Advanced Characterization of Monofloral Honeys from Romania

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Abstract: Honey’s authenticity is a major concern for producers and consumers, and this prompts research into reliable methods to determine the source of honey (botanical and geographical). This study aimed to find the botanical origin of seven samples of monofloral honey (acacia, thyme, tilia, rape, raspberry, mint and sunflower) based on pollen analysis and identification of the physicochemical characteristics of these types of honey. For these types of honey, the following parameters were determined: color, electrical conductivity, free acidity, moisture content, pH, hydroxymethylfurfural content and sugar content. Alongside pollen analysis, these methods succeeded in classifying the analyzed samples as monofloral honey. Non-destructive methods of analysis such as Fourier transform infrared spectroscopy, the determination of the rheological behavior of honey in the negative domain and the determination of the thermal behavior were also employed to characterize the honey samples. The best differentiation between samples was achieved in the spectral region between 950 and 750 cm$^{-1}$, which is of interest for carbohydrate analysis in IR spectroscopy. In the negative interval, the viscous and elastic modules intersected at different temperatures, these temperatures being influenced both by the moisture of the analyzed samples and the botanical origin.

Keywords: honey; physicochemical parameters; rheological properties; differential scanning calorimetry

1. Introduction

Honey is a natural product produced by honey bees that has many health benefits, which make it valuable among consumers. To ensure consumer safety, there must be standardized methods to identify the origin and quality of this product [1]. Honey contains a high level of carbohydrates, about 80%, most of which constitute glucose and fructose. Depending on the floral origin, honey contains small amounts of other sugars in addition to glucose and fructose [2]. Apart from sugars, honey contains about 200 other constituents such as amino acids, vitamins, minerals, enzymes, organic acids and phenolic compounds [3]. The quality of honey depends on its sensory, physicochemical and microbiological properties [4]. Sensory analysis can establish the organoleptic profiles of different types of honey and is an indicator in monitoring the impact of food on consumers [5]. Monofloral honey is often preferred by consumers, being a high-quality product with a special aroma and a refined, unique taste. Due to its high price and consumer preferences, honey is often not monofloral, either due to mixing with inferior-quality honey or incorrect labeling [6]. To confirm the authenticity of honey, its botanical and geographical origins must be identified. The geographical origin and the botanical source of honey, together with external conditions such as the methods of processing, packaging and storage, influence the physicochemical properties of this product [7]. Traditional analytical methods have shown that determining physicochemical parameters (free acidity, pH, conductivity) has limited scope for authenticating honey samples [7]. Therefore, there is a demand for alternative methods of analysis, besides traditional ones, with which to authenticate honey samples.
Fourier transform infrared spectroscopy (FT-IR) is one of the practical and fast methods for distinguishing honeys with different floral origins [8]. FT-IR spectroscopy can provide information about the chemical compounds in honey as it is a highly sensitive method of analysis, which obtains results that identify the product’s botanical origin.

The aim of this study was to authenticate seven honey samples from Romania using destructive methods (analysis of physicochemical parameters) and non-destructive methods including determination of the FT-IR spectra, determination of the rheological properties of authentic honey in the negative domain and analysis of the thermal behaviors of these types of honey.

2. Materials and Methods

2.1. Honey Samples

Acacia (Robinia pseudoacacia), thyme (Thymus spp.), sunflower (Helianthus spp.), rape (Brassica spp.), tilia (Tilia europaea), mint (Mentha piperita) and raspberry (Rubus idaeus) honey samples were collected from Romania and analyzed in this study. The honey samples were from 2018 and 2019 production and were collected from the northeast region of Romania. The harvesting period was chosen according to the flowering period of the botanical sources used by the bees. Honey samples were kept away from sunlight at room temperature prior to the analysis.

2.2. Melissopalynological Analysis

To identify their botanical origins, the seven honey samples were subjected to melissopalynological analysis using the method recommended by the International Commission for Bee Botany [9].

2.3. Physicochemical Analysis

2.3.1. Electrical Conductivity, Moisture Content, pH, Free Acidity and Hydroxymethylfurfural (HMF) Content

The physicochemical parameters of honey were determined using the methods proposed by Bogdanov et al. [10,11]. The devices used in this study were: HQ14d portable conductometer (Hach, Loveland, CO, USA) (electrical conductivity), Leica Mark II Abbé refractometer (Leica, Austin, TX, USA) (moisture content), Titroline easy device (Schott Instruments, Mainz, Germany) (free acidity), Mettler Toledo FiveGo pH meter (Mettler Toledo, Columbus, OH, USA) (pH) and Schimadzu UV-3600 UV-VIS-NIR spectrophotometer (Schimadzu Corporation, Tokyo, Japan) (HMF content).

