile of volatile compounds, which suggest possible influence especially of the geographical location.

In honeydew honeys (unknown origin) from Turkey, Senyuva et al. (2009) propose the nonanal compound in combination with α,α-dimethyl phenyl acetate as markers. In *Salix* spp. honeydew honey from Croatia, a high percentage of benzoic acid, phenylacetic acid, 2-hydroxybenzoic acid, and 4-hydroxyphenylacetic acid, and a low percentage of 4-hydroxybenzoic acid, 4-hydroxyphenylethanol, and 4-methoxybenzoic acid, as well as the presence of methyl salicylate were highlighted as biomarkers of this type of honey, which are probably derived from *Salix* spp. tree (Jerković, Marijanović, Tuberoso, Bubalo, & Kezić, 2010). In honeydew honeys from *Nothofagus* spp., significantly higher amounts of phenol and linalool were observed, but these were not considered markers of this honey for being found in the other New Zealand honeys evaluated (Revell, Morris, & Manley-Harris, 2014).

In *Pinus* honeydew honeys from Turkey and Greece, 3-carene and an unknown compound were considered as markers of *Pinus* honeydew honey from Turkey. Also, 1-chloro-octane and tridecane were found in all pine honeydew honeys from Greece and Turkey, been potential markers of these honeys (Tananaki et al., 2007). In *Pinus brutia* Ten. honeydew honeys from Turkey, the compounds nonanal, benzene, 4-hexen-3-ol, α-pinene, and 2-heptanone were cited as botanical markers of this honey (Silici, 2011). In pine honeydew honeys from Greece, the compounds hexanoic acid ethyl ester, 2,3-butanediol, decane, β-thujone, 1-methyl-4-(1-methylethenyl) benzene, nonanal, heptanoic acid ethyl ester, and 2-ethyl-1-hexanol were found to be significant for the geographical differentiation of this honey (Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). It is observed that few compounds, such as nonanal and pinene, are highlighted as abundant compounds in pine honeydew honeys from different geographical locations, showing the possible influence of other conditions in the volatile profile.

Phenolic and benzene compounds were considered characteristics of holm-oak, oak, and forest honeydew honeys from Spain evaluated by Castro-Vázquez et al. (2006). For holm-oak honeydew honeys, some compounds such as aminoacetophenone and propylanisol were suggested as characteristics (Castro-Vázquez et al., 2006). However, benzene compounds, including benzeneacetaldehyde and phenylethyl-alcohol, were not found in considerable levels in another study in Spanish honeydew honeys (forest origin), in addition to not being considered exclusive of this type of honey (Escrache et al., 2009). Furthermore, the trans-β-methyl-γ-octalactone was suggested as a marker for oak honeydew honey, for being only found in oak wood (Castro-Vázquez et al., 2006). However, this compound was not identified in another study with oak (*Quercus frainetto* Ten.) honeydew honeys, where no compound was suggested as a specific marker (Jerkovic & Marijanovic, 2010).

Furan and pyran derivatives were also identified in honeydew honeys, such as in *Quercus frainetto* Ten. (Jerkovic & Marijanovic, 2010), holm-oak, oak, and forest honeydew honeys (Castro-Vázquez et al., 2006; Escrache et al., 2009), which assists in the evaluation of the quality of honeydew honeys.

Therefore, the profile and amount of volatile compounds are a potential tool for the discrimination of honeydew honeys. However, advances still must be made since many compounds (or quantities) cited as characteristics of honeydew honey or markers of specific honeydew honey have already been reported in blossom honeys or different honeydew honeys. In this sense, the continuous investigation of volatile compounds in the representative quantity of honeydew honeys of different botanical and geographical origin, as well as in blossom honeys, will contribute to that specific volatile compounds and/or quantities of these being indicated with greater reliability as specific markers of

honeydew honeys.

### 3. Functional and health-promoting properties

Honey had been considered a healthy food due to the range of studies that have been taking place around the composition of honey and its benefits (Beretta, Granata, Ferrero, Orioli, & Facino, 2005). Honey has been used in the treatment of several disorders due to properties as antioxidant, antimicrobial, anti-inflammatory (Escuredo et al., 2013; Lukaszewicz, Kowalski, & Makarewicz, 2015; Majtan et al., 2013).

#### 3.1. Antioxidant property

Some authors that evaluated the antioxidant activity of honeys from different origins, reported that honeydew honeys are richest in phenolic compounds amount and consequently have higher antioxidant activity, as shown by Kowalski (2013), Vela, Lorenzo, and Pérez (2007) and Al et al. (2009) in honeydew honeys.

Because of the different purposes and responses obtained, studies in honeydew honeys have used different methods, such as ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical), DPPH (2,2-diphenyl-picrylhydrazyl radical), FRAP (ferric reducing antioxidant power), and ORAC (oxygen radical absorbance capacity), among others tests.

The DPPH method has been used by Al et al. (2009), with 41 to 65% of radical inhibition by honeydew honeys from Romania, and a significant correlation of  $R^2 = 0.94$  with total phenolic content. Kowalski (2013) used both ABTS and DPPH radical assays and reported 63% and 87% of scavenging, respectively, in honeydew honeys (unknown origin). Flores et al. (2015) reported DPPH scavenging of 73% in oak (*Quercus pyrenaica*) honeydew honeys.

Beretta et al. (2005) evaluated the antioxidant properties of honeydew honey (unknown origin) through FRAP ( $772 \mu\text{mol L}^{-1}$  Fe (II)), DPPH ( $\text{IC}_{50}$  of  $8.5 \text{ mg mL}^{-1}$ ), and ORAC ( $6.3$  Trolox equivalent  $\text{g}^{-1}$ ). The authors found interdependence among the responses of these tests and the total phenolic content. Bertonecelj et al. (2007) used DPPH ( $\text{IC}_{50}$  of  $7.2 \text{ mg mL}^{-1}$ ) and FRAP assays ( $426.4 \mu\text{mol L}^{-1}$  Fe (II)), with the best responses for honeydew honey from coniferous trees and high correlations between these tests and total phenolic content.

DPPH assay was also used in honeydew honeys from forest origin by Lukaszewicz et al. (2015) with scavenging activity of 58%, by Meda, Lamien, Romito, Millogo, and Nacoulma (2005) with 4.9%, and by Vela, de Lorenzo, and Pérez (2007) with 67% of scavenging activity. In honeydew honeys from pine (*Pinus* spp.) and fir (*Abies cephalonica* Loudon), Karabagias, Dimitriou, Kontakos, and Kontominas (2016) found 50 and 38% of DPPH scavenging activity, respectively, and Nayik and Nanda (2016) found 55% for pine honeydew honey, and correlated with the mineral content. Pichichero et al. (2009) used FRAP and DPPH assays in honeydew honey from thymus (*Thymus vulgare*) ( $1800 \mu\text{mol L}^{-1}$  Fe (II) and  $\text{IC}_{50}$  of  $31 \text{ mg mL}^{-1}$ , respectively). Tuberoso et al. (2011) used the FRAP assay in honeydew honey from willow (*Salix* spp.) with  $3200 \mu\text{mol L}^{-1}$  Fe (II) and Lukaszewicz et al. (2015) evaluated antioxidant activity of honeydew honeys from forest origin through the ABTS assay with 35% of inhibition.

The antioxidant activity is highly correlated with phenolic compounds. However, honeydew honeys also present high contents of minerals, proteins, organic acids, enzymes and amino acids, as presented in this review. These constituents are also responsible for the antioxidant activity of honeydew honeys that is strongly affected by the botanical source.

#### 3.2. Antimicrobial property

The antimicrobial activity of honey is mainly credited to its acidity, osmolality, and generation of hydrogen peroxide via glucose oxidase

enzyme (Mundo, Padilla-Zakour, & Worobo, 2004; Szwed, 2017). This enzyme produces gluconic acid from glucose. As a by-product of the reaction, hydrogen peroxide is generated. Hydrogen peroxide activates nuclear transcription factors that activate genes responsible for producing a response to inflammation generated by bacteria (Vandamme, Heyneman, Hoeksema, Verbelen, & Monstrey, 2013).

Lukaszewicz et al. (2015) reported that honeydew honeys with low glucose oxidase activity showed the best antimicrobial action. It means that the antimicrobial efficiency of each honey is not only dependent on glucose oxidase activity, but also dependent on its profile and concentration of honey constituents as the phenolic compounds.

Osés et al. (2016) evaluated antimicrobial activity and founded lower minimum inhibitory concentration (MIC) for honeydew honeys over blossom honeys against *Staphylococcus aureus*. Majtan, Bohova, Horniackova, and Kludiny (2013) evaluated antimicrobial and anti-biofilm effects and founded that honeydew honey had lower MIC values than blossom honeys against *Enterobacter cloacae* (12.5%) and *Proteus mirabilis* (12.5%) isolates.

Salonen, Virjamo, Tammela, Fauch, and Julkunen-Tiitt (2017) evaluated honeys against *Pseudomonas aeruginosa*, *S. aureus*, and reported that 15% dilution of honeydew honey (unknown origin) inhibited over 85% of *P. aeruginosa*, the lowest MIC among other honeys (blossom honeys).

Antimicrobial activities of honeydew honeys have been not widely studied and there is a need to highlight this potential, because these honeys usually have higher phenolic compounds content and acidity, besides the greatest performance against bacterial when compared with blossom honeys.

#### 3.3. Anti-inflammatory property

The healing effect of honey is mainly due to the antimicrobial properties of hydrogen peroxide and bioactive compounds against this microbial flora of the wound (Mayer et al., 2014; Silici, Sagdic, & Ekici, 2010). Honey also provides an appropriate moist environment for proper wound healing, due to the physical property of osmosis (Majtan, Bohova, Garcia-Villalba, et al., 2013).

Some reports about honeydew honey performance against inflammatory responses had already been published. Majtan, Majtanova, Bohova, and Majtan (2011) tested Slovak honeydew honey (unknown origin) and Manuka honey as antimicrobial to *S. maltophilia* isolates from venous leg ulcers in cancer patients. The MICs for honeydew honey ranged from 6.25 to 17.5%, while those for Manuka honey ranged from 7.5 to 22.5%.

Majtan, Bohova, Garcia-Villalba, et al. (2013) evaluated *in vitro* the action of fir (*Abies cephalonica* Loudon) honeydew honey aqueous extracts in the regulation of the expression of the matrix metalloproteinase-9, a protease key inflammatory mediator. According to the authors, the flavonoid content of honey can down-regulate the expression of this protease. Mayer et al. (2014) treated with fir (*Abies cephalonica* Loudon) honeydew honey from Slovak twenty-five patients with leg ulcers for six weeks. After the treatment, 72% of the patients reported a decrease in the pain levels.

Honeydew honey has already been used by Majtanova et al. (2015) for the treatment of contact lens-induced corneal ulcer. Honeydew honey (unknown origin) was shown to be highly effective against tested ocular bacterial isolates, in particular *S. maltophilia*, with MIC of 10%.

Fir (*Abies cephalonica* Loudon) honeydew honey was used by Cernak et al. (2012) to avoid endophthalmitis, a disease that represents a complication of eye surgery, particularly cataract. The honeydew honey completely eradicated *Staphylococcus aureus*, *Corynebacterium* spp. and *Enterococcus* spp. isolates after treatment.

Vlcekova et al. (2012) applied honeydew honey (unknown origin) to treat a patient with gluteofemoral fistulas (infection of the anal crypts and glands). Cultures of fistula secretions were positive for *Staphylococcus aureus*, *Staphylococcus haemolyticus* and *Escherichia coli*.