2.3.2. Color

Two devices were used to determine the color of the honey samples, namely: CR-400 portable chroma meter (Konica Minolta, Tokyo, Japan) and Pfund HI portable photometer (Hanna instruments, Woonsocket, RI, USA). Color was analyzed with a CR-400 colorimeter using the CIEL*a*b* color space. The color examination was performed on a white background. This measures the color parameters L* (brightness) with values ranging from 0 (indicates black) to 100 (indicates white), a* (negative values indicate green and positive values indicate red) and b* (negative values indicate blue and positive ones indicate yellow). Brightness, chroma (relative saturation) and hue angle are the chromaticity attributes used by this technique to describe the color of honey [12]. The Pfund HI 96785 photometer was also used to determine the color of the samples, and for this device, the measured value was indicated on the Pfund scale. The Pfund photometer measures the intensity of the color of the honey samples, and the degrees of color are expressed in mm Pfund compared to a glycerol standard of analytical quality.

2.3.3. Determination of Sugar Composition

The sugar content was determined using 5 g of each honey sample. After weighing, 5 g honey was mixed with 40 mL water and stirred until dissolved, and then the solution
was transferred to a 100-mL volumetric flask containing 25 mL methanol. The solution was then brought to volume using distilled water. The apparatus used for this analysis was an HPLC instrument (Shimadzu, Kyoto, Japan), which was described along with the working method in the study by Pauliuc et al. [13].

2.4. FT-IR Analysis

The FT-IR analysis of honey samples was made in the mid-infrared region of 4000–650 cm\(^{-1}\). This analysis was made with a resolution of 4 cm\(^{-1}\) using a Nicolet iS-20 spectrometer (Thermo Scientific, Karlsruhe, Germany). The temperature at which the spectra were collected was 25 °C. For each of the seven samples that were placed on the ATR surface, the spectrum was collected using OMNIC software (version 32, Thermo Scientific).

2.5. Rheological Properties

The rheological properties of honey samples in the negative domain were determined using a Mars 40 rheometer (Thermo Haake, Karlsruhe, Germany). Ethanol (96%, v/v) was used as a recirculation solution for rapid cooling. A parallel plate system of 40 mm diameter was used at a gap of 1 mm. The temperature range in which measurements were made was −15 to −40 °C and the aim was to determine the point of intersection between the viscous and elastic moduli.

2.6. Differential Scanning Calorimetry

This thermal analysis method was performed with a TA Q100 differential scanning calorimeter (DSC) (TA Instruments, Newcastle, DE, USA). The temperature range between −60 °C and 100 °C was set to perform DSC measurements, using nitrogen as the purge gas at a flow rate of 20 mL/min. The heating rate was 10 °C/min and the analysis was performed as follows: after the samples were weighed in aluminum pans with a hole in the lid, they were hermetically sealed (1.5–1.8 mg) and left to balance at 25 °C. DSC runs were carried out to determine a range of thermal and thermodynamic parameters (\(T_g\), \(\Delta C_p\), mid-point glass transition temperature).

2.7. Statistical Analysis

The results were submitted to analysis of variance (ANOVA) using Statgraphics Centurion XIX software (trial version, Statgraphics Technologies, Inc., The Plains, VA, USA). Fisher’s least significant difference (LSD) procedure was used at the 95% confidence level.

3. Results and Discussion

3.1. Pollen Analysis

A honey sample must contain 45% pollen granules to be classified as authentic, with a known botanical source [14]. Pollen analysis was proposed by the International Commission on Bee Botany (IBBC) in 1970 and revised in 1978 [9]. This method is used to identify monofloral honey, the honey type that is preferred by consumers for its special properties. In this study, the highest percentage of pollen grains was identified in sunflower honey (90%), followed by acacia honey with a percentage of Rubus pollen (78%). The rape honey also had a high content of pollen grains (75%), followed by mint honey (68%). The lowest percentage of pollen grains was identified in thyme honey (35%; in the case of thyme honey, the minimum percentage of pollen grains allowed for its classification is 18%) [15].

3.2. Moisture Content

A 20% moisture content is the maximum value that Codex Alimentarius allows for honey [16]. Moisture affects the quality of honey because the amount of water present determines its stability. Moisture depends on the season, environmental conditions and way of harvesting, and it influences the shelf life of honey. A high moisture content leads to the fermentation of honey and a low content affects the viscosity and crystallization [17].
In this study, the values varied between 15.25 and 17.31%, as shown in Table 1. These values were in agreement with those obtained for honey samples from Serbia (14.2 and 20.2%), with an average of 16.5 ± 1.01% [18]. Albu et al. [19], meanwhile, reported values between 16.20 and 20.52% for acacia honey from Romania.

Table 1. Physicochemical parameters of seven types of honey (acacia, mint, raspberry, rape, sunflower, thyme, and tilia).