**Table 6**  
Phenolic compounds reported in honeydew honeys.

| Phenolic compound     | Geographical origin / botanical origin   | Range (mg kg <sup>-1</sup> ) | References   |
|-----------------------|--|------------------------------|--|
| Acacetin              | Turkey / <i>Quercus robur</i> L. (n = 1)   | 0.04 <sup>a</sup>            | Haroun, Poyrazoglu, et al. (2012)  |
| Apigenin              | Italy / <i>Thymus vulgare</i> (n = 3), Turkey / <i>Pinus</i> sp. (n = 7), Czech Republic / nr (n = 3)  | nd – 0.16                    | Halouzka et al. (2016); Haroun, Poyrazoglu, et al. (2012); Pichichero et al. (2009)  |
| Benzoic acid          | Brazil / <i>Mimosa scabrella</i> Benth (n = 18), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), France / Pyrenean forestry (n = 2), France, Italy / <i>Abies alba</i> Miller (n = 2)  | 1.18–15.20                   | Daher and Gülaçar (2008); Seraglio et al. (2016); Silva et al. (2018)  |
| Caffeic acid          | Italy / <i>Thymus vulgare</i> (n = 3), Spain / Forest (n = 1), Poland / nr (n = 1), Turkey / <i>Quercus robur</i> L. (n = 3), Turkey / <i>Pinus brutia</i> L. (n = 3), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Turkey / <i>Quercus robur</i> L. (n = 1), Turkey / <i>Pinus</i> sp. (n = 7), Czech Republic / nr (n = 3), Turkey / nr (n = 17)  | nd – 26,780.00               | Can et al. (2015); Escriche et al. (2014); Halouzka et al. (2016); Haroun, Poyrazoglu, et al. (2012); Pichichero et al. (2009); Seraglio et al. (2016); Silici et al. (2013); Socha et al. (2011)                      |
| Catechin              | Turkey / <i>Pinus brutia</i> L. (n = 3), Turkey / nr (n = 17)  | nd – 21,750.00               | Can et al. (2015); Silici et al. (2013)  |
| Cinnamic acid         | France, Italy / <i>Abies alba</i> Miller (n = 2)   | 0.59–0.79                    | Daher and Gülaçar (2008)   |
| Chlorogenic acid      | Italy / <i>Thymus vulgare</i> (n = 3), Poland / nr (n = 1), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Czech Republic / nr (n = 3), Turkey / nr (n = 17)  | nd – 28.34                   | Halouzka et al. (2016); Pichichero et al. (2009); Seraglio et al. (2016); Silici et al. (2013); Socha et al. (2011)  |
| Coniferaldehyde       | Brazil / <i>Mimosa scabrella</i> Benth (n = 9)   | nd – 0.09                    | Seraglio et al. (2016)   |
| Chrysin               | Italy / <i>Thymus vulgare</i> (n = 3), Spain / Forest (n = 1), Turkey / <i>Quercus robur</i> L. (n = 1), Turkey / <i>Pinus</i> sp. (n = 7), France / Pyrenean forestry (n = 2), France, Italy / <i>Abies alba</i> Miller (n = 2)   | 0.02–19.00                   | Daher and Gülaçar (2008); Escriche et al. (2014); Haroun, Poyrazoglu, et al. (2012); Pichichero et al. (2009)  |
| Ellagic acid          | Turkey / <i>Quercus robur</i> L. (n = 1)   | 3.36 <sup>a</sup>            | Haroun, Poyrazoglu, et al. (2012)  |
| Epicatechin           | Turkey / <i>Quercus robur</i> L. (n = 3), Turkey / nr (n = 17)   | nd – 10,320.00               | Can et al. (2015); Silici et al. (2013)  |
| Ferulic acid          | Poland / nr (n = 1), Turkey / <i>Quercus robur</i> L. (n = 3), Turkey / <i>Pinus brutia</i> L. (n = 3), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Turkey / <i>Quercus robur</i> L. (n = 1), Turkey / <i>Pinus</i> sp. (n = 7), Czech Republic / nr (n = 3), Turkey / nr (n = 17), France / Pyrenean forestry (n = 2), France, Italy / <i>Abies alba</i> Miller (n = 2)                           | nd – 4190.00                 | Can et al. (2015); Daher and Gülaçar (2008); Halouzka et al. (2016); Haroun, Poyrazoglu, et al. (2012); Seraglio et al. (2016); Silici et al. (2013); Socha et al. (2011)  |
| Galangin              | Italy / <i>Thymus vulgare</i> (n = 3), Spain / Forest (n = 1)  | 0.32–14.00                   | Escriche et al. (2014); Pichichero et al. (2009)   |
| Gallic acid           | Italy / <i>Thymus vulgare</i> (n = 3), Poland / nr (n = 1), Turkey / <i>Quercus robur</i> L. (n = 3), Brazil / <i>Mimosa scabrella</i> Benth (n = 18), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Czech Republic / nr (n = 3), Turkey / nr (n = 17)   | nd – 82,490.00               | Can et al. (2015); Halouzka et al. (2016); Pichichero et al. (2009); Seraglio et al. (2016); Silici et al. (2013); Silva et al. (2018); Socha et al. (2011)  |
| Genistein             | Italy / <i>Thymus vulgare</i> (n = 3)  | 0.13 <sup>a</sup>            | Pichichero et al. (2009)   |
| Genistic acid         | Czech Republic / nr (n = 3)  | 0.24–0.65                    | Halouzka et al. (2016)   |
| Hesperidin            | Brazil / <i>Mimosa scabrella</i> Benth (n = 9)   | 0.02–0.19                    | Seraglio et al. (2016)   |
| Isoferulic acid       | Czech Republic / nr (n = 3)  | 0.78–0.88                    | Halouzka et al. (2016)   |
| Isorhamnetin          | Brazil / <i>Mimosa scabrella</i> Benth (n = 9)   | 0.09–0.11                    | Seraglio et al. (2016)   |
| Kaempferol            | Italy / <i>Thymus vulgare</i> (n = 3), Spain / Forest (n = 1), Turkey / <i>Pinus brutia</i> L. (n = 3), Brazil / <i>Mimosa scabrella</i> Benth (n = 9)   | 0.08–7500.00                 | Can et al. (2015); Escriche et al. (2014); Pichichero et al. (2009); Seraglio et al. (2016)  |
| Kynurenic acid        | Croatia / <i>Salix</i> spp. (n = 2)  | 46.5 <sup>a</sup>            | Tuberoso et al. (2011)   |
| Luteolin              | Brazil / <i>Mimosa scabrella</i> Benth (n = 18), Brazil / <i>Mimosa scabrella</i> Benth (n = 9)  | 0.06–16.05                   | Seraglio et al. (2016); Silva et al. (2018)  |
| Methyl syringate      | Turkey / <i>Pinus</i> sp. (n = 7), France / Pyrenean forestry (n = 2), France, Italy / <i>Abies alba</i> Miller (n = 2)  | nd – 26.87                   | Daher and Gülaçar (2008); Haroun, Poyrazoglu, et al. (2012)  |
| Myricetin             | Italy / <i>Thymus vulgare</i> (n = 3)  | 0.09 <sup>a</sup>            | Pichichero et al. (2009)   |
| m-Hydroxybenzoic acid | Czech Republic / nr (n = 3)  | 0.03–0.05                    | Halouzka et al. (2016)   |
| Naringenin            | Spain / Forest (n = 1), Brazil / <i>Mimosa scabrella</i> Benth (n = 18), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Czech Republic / nr (n = 3)   | nd – 18.00                   | Escriche et al. (2014); Halouzka et al. (2016); Seraglio et al. (2016); Silva et al. (2018)  |
| Naringin              | Brazil / <i>Mimosa scabrella</i> Benth (n = 9)   | 0.08–0.05                    | Seraglio et al. (2016)   |
| Pinobanksin           | Brazil / <i>Mimosa scabrella</i> Benth (n = 9)   | nd – 0.03                    | Seraglio et al. (2016)   |
| Pinocembrin           | Spain / Forest (n = 1), Turkey / <i>Quercus robur</i> L. (n = 1), Turkey / <i>Pinus</i> sp. (n = 7), Czech Republic / nr (n = 3), Turkey / nr (n = 17), France / Pyrenean forestry (n = 2), France, Italy / <i>Abies alba</i> Miller (n = 2)   | nd – 19.00                   | Daher and Gülaçar (2008); Escriche et al. (2014); Halouzka et al. (2016); Haroun, Poyrazoglu, et al. (2012); Silici et al. (2013)  |
| Protocatechuic acid   | Turkey / <i>Quercus robur</i> L. (n = 3), Turkey / <i>Pinus brutia</i> L. (n = 3), Brazil / <i>Mimosa scabrella</i> Benth (n = 18), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Turkey / <i>Quercus robur</i> L. (n = 1), Turkey / <i>Pinus</i> sp. (n = 7), Czech Republic / nr (n = 3)   | nd – 744,600.00              | Can et al. (2015); Halouzka et al. (2016); Haroun, Poyrazoglu, et al. (2012); Seraglio et al. (2016); Silva et al. (2018)  |
| p-Coumaric acid       | Italy / <i>Thymus vulgare</i> (n = 3), Spain / Forest (n = 1), Poland / nr (n = 1), Turkey / <i>Quercus robur</i> L. (n = 3), Turkey / <i>Pinus brutia</i> L. (n = 3), Brazil / <i>Mimosa scabrella</i> Benth (n = 18), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Turkey / <i>Quercus robur</i> L. (n = 1), Turkey / <i>Pinus</i> sp. (n = 7), Czech Republic / nr (n = 3), Turkey / nr (n = 17) | nd – 15,950.00               | Can et al. (2015); Escriche et al. (2014); Halouzka et al. (2016); Haroun, Poyrazoglu, et al. (2012); Pichichero et al. (2009); Seraglio et al. (2016); Silici et al. (2013); Silva et al. (2018); Socha et al. (2011) |
| p-Hydroxybenzoic acid | Czech Republic / nr (n = 3)  | 1.65–4.25                    | Halouzka et al. (2016)   |
| p-OH Benzoic acid     | Turkey / <i>Quercus robur</i> L. (n = 3), Turkey / <i>Pinus brutia</i> L. (n = 3)  | 29,240.00–50,191.00          | Can et al. (2015)  |