| Parameter                        | Acacia  | Mint    | Rape    | Raspberry | Sunflower | Thyme   | Tilia   | F Value |
|----------------------------------|---------|---------|---------|-----------|-----------|---------|---------|---------|
| L*                               | 44.01(3.9) a | 42.50(3.9) d | 36.44(3.9) b | 38.39(3.9) c | 33.60(3.9) a | 35.23(3.9) b | 37.76(3.9) c | 95.41 *** |
| h*ab                             | 84.48(0.83) c | 78.24(0.83) b | 92.87(0.83) d | 74.19(0.83) a | 82.37(0.83) c | 61.07(0.83) c | 83.21(0.83) a | 47.50 *** |
| C*ab                             | 24.99(0.25) d | 21.08(0.25) b | 18.33(0.25) a | 25.19(0.25) d | 23.09(0.25) c | 26.95(0.25) e | 29.04(0.25) f | 215.22 *** |
| Pfund, mm                        | 12.87(0.44) a | 63.86(0.44) b | 36.14(0.44) c | 63.36(0.44) e | 33.66(0.44) b | 39.60(0.44) d | 35.64(0.44) c | 1623.21 *** |
| pH                               | 4.31(0.04) b | 4.52(0.04) c | 4.11(0.04) a | 4.27(0.04) b | 4.04(0.04) a | 4.03(0.04) a | 4.05(0.04) a | 19.17 ** |
| Free Acidity, meq/kg             | 3.86(0.21) a | 33.17(0.21) b | 17.33(0.21) c | 24.06(0.21) f | 18.32(0.21) d | 19.51(0.21) e | 14.55(0.21) b | 1874.80 *** |
| Electrical conductivity, µS/cm   | 122.27(3.70) a | 601.92(3.70) b | 146.67(3.70) b | 518.76(3.70) f | 314.82(3.70) d | 242.53(3.70) c | 333.63(3.70) a | 2368.37 *** |
| Moisture, %                      | 15.96(0.17) b | 16.24(0.17) c | 17.31(0.17) d | 17.27(0.17) c | 16.95(0.17) d | 15.25(0.17) a | 16.75(0.17) c | 20.45 *** |
| HMF, mg/kg                       | 18.97(0.14) e | 10.52(0.14) f | 9.48(0.14) e | 6.51(0.14) a | 10.96(0.14) c | 19.12(0.14) e | 13.34(0.14) d | 1239.39 *** |
| Fructose, %                      | 37.19(0.36) f | 34.20(0.36) e | 35.23(0.36) d | 34.62(0.36) b | 34.53(0.36) d | 39.87(0.36) d | 33.60(0.36) e | 28.20 *** |
| Glucose, %                       | 25.93(0.28) b | 24.41(0.28) a | 36.09(0.28) a | 25.79(0.28) c | 26.15(0.28) c | 25.17(0.28) a | 30.61(0.28) d | 219.29 *** |
| Sucrose, %                       | 0.450(0.006) e | 0.300(0.006) d | ND           | 0.130(0.006) b | 1.37(0.006) f | 0.17(0.006) c | ND           | 7125.60 *** |
| Turanose, %                      | 0.17(0.002) d | 0.07(0.002) a | 0.29(0.002) f | 0.11(0.002) b | 0.12(0.002) c | 0.20(0.002) e | 0.36(0.002) f | 2270.48 *** |
| Maltose, %                       | 2.19(0.02) e | 2.25(0.02) f | 0.94(0.02) a | 1.84(0.02) e | 1.99(0.02) d | 2.34(0.02) f | 1.15(0.02) e | 849.50 *** |
| Trehalose, %                     | 1.15(0.02) c | 2.62(0.02) a | 0.99(0.02) e | 2.31(0.02) d | 3.17(0.02) f | 2.63(0.02) e | 1.45(0.02) c | 1452.81 *** |
| Melezitose, %                    | 1.32(0.01) a | 1.46(0.01) f | 0.63(0.01) a | 1.24(0.01) a | 0.98(0.01) e | 1.63(0.01) e | 0.75(0.01) b | 941.37 *** |
| Raffinose, %                     | 0.510(0.005) e | 0.620(0.005) f | 0.11(0.005) a | 0.44(0.005) d | 0.42(0.005) c | 0.73(0.005) f | 0.18(0.005) b | 2111.83 *** |
| F/G                              | 1.42(0.01) d | 1.39(0.01) d | 0.97(0.01) a | 1.33(0.01) c | 1.31(0.01) e | 1.53(0.01) b | 1.09(0.01) a | 223.58 *** |

Mean values and standard deviation in brackets, ND—not detected, **—p < 0.01, ***—p < 0.001, a–g—different letters in the same row indicate significant differences between samples (p < 0.001).

### 3.3. pH

Honey contains a certain level of microorganisms [20], and an indicator of possible microbial growth is its pH. Honey is an acidic product but a pH greater than 7 is favorable for microorganisms’ growth. A normal pH value for honey is between 3.2 and 4.5 [21]. However, this value depends on many factors, primarily on the chemical composition of honey. The physicochemical properties of honey depend on the botanical source and geographical origin; for instance, the pH of honey may depend on the pH of plant nectar visited by bees in different geographical regions, as well as soil pH [22]. In this study, the pH values of the honey samples ranged from 4.03 (thyme honey) to 4.52 (mint honey) (Table 1). Krishnasree and Ukkuru [23] reported values between 3.73 and 3.83 for Indian honey, while Ciulu et al. [24] reported a mean value of 4 for 40 samples of strawberry tree honey and Ciursă et al. [25] reported a mean value of 4.4 for authentic honey from Romania.