(continued on next page)

Table 6 (continued)

| Phenolic compound   | Geographical origin / botanical origin   | Range (mg kg <sup>-1</sup> ) | References  |
|---------------------|--|------------------------------|---|
| Quercetin           | Italy / <i>Thymus vulgare</i> (n = 3), Spain / Forest (n = 1), Turkey / <i>Quercus robur</i> L. (n = 1), Brazil / <i>Mimosa scabrella</i> Benth (n = 18), Turkey / <i>Quercus robur</i> L. (n = 1), Turkey / <i>Pinus</i> sp. (n = 7), Turkey / nr (n = 17)      | nd – 11,770.00               | Can et al. (2015); Escriche et al. (2014); Haroun, Poyrazoglu, et al. (2012); Pichichero et al. (2009); Silici et al. (2013); Silva et al. (2018) |
| Rutin               | Turkey / <i>Quercus robur</i> L. (n = 3), Turkey / <i>Pinus brutia</i> L. (n = 3), Brazil / <i>Mimosa scabrella</i> Benth (n = 9)  | 0.09–538,620.00              | Can et al. (2015); Seraglio et al. (2016); Silva et al. (2018)  |
| Salicylic acid      | Croatia / <i>Salix</i> spp. (n = 2), Brazil / <i>Mimosa scabrella</i> Benth (n = 18), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Czech Republic / nr (n = 3), France / Pyrenean forestry (n = 2), France, Italy / <i>Abies alba</i> Miller (n = 2)          | 0.82–20.10                   | Daher and Gülaçar (2008); Halouzka et al. (2016); Seraglio et al. (2016); Silva et al. (2018); Tuberoso et al. (2011)                             |
| Synapic acid        | Poland / nr (n = 1), Czech Republic / nr (n = 3)   | 0.04                         | Halouzka et al. (2016); Socha et al. (2011)   |
| Syringaldehyde      | Brazil / <i>Mimosa scabrella</i> Benth (n = 9)   | nd – 0.03                    | Seraglio et al. (2016)  |
| Syringic acid       | Poland / nr (n = 1), Turkey / <i>Quercus robur</i> L. (n = 3), Turkey / <i>Pinus brutia</i> L. (n = 3), Brazil / <i>Mimosa scabrella</i> Benth (n = 9), Turkey / <i>Quercus robur</i> L. (n = 1), Turkey / <i>Pinus</i> sp. (n = 7), Czech Republic / nr (n = 3) | nd – 18,780.00               | Can et al. (2015); Halouzka et al. (2016); Haroun, Poyrazoglu, et al. (2012); Seraglio et al. (2016); Socha et al. (2011)                         |
| Taxifolin           | Czech Republic / nr (n = 3)  | nd – 0.04                    | Halouzka et al. (2016)  |
| trans-Cinnamic acid | Czech Republic / nr (n = 3)  | 0.04–0.53                    | Halouzka et al. (2016)  |
| Vanillic acid       | Turkey / <i>Pinus brutia</i> L. (n = 3), Czech Republic / nr (n = 3)   | 0.36–20,000.00               | Can et al. (2015); Halouzka et al. (2016)   |

nd – not detected; nr – not reported.

<sup>a</sup> Data presented as mean, not as the range of values.

After five months all regions associated with fistulae not presented inflammation and fistulas in gluteofemoral left region were completely healed and closed.

Further detailed studies are needed to identify the components responsible for the anti-inflammatory properties of honeydew honey. The current review made it clear that there is much to be discovered about the effects of honeydew honey on wounds and the study of the mechanisms of action of the honeydew honey compounds.

#### 4. Contamination

Honey may present chemical contamination by pesticide, antibiotic residues, inorganic matter or be microbiologically contaminated by microorganisms from the soil, nectar, pollen, wax, honey bees and practices of the beekeeper (Al-Waili, Salom, Al-Ghamdi, & Ansari, 2012). Table 8 presents works regarding honeydew honeys microbiological and chemical contamination.

##### 4.1. Microbiological contamination

Naturally, honeydew honey has conditions (acidic pH, low water activity, high viscosity, high sugar concentration, and osmotic pressure) that hamper bacterial development (Mandal & Mandal, 2011). The microorganisms that may be present are those that support the high concentration of sugar and acidity, mainly yeasts, fungi, and spore-forming bacteria (Sinacori et al., 2014).

The *Clostridium* spores are especially dangerous for children that do not have fully developed immunological systems (Migdal, Owczarczyk, Kędzia, Holderna-Kędzia, & Madajczyk, 2000). Ingested spores multiply and produce botulinum toxin in the digestive tract of newborns and infants (Al-Waili et al., 2012). Honey is known as a source of spores without the pre-formed botulinum toxin (Desurkar, 2015). A scientific committee of the European Union has examined the hazard of *Clostridium botulinum* in honey (European Commission, 2002b). It has concluded that no microbiological examinations of honey, both blossom and honeydew are necessary, as the incidence of *Clostridium botulinum* is relatively low (Al-Waili et al., 2012). This explains why so few works are found with honeydew honeys. Migdal et al. (2000) evaluated for the first time the decontamination of blossom and honeydew honeys naturally contaminated with *Clostridium botulinum* by irradiation. The authors analyzed one honeydew honey (unknown origin) and found 100 *Clostridium* spores, that reduced to < 10 after irradiation. The sampling is not representative of honeydew honeys, but

warns about microbiological contamination of these honeys. After that, Küplülü, Göncüoğlu, Özdemir, and Koluman (2006) evaluated the presence of *Clostridium* spores in eight honeydew honeys (unknown origin) and 1 out of the eight samples was positive for *Clostridium* presence.

Honeydew honeys data like those of Migdal et al. (2000) and Küplülü et al. (2006), shows that there is a need for attention about the hygienic quality of these honeys, which demonstrates the potential of the study of microbiological contaminants.

##### 4.2. Chemical contamination

###### 4.2.1. Heavy metals

Heavy metals are regularly found in air, water, and soil, so honey bees are exposed to them either directly, retaining atmospheric particles on their body hairs, or indirectly through pollen, nectar, honeydew or water (Johnson, 2014; Nikolić et al., 2016).

Chudzinska and Baralkiewicz (2010) and González-Miret et al. (2005) reported Cd contamination in honeydew honeys (unknown origin), as shown in Table 8. Soil contamination by Cd is commonly due to the application of phosphate fertilizers, the combustion of fossil fuels, the incineration of waste, among others (Belas et al., 2014) and the sap of trees can be contaminated and consequently, the honeydew.

The European Food Safety Authority established a maximum of 1.0 mg kg<sup>-1</sup> of Cd in food and the recommended daily dose of 5 mg of Cu in an adult subject (Alexander et al., 2009). Although some works have reported the presence of Cd and Cu in honeydew honeys (Chudzinska & Baralkiewicz, 2010; González-Miret et al., 2005; Madejczyk & Baralkiewicz, 2008; Pisani et al., 2008), maximum levels are not established.

Dark honeys as the honeydew honeys contain higher amounts of a certain major, minor and trace metals, e.g., Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn; than light honeys (Pohl, 2009). According to González-Miret et al. (2005), the color of dark honeys from avocado, chestnut, honeydew, and heather is greatly correlated with the concentration of the trace elements such as As, Cd, Fe, S, Pb and Ca. These results bring up the care with heavy metals presence in honeydew honeys.

###### 4.2.2. Pesticides and veterinary drugs

The honey bees can be directly treated with pesticides to control some disease, or the pesticides residue came from indirect contamination, i.e. those used in the agriculture, because they are distributed in