### 3.4. Free Acidity

The freshness of honey samples can be determined by analyzing the acidity, which is influenced by the organic acid content [1]. An increase in free acidity can occur over time with the deterioration of honey by fermenting carbohydrates, resulting in organic acids [26]. A value of free acidity above the limit allowed by law (50 meq/kg) may also occur in the case of honey fermentation, when in the presence of honey yeasts, acetic acid is produced from ethyl alcohol [16]. From the results presented in Table 1, it can be observed that all the honey samples were fresh, with values of free acidity below the limit of 50 meq/kg. Similar values, ranging from 12 to 24 meq/kg, were previously reported for acacia, clover, rape, linden and sunflower honey [27].
3.5. Electrical Conductivity

Electrical conductivity (EC) is a parameter that correlates with the pollen content of monofloral honey [28] and is often used in determining the botanical origin [29]. The electrical conductivity of honey is related to its botanical origin, acidity and mineral content [30]. According to an EU Council Directive [31], flower honey, with some exceptions, must have values \( \leq 0.800 \text{ mS/cm} \), while chestnut honey and honeydew honey must have values \( >0.800 \text{ mS/cm} \). The specific electrical conductivity range for mixed honey is \( 0.5\text{–}0.8 \text{ mS/cm} \), while a value less than \( 0.5 \text{ mS/cm} \) indicates a pure floral honey [32]. As can be seen in Table 1, the analyzed honey samples had an electrical conductivity that varied between 122.27 (acacia honey) and 601.92 \( \mu \text{S/cm} \) (mint honey). The findings showed that light-colored honey has a lower electrical conductivity than dark honey. In the literature, Chirsanova et al. [33] reported values for electrical conductivity between 161 and 775 \( \mu \text{S/cm} \) for honey samples from the Republic of Moldova, while Alygizou et al. [34] reported an electrical conductivity of 480 \( \mu \text{S/cm} \) for thyme honey and 260 \( \mu \text{S/cm} \) for orange honey from Greece.

3.6. Color

The visual impact that food has on consumers is very important, and therefore, the color of monofloral honey is a decisive parameter in establishing its quality [5]. Honey color presents variations depending on the contents of beta-carotene, chlorophyll and its derivatives, xanthophyll pigments, flavonoids and anthocyanins, and thus there is a correspondence between color and the total phenolic content and antioxidant activity. The color of honey depends on its botanical source [35,36], with dark honey richer in pollen, pigments, phenolics and minerals [37]. A brightness value (L*) of less than 50 indicates a dark honey, while honey with an L* value of more than 50 is considered lighter [38]. In this study, the lowest value of L* was identified in the case of sunflower honey (33.60) and the highest value with acacia honey (44.01) (Table 1). Kanbur et al. [39] reported that in chestnut honey samples, the L* values were between 38.39 and 47.03, and in highland honey, these varied between 65.58 and 80.37, while for sunflower honey, the L* value was 33.60. The color of honey samples varied between extra-white (acacia honey), white (sunflower honey), extra-light amber (tilia honey, thyme and rape) and light amber (mint and raspberry honey). In a study of 85 samples of honey from Spain, Manzanares et al. [40] reported values between 24 (white) and 140 mm Pfund (dark amber).

3.7. HMF

5-Hydroxymethyl-2-furaldehyde, or hydroxymethylfurfural (HMF), is a cyclic aldehyde produced by the acidic decomposition of monosaccharides that occurs naturally in products where water coexists with acidic monosaccharides. Another way in which it can form naturally is by condensing carbohydrates that have free amino groups, according to Maillard reactions. These reactions destroy amino acids, which form products without nutritional activity, sometimes with a toxic action [41]. HMF and its compounds occur in foods that contain carbohydrates either by acid-catalyzed dehydration of hexoses or by Maillard-type reactions (non-enzymatic browning reaction) [42]. The hydroxymethylfurfural content is the most known indicator of the quality and freshness of honey. The formation of this compound is due to a high temperature that causes the breakdown of reducing sugars in honey. When combined with an acid, fructose disintegrates and HMF is formed. This component forms slowly during honey storage, but forms very quickly if the honey is heated [43]. Hydroxymethylfurfural is closely related to the chemical properties of honey, such as the mineral content, pH and total acidity [44].

As can be seen in Table 1, the HMF content of the samples was between 6.51 (raspberry honey) and 19.12 mg/kg (thyme honey). This parameter was significantly influenced \((p < 0.001)\) by the botanical source. For all samples analyzed in this study, the HMF content was within the limit \((40 \text{ mg/kg})\) allowed by European legislation [31]. In the Greek thyme
honey studied by Alygizou et al. [34], the HMF content was between 2.4 and 13.9 mg/kg, and in orange honey, between 7.8 and 36.9 mg/kg.

3.8. Sugars

Honey contains large quantities of sugars, where the main monosaccharides represent about 75% of the sugar content and disaccharides 10–15%. Honey sugars result from the activity of various enzymes in nectar, and the predominant ones are glucose and fructose, but in addition to these, there are at least other 22 different sugars [45]. The content and composition of honey sugars are influenced by the botanical source, geographical origin, processing and storage conditions and also the climate [7]. Sugars influence the energy value and viscosity of honey. Honey sucrose is an indicator of honey’s maturity but also of its adulteration [1]. A high concentration of sucrose may be the result of an early harvest, where the sucrose has not been completely converted to glucose and fructose, or it may indicate the feeding of bees with sucrose syrup for a long time [1,38].

The fructose and glucose concentrations, as well as the F/G ratio, are used to classify honey by botanical origin. Fructose is the carbohydrate with the highest concentration in honey, but in the case of rape and dandelion honey, which crystallize faster, the concentration of glucose is higher than that of fructose [1]. Thyme honey has the highest fructose content, as can be seen in Table 1 (38.97%), followed by acacia honey (37.19%). The lowest fructose content was identified in tilia honey (33.65%), and rape honey had the highest glucose content (36.09%). The analyzed honey samples had a small percentage of sucrose (maximum 1.37%), which was below the 5% limit specified by Codex Alimentarius [16]. Turanose, maltose, trehalose, melezitose and raffinose were identified in all types of honey in small quantities. Anjos et al. [46] reported 37.96% fructose and 29.30% glucose contents for sunflower honey in Protogalia, along with 31.87% and 19.98% for thyme honey, respectively. Small amounts of turanose, maltose, trehalose, melezitose and raffinose were also identified in their study.

3.9. FT-IR Analysis

Medium infrared spectroscopy is a non-destructive analysis method by which, based on molecular vibrations, samples with different compositions or concentrations can be distinguished. The region between 4000 and 650 cm\(^{-1}\) is important in medium infrared spectroscopy because it provides information about the physical and chemical properties of the analyzed sample. IR radiation passes through the sample to be analyzed; part of the radiation is absorbed by it and another part is transmitted, resulting in a unique spectrum also known as the “molecular footprint” of the sample. Thus, this technique is useful for a wide range of analyses because no sample to be analyzed produces the same infrared spectrum [47]. The FT-IR spectra collected for the honey samples analyzed in this study are presented in Figure 1. Overall, the Romanian honey samples showed relatively similar spectra with small differences depending on the botanic origin. In the region of 3700 and 3000 cm\(^{-1}\), the absorption bands were due to the vibrations of the O–H functional group from the carbohydrates present in honey [48]. Between 3300 and 2800 cm\(^{-1}\), the spectra showed characteristic peaks at 3297 cm\(^{-1}\), which indicates the presence of water in the honey samples. Gok et al. [49] also identified in their study the presence of these peaks in the FT-IR spectrum of honey. The absorption peaks at 3000–2700 cm\(^{-1}\) corresponded to vibration of the C–H bonds that constitute the chemical backbone of the sugars. The peaks between 2400 and 2200 cm\(^{-1}\) that were recorded for the samples of rape and raspberry honey were attributed to changes in the amino group. The best differentiation between samples can be achieved in the spectral region of 1800–750 cm\(^{-1}\) because this region includes the bands between 950 and 750 cm\(^{-1}\), which are of interest for the analysis of carbohydrates in IR spectroscopy. Anjos et al. [46] and Gok et al. [49] reported that the peak between 1700 and 1600 cm\(^{-1}\) was present due to the bending vibrations of O–H in water, and the stretching vibrations of the functional groups C=O of fructose and the aldehyde H–C=O of glucose. Furthermore, the vibrations specific to the chemical structure
of carbohydrates (stretching vibrations of C–O, C–C and C–H and bending vibrations of C–H) were attributed to peaks between 1470 and 700 cm$^{-1}$. The peak at 1148 cm$^{-1}$ was specific to sucrose, 1087 and 1043 cm$^{-1}$ were correlated with both glucose and fructose and the peaks at 983 and 965 cm$^{-1}$ were specific to fructose [50].

Figure 1. FT-IR spectra of honey samples of different botanical origins: raspberry, mint, rape, sunflower, thyme, acacia and tilia.

3.10. Rheological Properties

Ciursă and Oroian [51] and Nguzen et al. [52] investigated the viscoelastic behavior of honey at temperatures in the negative range. Both studies had comparable results, and the authors identified an increase of $G'$ (elastic modulus) and $G''$ (viscous modulus). The temperature at which the viscous component dominates the elastic component is called the glass transition temperature. Glass transition is a reversible state that occurs within a certain temperature range. The viscous and the elastic moduli intersected at different temperatures depending on the moisture of the analyzed samples but also on the botanical origin. Following the intersection of the two moduli, a dominant elastic behavior was identified, the glassy state being characterized by a reduction of physicochemical reactions [52] because molecular movements were limited to rotations and vibrations, resulting in solid characteristics of the material [53]. Roos [54] observed that in the glass
transition state, changes were identified in terms of elastic and viscous moduli, volume, thermal expansion and dielectric properties. As can be seen in Figure 2, the glass transition temperature varied depending on the botanical origin of the analyzed honey sample but also on the moisture content. Thus, for acacia honey, the glass transition temperature was −19.8 °C, for tilia honey −23.2 °C, for rape honey −22.82 °C, for thyme honey −22.89 °C, for raspberry honey −22.16 °C, for mint honey −24.5 °C and for sunflower honey −31.38 °C. It can also be observed that, with the decrease of the applied temperature, there was an increase in the moduli from $10^3$ to $10^9$ Pa.