**Table 7**  
Main volatile compounds reported in honeydew honeys.

| Geographical origin / botanical origin          | Number of volatile compounds found | Main volatile compounds  | Analytical technique / extraction method | Reference                       |
|---|------------------------------------|--|--|---------------------------------|
| Brazil / nr (n = 6)                             | > 20                               | Acetic acid  | GC-MS / Stream of hydrogen               | Campos et al. (2000)            |
| Spain / nr (n = 16)                             | > 5                                | Borneol, 2,3-butanediol ( <i>threo</i> - and <i>erythro</i> -), 1-hydroxy-2-propanone, 3-hydroxy-2-butanone  | GC-MS / SPME                             | Soria et al. (2004)             |
| Spain / nr (nr)                                 | > 5                                | 2,3-Butanediol ( <i>threo</i> - and <i>erythro</i> -), 3-hydroxy-2-butanone, 1-hydroxy-2-propanone, acetic acid  | GC-MS / SPME                             | Soria et al. (2005)             |
| Spain / Holm-oak, oak, and forest (n = 9)       | 66                                 | Phenylacetaldehyde, guaiacol, <i>p</i> -anisaldehyde, propylanisole, linalool oxide, hotrienol, $\alpha$ -terpineol, eugenol, car-2-en-4-one, epoxylinalool, <i>p</i> -cymen-8-ol, 2-hydroxycineol, aminoacetophenone, isophorone, ketoisophorone, 3-hydroxy-2-butanone, <i>trans</i> - $\beta$ -methyl- $\gamma$ -octalactone   | GC-MS / SDE                              | Castro-Vázquez et al. (2006)    |
| Croatia / <i>Abies alba</i> (n = 3)             | 45                                 | Methyl-2-buten-1-ol, <i>n</i> -hexanol, 3-hexanol, 1-propyne, 2-furanmethanol, 5-methyl-2(5H)-furanone, 4-methylphenol, hexadecanoic acid, methylheptanoate, acetonitrile  | GC-MS / HS-SPME                          | Lušić et al. (2007)             |
| Turkey and Greece / <i>Pinus</i> (n = 42)       | 77                                 | Octanal, 3-carene, camphene, octane, nonanal, decanal, $\alpha$ -pinene, $\beta$ -pinene, toluene, 1,2,3-trimethylindene, unknown ( <i>m/z</i> 55, 79, 91, 107, 123, 165), 1-chloro-octane, tridecane  | GC-MS / P&T                              | Tananki et al. (2007)           |
| Spain / Forest (n = 10)                         | 47                                 | 2-Methyl-1-butanol, 3-hydroxy-2-butanone, 3-methyl-3-buten-1-ol, ethanol, acetic acid, 2-methyl-propanoic acid, propanoic acid, 1,3-butanediol, 1-hexanol, 2,3-butanediol, 3-hexen-1-ol, 3-methyl-2-butanol, 3-pentanol, 1-cyclohexene-1-carboxaldehyde-5,5-dimethyl-3-oxo, 1-hydroxy-2-butanone, 2-hydroxy-3-pentanone, 2-methyl-4-hexyne-3-one, 3,5,5-trimethyl-2-ciclohexen-1-one, 1,1-bicyclopropyl-2-octanoic acid-2-hexyl-methyl ester, ethyl acetate, 2-furanmethanol, 2-furanmethanol-5-ethenyltetrahydro- $\alpha,\alpha,5$ -trimethyl- <i>trans</i> ; dihydro-2(3H)-furanone, dihydro-2-methyl-3(2H)-furanone, 2,4,5-trimethyl-1,3-dioxolane | GC-MS / P&T                              | Escriche et al. (2009)          |
| Turkey / nr (n = 6)                             | $\geq 5$                           | Nonanal, $\alpha,\alpha$ -dimethyl phenyl acetate, 5-hydroxymethylfurfural, 1-(2-furyl)-2-hydroxyethanone, 2-furanmethanol, <i>n</i> -decane, nonanol, 2-methyl heptanoic acid   | GC-MS / SPME                             | Senyuva et al. (2009)           |
| Croatia / <i>Quercus frainetto</i> Ten. (n = 2) | > 60                               | In HS-SPME: linalool oxides ( <i>cis</i> - and <i>trans</i> ), hotrienol, epoxylinalool, octanoic acid, nonanoic acid, decanoic acid, hexadecanoic acid, 2-phenylethanol, phenylacetaldehyde, benzyl alcohol, 2-furan-carboxaldehyde, 2-furanmethanol, 1-(2-furanyl)-ethanone, 5-methyl-2-furfural   | GC-FID, GC-MS / HS-SPME, USE             | Jeršković and Marjanović (2010) |
| Croatia / <i>Salix</i> spp. (nr)                | > 45                               | In USE: phenylacetic acid, benzoic acid, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, 4-hydroxycinnamic acid, methyl syringate, (Z)-octadec-9-en-1-ol, hexadecan-1-ol, octadecan-1-ol, hexadecanoic acid, tetradecane, hexadecane, tricosane  | GC-FID, GC-MS / HS-SPME, USE             | Jeršković et al. (2010)         |
| Turkey / <i>Pinus brutia</i> Ten. (n = 13)      | 42                                 | In HS-SPME: nonan-1-ol, hotrienol, dimethyl sulfide, benzaldehyde, 2-phenylethyl acetate, 3-methylbutanoic acid, isophorone, hexanoic acid, benzyl alcohol, 2-phenylethanol  | GC-MS / SPME                             | Silici (2011)                   |
| Slovakia / nr (n = 35)                          | > 300                              | In USE: benzoic acid, phenylacetic acid, 2-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, (Z)-octadec-9-enoic acid, (Z)-octadec-9-en-1-ol, hexadecan-1-ol  | GC x GC-TOF-MS / SPME                    | Jánošková et al. (2014)         |
| Greece / Pine (n = 39)                          | 55                                 | Nonanal, benzene, 4-hexen-3-ol, $\alpha$ -pinene, 2-heptane, octane, <i>n</i> -decane, benzophenone, methyl dihydrojasmonate, benzeneacetaldehyde  | GC-MS / HS-SPME                          | Karabagias et al. (2014)        |
| New Zealand / <i>Nothofagus</i> spp. (n = 28)   | 23                                 | 2,3-Butanediol, 3-hydroxy-2-butanone, acetic acid, 2-oxooctanoic acid, 4-oxapentanoic acid, allyl ester of acetic acid, methyl ester of 2,6-dihydroxybenzoic acid  | GC-MS / SPME                             | Revell et al. (2014)            |
| Poland / <i>Abies alba</i> M. (n = 5)           | > 19                               | Hexanoic acid ethyl ester, 2,3-butanediol, decane, $\beta$ -thujone, 1-methyl-4-(1-methylethenyl) benzene, nonanal, 2-ethyl-1-hexanol, heptanoic acid ethyl ester  | GC-MS / HS-SPME, USE                     | Kuš et al. (2017)               |
| European countries / Forest (n = 24)            | 18                                 | Phenol, hotrienol, linalool, 3-methylpentanoic acid, benzaldehyde, <i>o</i> -methoxyacetophenone, Nonanal, 2-phenylethanol, benzoic acid, 1-(2-methoxy-phenyl)ethanol  | GC-IMS / HS                              | Gerhardt et al. (2018)          |
|   |                                    | In HS-SPME: benzaldehyde, borneol, linalool derivatives  |  |                                 |
|   |                                    | In USE: methyl syringate, 4-hydroxy-3,5,6-trimethyl-4-(3-oxobut-1-enyl)cyclohex-2-en-1-one, comiferyl alcohol isomers  |  |                                 |
|   |                                    | <i>cis</i> -Linalool oxide, limonene, 3-hydroxy-2-butanone, <i>trans</i> -2-pentenal, 3-methylbutanol  |  |                                 |

FID – Flame-Ionization Detector; GC X GC – Two-Dimensional Gas Chromatography; HS – Headspace; IMS – Ion Mobility Spectrometry; MS – Mass Spectrometry; P&T – Purge & Trap; SDE – Simultaneous Distillation-Extraction; SPME – Solid Phase Micro-Extraction; TOF – Time-Of-Flight; USE – Ultrasonic Solvent Extraction; nr – not reported.