Figure 2. Rheological behaviors of acacia, rape, raspberry, sunflower, thyme, tilia and mint honey in negative temperatures.
3.11. Differential Scanning Calorimetry (DSC)

From the thermoanalytic curves of honey, two thermal phenomena can be observed. The first phenomenon takes place in the temperature range from −44 to −36 °C and is represented by a deviation of the baseline. This deviation is due to the change in heating capacity. It is a glass transition characterized by a glass transition temperature ($T_g$) taken at the beginning of the thermal effect. The second (relatively weak) endothermic phenomenon occurs in the temperature range from 40 to 90 °C and is called transition 2 [55]. $T_g$ values are mainly determined by the levels of glucose and fructose in honey [56]; the ratio of sugar depends on the floral source of honey and environmental conditions [56]. While the glass transition temperature is greatly influenced by the carbohydrate content, proteins and fats have minimal effects on $T_g$ [57]. The glass transition is related to the relaxation effect, the amplitude of which depends on the thermal history of the samples. When a substance does not crystallize, glassy transition is observed at low temperatures. The endothermic phenomenon that can occur in the temperature range of 40–90 °C may be due to melting of the water/sugars/starch complex or could be caused by a polymorphic form of sugars. This phenomenon disappears systematically after a heating/cooling/heating sequence [55]. As the temperature rises above $T_g$, some of the physical properties change: the free molecular volume increases, the heat capacity ($C_p$) increases, the thermal expansion coefficient increases, the dielectric coefficient increases, and the viscoelastic properties change [58].

In our work, $T_g$ values ranged from −38.54 °C (raspberry honey) to −45.82 °C (sunflower honey) (Table 2). By comparison, Nguyen et al. [52] reported in their study values for $T_g$ between −47 and −51 °C for four types of honey (Tulsi, Alfalfa and two varieties of Manuka honey derived from medicinal plants). Kim and Yoo [59], meanwhile, reported for Korean acacia honey a $T_g$ variation between −42.7 and −50.0 °C for a moisture content between 18.3 and 20.1%. The $T_g$ value is influenced by the moisture content of the honey due to the plasticizing effect of the water. As a result, $T_g$ generally decreases with increasing moisture content. Transition 2 took place in the temperature range between 43.14 °C (mint honey) and 47.14 °C (sunflower honey). Acacia honey does not show transition 2 due to the highly uneven ratio of fructose to glucose in this type of honey and the low crystallization rate (Figure 3). $\Delta C_p$ is a measure that characterizes amorphous material in terms of its fragility (or sensitivity) to thermal changes, and the moisture content influences this value [60]. Thus, strong liquids, which are not susceptible to thermal changes, have a low value for $\Delta C_p$, as opposed to fragile liquids, which are characterized by a high value. In this study, the lowest value for $\Delta C_p$ was in mint honey (0.27 J/(g·°C)) and the highest in thyme honey (0.70 J/(g·°C)). In the literature, Kim and Yoo [59] studied nine honey samples and reported values for $\Delta C_p$ between 0.52 and 0.66 J/(g·°C).

### Table 2. Thermal characteristics of honey samples.

| Honey Type | Transition 1 | Transition 2 |
|------------|--------------|--------------|
|            | $T_{Onset}$ (°C) | Heat Flow (W/g) $T_{Onset}$ | Heat Flow (W/g) $T_{End}$ | $T_{Mid}$-point (°C) | $\Delta C_p$ (J/(g·°C)) | $\Delta H$/g | $T_{Onset}$ (°C) | Heat Flow (W/g) $T_{Onset}$ | Heat Flow (W/g) $T_{End}$ | $T_{Mid}$-point (°C) | $\Delta C_p$ (J/(g·°C)) | $\Delta H$/g |
| Sunflower  | −45.82       | −0.24        | −40.48        | −0.32        | −43.13            | 0.47            | 1.06          | 41.98 | −0.47            | 52.31          | −0.56          | 47.14            | 0.58            | 4.38            |
| Raspberry  | −38.54       | −0.18        | −33.18        | −0.25        | −35.86            | 0.41            | 3.46          | 40.39 | −0.39            | 48.89          | −0.45          | 44.58            | 0.35            | 3.06            |
| Mint       | −41.03       | −0.17        | −38.35        | −0.22        | −39.66            | 0.27            | 1.88          | 40.98 | −0.37            | 45.38          | −0.48          | 43.14            | 0.65            | 5.20            |
| Thyme      | −41.11       | −0.23        | −35.18        | −0.35        | −38.16            | 0.70            | 0.90          | 40.66 | −0.44            | 48.79          | −0.62          | 44.72            | 1.06            | 10.16           |
| Rape       | −43.24       | −0.17        | −39.81        | −0.23        | −41.52            | 0.34            | 1.92          | 40.67 | −0.36            | 47.80          | −0.44          | 44.22            | 0.50            | 4.74            |
| Acacia     | −41.74       | −0.18        | −38.31        | −0.28        | −40.08            | 0.60            | 4.07          | −     | −                | −              | −             | −                | −              | −              |
| Tilia      | −43.14       | −0.23        | −39.30        | −0.29        | −41.22            | 0.32            | 1.53          | 39.84 | −0.40            | 49.40          | −0.51          | 44.62            | 0.66            | 5.03            |