**Table 8**  
Microbiological and chemical contamination in honeydew honeys.

| Microbiological contamination          |  | Chemical contamination              |                        |                        |      |           |                        |                        |                        |                         |        |   | Reference             |
|--|--|-------------------------------------|------------------------|------------------------|------|-----------|------------------------|------------------------|------------------------|-------------------------|--------|---|-----------------------|
| Geographical origin / botanical origin |  | <i>Clostridium botulinum</i> spores |                        |                        |      |           |                        |                        |                        |                         |        |   |                       |
| Poland / nr (n = 1)                    |  | 100                                 |                        |                        |      |           |                        |                        |                        |                         |        |   | Migdal et al. (2000)  |
| Turkey / nr (n = 8)                    |  | + <sup>b</sup>                      |                        |                        |      |           |                        |                        |                        |                         |        |   | Kıptılı et al. (2006) |
| Chemical contamination                 |  | Heavy metals (mg kg <sup>-1</sup> ) |                        |                        |      |           |                        |                        |                        |                         |        |   | Reference             |
| Geographical origin / botanical origin |  | Cd                                  | Cu                     | Zn                     | As   | Pb        | Mn                     | Ba                     | Ni                     | Al                      | Co     |   |                       |
| Poland / nr (n = 2)                    |  | 0.03                                | –                      | 4.31                   | –    | –         | –                      | –                      | –                      | –                       | –      | Przybyłowski and Wilczyńska (2001)        |                       |
| Spain / Quercus sp. (n = 33)           |  | 0.01                                | 0.94                   | 4.73                   | 0.05 | 0.28      | 8.73                   | –                      | –                      | –                       | –      | González-Miret et al. (2005)              |                       |
| Czech Republic / nr (n = 9)            |  | –                                   | 0.27–0.66 <sup>a</sup> | 1.86–3.42 <sup>a</sup> | –    | –         | 4.83–8.99 <sup>a</sup> | –                      | 0.32–1.53 <sup>a</sup> | –                       | –      | Lachman et al. (2007)                     |                       |
| Poland / nr (n = 19)                   |  | –                                   | 0.26–1.82 <sup>a</sup> | 1.38–9.93 <sup>a</sup> | –    | –         | –                      | –                      | –                      | –                       | –      | Madejczyk and Baralkiewicz (2008)         |                       |
| Italy / nr (n = 4)                     |  | < 0.01                              | 4.40                   | 1.76                   | 0.01 | 0.09      | 1.70                   | –                      | 0.53                   | –                       | 0.04   | Pisani et al. (2008)                      |                       |
| Poland / nr (n = 21)                   |  | 0.02–0.10 <sup>a</sup>              | nd – 2.63 <sup>a</sup> | 1.28–39.7              | –    | nd – 0.89 | 2.70–7.37 <sup>a</sup> | 0.03–0.31 <sup>a</sup> | 0.35–1.87 <sup>a</sup> | 5.14–46.70 <sup>a</sup> | –      | Chudzińska and Baralkiewicz (2010)        |                       |
| Bulgaria / nr (n = 1)                  |  | < 0.01                              | 0.45                   | 0.47                   | 0.32 | < 0.08    | 12.70                  | –                      | 0.14                   | –                       | < 0.01 | Atanassova, Yurukova, and Lazarova (2012) |                       |

Al – Aluminium; As – Arsenic; Ba – Barium; Cd – Cadmium; Co – Cobalt; Cu – Copper; Mn – Manganese; Ni – Nickel; Pb – Lead; Zn – Zinc. nr – not reported; nd – not detected.

<sup>a</sup> Data presented as range of values, not as mean.

<sup>b</sup> Reported as the presence of *Clostridium* spores.

the environment (soil, air, water) and are often deposited on plants pollinated by bees (Rial-Otero, Gaspar, Moura, & Capelo, 2007). Bee's exposure to pesticides contamination is a worldwide problem, and due to that, has become worrying.

The neonicotinoids became the most commonly used class of insecticides on a large variety of crops (Benuszek, Laurent, & Chauzat, 2017). They are very toxic for bees, furthermore, are persistent in the environment, due to their systemic capacity for translocation into treated plants, create the potential for specific exposure routes for pollinators, including pollen, nectar, and honeydew fluids (Benuszek et al., 2017; Domenica et al., 2016).

The searches about pesticide residues in honey are a few limited. The honeydew honey scenery about pesticides contamination is still scarcer. Jovanov et al. (2015) evaluated the presence of neonicotinoids in sunflower honey, wildflower honey, linden honey and acacia honeydew honey. Only sunflower and wildflower honeys were contaminated with the commercial neonicotinoids thiacloprid, imidacloprid and thiamethoxam. The lack of reports on the pesticide residues in honeydew honeys brings up the question of how the accumulation of residue happens in honeydew, and if this accumulation does not occur in a harder way than in flowers.

Veterinary drugs, mainly antibiotics are used to fight disease in bees caused by bacterial and fungal. Sulphathiazole and oxytetracycline are probably the first antibiotics used to fight honeybee diseases (Bargańska, Namieśnik, & Ślebioda, 2011; Moretti, Saluti, & Galarini, 2017). The treatment of bees with antibiotics is prohibited in the European Union.

Screening about antibiotics residues in honeydew honey is not able in the literature. The lack of studies does not allow ensure the quality in the absence of antibiotic and pesticides residues. A second question emerges about honeydew honeys: if the insects that produce honeydew may be affected by residues of pesticides or veterinary drugs at some point in its life cycle.

## 5. Adulteration and authenticity

For honeys, in general, the authenticity is a parameter related to a botanical and geographical origin that directly affects its commercial value (Fechner et al., 2016; Madejczyk & Baralkiewicz, 2008; Siddiqui, Musharraf, Choudhary, & Rahman, 2017). Until recently, consumers had no habit of consuming honeydew honey because it came from insect secretion (Karabagias et al., 2014). Over the years, the properties of honeydew honey have been studied and highlighted, making this product increasingly valued by consumers mainly from European countries, and this appreciation makes the product susceptible to fraud (Azevedo, Seraglio, et al., 2017).

According to Codex Alimentarius Commission (2001), honeys must be unadulterated by materials, organic or inorganic, foreign to its natural composition. While in blossom honeys the most common adulterations are by direct addition of sugar beet syrups, corn syrup, inverted syrup and sucrose syrup into honey (Ribeiro et al., 2014; Ruiz-Matute et al., 2007) or excessive feeding of the bees with industrial syrups like maize (Guler, Bek, & Kement, 2008), the main form of adulteration of honeydew honey is by the addition of commercial low value blossom honeys.

Currently, the melissopalynology is the most widely used method for the determination of botanical origin and possible frauds caused by the mixture of honeys (Soria et al., 2004). The melissopalynological analysis evaluates the botanical origin of honey by microscopic analysis. To be considered honeydew honey, the presence of honeydew elements - microalgae, fungal mycelia, and spores - in the minimum proportion of 3 parts for a part of pollen is evaluated (Louveau, Maurizio, & Vorwohl, 1978). However, this method may not properly discriminate honeydew honeys or detect frauds (Soria et al., 2004). Thus, many studies have been developed with the objective to find parameters and develop techniques that differentiate honeydew honeys