Onset—start temperature with phenomena of glass transition, melting. Midpoint—temperature measured according to international graphical norm with phenomenon of glass transition. $\Delta H$—value of absorbed or emitted energy by phenomenon, characterized by phenomena of melting, crystallization and gelatinization. $\Delta C_p$—measure of baseline deviation of curve due to physical and chemical property modifications, characterized by phenomenon of glass transition [55].
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Figure 3. Typical thermograms for thyme, sunflower, mint, rape, acacia, tilia and raspberry honey samples, showing glass transition temperatures.

4. Conclusions

The analysis of pollen, together with the results obtained for the physicochemical parameters of the samples of tilia, raspberry, acacia, mint, sunflower, thyme and rape honey from Romania, classified the honey samples according to their botanical origin, as follows: the percentages of pollen grains above the limit of 45%, and of 35% in the case of thyme honey, confirmed that the samples were of monofloral honey. The color parameters, meanwhile, were in line with the botanical origin of the honey. The electrical conductivity below 0.5 mS/cm was an indicator of pure floral honey, while the content and composition of honey sugars corroborated the values reported by other authors for honeys of the same botanical source. Thus, the usefulness of determining physicochemical parameters, along with the sugar content, when classifying honey according to its botanical origin, was demonstrated. For all seven types of honey, the values determined for the physicochemical parameters were in accordance with the European standards for honey.

The characterization of the seven monofloral honey types was also completed by an investigation of the rheological and thermal properties, along with an analysis of the chemical composition by FT-IR. The analyzed honey samples showed relatively similar spectra with small differences depending on the botanical origin. The main sugars were identified in the region between 1470 and 700 cm⁻¹. In terms of rheological properties, the glass transition temperature varied depending on the botanical origin and the moisture content of the analyzed honey sample. DSC analysis also confirmed that $T_g$ is influenced by the moisture content of honey. Furthermore, this thermal analysis method showed that in the case of acacia honey, there is no transition 2, probably due to the highly uneven ratio of fructose to glucose and the low crystallization rate.
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References