**Table 9**  
Main studies related to the differentiation between honeydew honeys and blossom honeys.

| Botanical origin / Geographical origin  | Evaluated parameter   | Statistical analysis | Reference   |
|---|---|----------------------|---|
| Rape blossom honey and honeydew honey (unknown origin) / Poland (n = 30)  | Minerals  | CA                   | Majejczyk and Baralkiewicz (2008)                         |
| <i>Robinia pseudoacacia</i> , <i>Tilia</i> spp., <i>Castanea sativa</i> , multiflower, <i>Abies alba</i> Mill., and <i>Picea abies</i> (L.) blossom honeys, and forest honeydew honey <sup>a</sup> / Slovenia (n = 40)  | Flavonoids and abscisic acid  | PCA and LDA          | Bertoncelj, Polak, Kropf, Korošec, and Golob (2011)       |
| Multiflower, rosemary, heather, citrus, echium, eucalyptus, rosaceae, chestnut, cornflower ( <i>Centaurea cyanus</i> ), sunflower, and thyme blossom honeys and honeydew honey (unknown origin) / Spain (n = 109)   | Carbohydrate  | DA                   | de la Fuente et al. (2011)                                |
| Multiflower honey and honeydew honey (unknown origin) / nr (n = 77)   | Carbohydrate profile and physicochemical parameters                       | LDA                  | Manzanares et al. (2011)                                  |
| Polyfloral, rapessed, and citrus blossom honeys, and oak, spruce, and fir honeydew honeys / Bulgaria, Czech Republic, Greece, Romania, Slovakia (n = 24)  | Signals of the protons and the carbon of the methylene group              | –                    | Simova et al. (2012)                                      |
| Polyfloral, anzer, organic, taurus flower, chestnut, cedar, rhododendron blossom honeys, honey adulterated with maple syrup, fructose syrup, and grape molasses, and pine honeydew honey / nr (n = nr)  | Attenuated total reflectance spectroscopic data                           | CA and PCA           | Gok, Severcan, Goormaghtigh, Kandemir, and Severca (2015) |
| Multiflower and acacia blossom honeys and pine honeydew honey / India (n = 30)  | Enzymatic properties, mineral content and physicochemical parameters      | PCA and LDA          | Nayik and Nanda (2015)                                    |
| <i>Mimosa scabrella</i> Benth honeydew honey and <i>Mimosa scabrella</i> Benth blossom honey / Brazil (n = 6)   | Proteins  | PCA                  | Azevedo, Valentim-Neto, et al. (2017)                     |
| <i>Sida</i> sp., <i>Mimosa scabrella</i> Benth, Multiflower, <i>Myrcia multiflora</i> , <i>Malus domestica</i> , <i>Clethra scabra</i> Pers., <i>Eucalyptus</i> sp., and <i>Citrus sinensis</i> (L.) blossom honeys, and <i>Mimosa scabrella</i> Benth honeydew honey / Brazil (n = 41) | Carbohydrate, reducing/scavenging capacity and physicochemical parameters | PCA                  | Bergamo et al. (2018b)                                    |

CA – Cluster Analysis; C&RT – Classification and Regression Tree; DA – Discriminant Analysis; LDA – Linear Discriminant Analysis; PCA – Principal Component Analysis; nr – not reported.

<sup>a</sup> – Honeydew honey from latifoliae and coniferous trees.

and blossom honeys, and the main ones are highlighted in Table 9.

Although there are controversies about the use of carbohydrates as a possible parameter of authenticity and differentiation between blossom honeys and honeydew honeys (de la Fuente, Ruiz-Matute, Valencia-Barrera, Sanz, & Martínez-Castro, 2011; Kaškonienė et al., 2010), the carbohydrates profile associated with other physicochemical parameters it seems to be a viable alternative for honey differentiation (Bergamo et al., 2018b; Nayik & Nanda, 2015). Also, other compounds such as quercitol and proto-quercitol, a cyclitol derived from the cyclohexanepentol, commonly studied with the carbohydrate group, were related as markers of oak (*Quercus* spp.) honeydew honeys produced in countries of the European continent (Sanz et al., 2005; Simova et al., 2012).

Besides the important role in the human diet, the profile and mineral content has been investigated as a possible marker of the botanical and geographical origin of honeys. In their study, Majejczyk and Baralkiewicz (2008) were able to differentiate Polish rape honeys and honeydew honeys (unknown origin) according to the mineral profile, and it was still possible to distinguish them geographically using chemometric analysis. Furthermore, the study developed by Bergamo, Seraglio, Gonzaga, Fett, and Costa (2018a), was able to distinguish bracinga honeydew honeys from blossom honeys, and also bracinga honeydew honeys adulterated with blossom honeys at 5% concentration using chemometric analysis associated with the analysis of five minerals (potassium, magnesium, sodium, calcium, and manganese).

The protein profile associated with chemometric analysis was successfully used for the differentiation of honeydew and blossom honeys from bracinga (Azevedo, Valentim-Neto, et al., 2017). The proteome profile investigated by the study showed 160 protein spots in the honeydew honeys and 84 spots in the blossom honeys, which provided the correct separation of the six samples analyzed. Additionally, based on the free amino acids profile, it was also possible to distinguish the geographical origin of bracinga honeydew honeys evaluated by Azevedo, Seraglio, et al. (2017).

Regarding the adulteration of honeydew honeys, few studies have been reported. In their work, Oroian, Ropciuc, et al. (2018) used the

Raman spectroscopy to detect honey adulterated with fructose, glucose, inverted sugar, hydrolyzed inulin syrup, and malt must. The total of 56 pure samples was evaluated, including honeydew honeys (unknown origin) and blossom honeys and 900 adulterated samples. The Raman spectra of honeys were analyzed with the aid of chemometric analysis and the proposed method can be considered easy and rapid for honey adulteration detection. In the study developed by Ruiz-Matute et al. (2007), carbohydrate composition of 20 samples (16 blossom honeys and four honeydew honeys) and six syrups were evaluated by GC – MS to detect differences between both honeys. The presence of difructose anhydrides was observed only in the syrup samples and the authors concluded that this compound could be considered as an adulteration marker when detected in samples of blossom and honeydew honeys.

The current challenge is to develop fingerprinting and profiling methods for the authentication of honey. In this way, some studies have already been published, being able to differentiate botanical origins for blossom honeys (Aliferis, Tarantilis, Harizanis, & Alissandrakis, 2010; Kuš & Van Ruth, 2015). However, there are no reports until the moment related to fingerprinting in honeydew honeys. This fact stimulates studies in this area, especially looking for fast, easy, and low-cost authenticity evaluation methods for honeydew honeys, since the authentication and traceability of food products are of great interest of consumers.

## 6. Conclusions

Honeydew honeys have become a commercially attractive product due to their peculiar physicochemical, sensory and therapeutic characteristics. In this review, the parameters of identity and quality, the nutritional and bioactive composition, the functional and health-promoting properties, contamination, adulteration and authenticity of honeydew honey have been described. The presence especially of phenolic compounds, minerals, amino acids and proteins in honeydew honeys suggest possible beneficial health effects of this product, reinforcing the importance of further research, especially *in vivo*, for a better understanding of the beneficial properties of this honey,

stimulating its consumption and application as a potential functional ingredient.

Therefore, further research on honeydew honeys and their physicochemical properties and composition are required to guarantee the quality, safety and authenticity of this product and to verify its functional and health properties. This work may contribute with knowledge about honeydew honey and consequently to encourage research within the subjects covered here, increasing the productive chain and providing greater quality control of these products for safe consumption.

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