1. Da Silva, P.M.; Gauche, C.; Gonzaga, L.V.; Costa, A.C.O.; Fett, R. Honey: Chemical Composition, Stability and Authenticity. Food Chem. 2016, 196, 309–323. [CrossRef] [PubMed]
2. Rahman, M.M.; Alam, M.N.; Fatima, N.; Shahjalal, H.M.; Gan, S.H.; Khalil, M.I. Chemical Composition and Biological Properties of Aromatic Compounds in Honey: An Overview. J. Food Biochem. 2017, 41, e12405. [CrossRef]
3. Kasprzyk, I.; Depciuch, J.; Grabek-Lejko, D.; Parlinska-Wojtal, M. FTIR-ATR Spectroscopy of Pollen and Honey as a Tool for Unifloral Honey Authentication. The Case Study of Rape Honey. Food Control 2018, 84, 33–40. [CrossRef]
4. do Nascimento, K.S.; Sattler, J.A.G.; Macedo, L.F.L.; González, C.V.S.; de Melo, I.L.P.; da Silva Araújo, E.; Granato, D.; Sattler, A.; de Almeida-Muradian, L.B. Phenolic Compounds, Antioxidant Capacity and Physicochemical Properties of Brazilian Apis Mellifera honeys. IWT-Food Sci. Technol. 2018, 91, 85–94. [CrossRef]
5. Carpenter, R.P.; Lyon, D.H.; Hasdell, T.A. Guidelines for Sensory Analysis in Food Product Development and Quality Control; Aspen Publishers, Inc.: Gaithersburg, MD, USA, 2000.
6. White, J.W. Isotope Ratio Testing of Honey: Demystifying the Internal Standard Test. Am. Bee J. 2000, 140, 318–321.
7. Escuredo, O.; Dobre, I.; Fernández-González, M.; Seijo, M.C. Contribution of Botanical Origin and Sugar Composition of Honeys on the Crystallization Phenomenon. Food Chem. 2014, 149, 84–90. [CrossRef] [PubMed]
8. Jandrić, Z.; Haughey, S.A.; Frew, R.D.; McComb, K.; Galvin-King, P.; Elliott, C.T.; Cannavan, A. Discrimination of Honey of Different Floral Origins by a Combination of Various Chemical Parameters. Food Chem. 2015, 189, 52–59. [CrossRef] [PubMed]
9. Louveaux, J.; Maurizio, A.; Vorwohl, G. Methods of Melissopalynology. Bee World 1978, 59, 139–157. [CrossRef]
10. Bogdanov, S.; Lullmann, C.; Martin, P.; Von Der Ohe, W.; Russmann, H.; Vorwohl, G. Honey Quality, Methods of Analysis and International Regulatory Standards: Review of the Work of the International Honey Commission; Swiss Bee Research Centre: Bern, Switzerland, 1999.
11. Bogdanov, S.; Martin, P. Honey Authenticity. Mitt. Lebensm. Hyg. 2002, 93, 232–254.
12. Tuberoso, C.I.G.; Jerković, I.; Sarais, G.; Congiu, F.; Marijanović, Z.; Kuš, P.M. Color Evaluation of Seventeen European Unifloral Honey Types by Means of Spectrophotometrically Determined CIE \( L^*C^*a^*b^* \) Chromaticity Coordinates. Food Chem. 2014, 145, 284–291. [CrossRef]
13. Pauliuc, D.; Dranca, F.; Oroian, M. Antioxidant Activity, Total Phenolic Content, Individual Phenolics and Physicochemical Parameters Suitability for Romanian Honey Authentication. Foods 2020, 9, 306. [CrossRef] [PubMed]
14. Siddiqui, A.J.; Musharraf, S.G.; Choudhary, M.I.; Rahman, A. Application of Analytical Methods in Authentication and Adulteration of Honey. Food Chem. 2017, 217, 687–698. [CrossRef] [PubMed]
15. Karabagias, I.K.; Badeka, A.; Kontakos, S.; Karabournioti, S.; Kontominas, M.G. Characterization and Classification of Thymus capitatus (L.) Honey According to Geographical Origin Based on Volatile Compounds, Physicochemical Parameters and Chemometrics. Food Res. Int. 2014, 55, 363–372. [CrossRef]
16. Codex Stan 12-1981; Codex Alimentarius Commission Standards. Codex Alimentarius Commission: Rome, Italy, 2001.
17. Singh, I.; Singh, S. Honey Moisture Reduction and Its Quality. I. Food Sci. Technol. 2018, 55, 3861–3871. [CrossRef] [PubMed]
18. Prica, N.; Živkov Baloš, M.; Jakšić, S.; Mihaljev, Ž.; Kartalović, B.; Babić, J.; Savić, M. Moisture and Acidity as Indicators of the Quality of Honey Originating from Vojvodina Region. Arch. Vet. Med. 2015, 7, 99–109. [CrossRef]
19. Albu, A.; Radu-Rusu, C.G.; Pop, I.M.; Frunza, G.; Nacu, G. Quality Assessment of Raw Honey Issued from Eastern Romania. Agriculture 2021, 11, 247. [CrossRef]
20. Snowdon, J.A.; Cliver, D.O. Microorganisms in Honey. Int. J. Food Microbiol. 1996, 31, 1–26. [CrossRef]
21. White, J.W. Spectrophotometric Method for Hydroxymethylfurfural in Honey. J. Assoc. Off. Anal. Chem. 1979, 62, 509–514. [CrossRef]
22. Gheldof, N.; Engeseth, N.J. Antioxidant Capacity of Honeys from Various Floral Sources Based on the Determination of Oxygen Radical Absorbance Capacity and Inhibition of in Vitro Lipoprotein Oxidation in Human Serum Samples. J. Agric. Food Chem. 2002, 50, 3050–3055. [CrossRef]
23. Krishnasree, V.; Ukkuru, P.M. Quality Analysis of Bee Honeys. Int. J. Curr. Microbiol. Appl. Sci. 2017, 6, 626–636. [CrossRef]
24. Ciuruș, P.; Pauliuc, D.; Dranca, F.; Rocpiciu, S.; Oroian, M. Detection of Honey Adulterated with Agave, Corn, Inverted Sugar, Maple and Rice Syrups Using FTIR Analysis. Food Control 2021, 130, 108266. [CrossRef]
25. Crăciun, M.E.; Pârvulescu, O.C.; Donise, A.C.; Dobre, T.; Stanciu, D.R. Characterization and Classification of Romanian Acacia Honey Based on Its Physicochemical Parameters and Chemometrics. Sci. Rep. 2020, 10, 20690. [CrossRef] [PubMed]
26. Ratiu, I.A.; Al-Suod, H.; Bukowska, M.; Ligot, M.; Buszewski, B. Correlation Study of Honey Regarding Their Physicochemical Properties and Sugars and Cyclitols Content. Molecules 2020, 25, 34. [CrossRef] [PubMed]
28. Kaškoniene, V.; Venskutonis, P.R.; Ėckstteryte, V. Carbohydrate Composition and Electrical Conductivity of Different Origin Honeys from Lithuania. LWT-Food Sci. Technol. 2010, 43, 801–807. [CrossRef]

29. Terrab, A.; González, A.G.; Díez, M.J.; Heredia, F.J. Characterisation of Moroccan Unifloral Honeys Using Multivariate Analysis. Eur. Food Res. Technol. 2003, 218, 88–95. [CrossRef]

30. De-Melo, A.A.M.; Estevinho, L.M.; Moreira, M.M.; Delerue-Matos, C.; de Freitas, A.d.S.; Barth, O.M.; de Almeida-Muradian, L.B. A Multivariate Approach Based on Physicochemical Parameters and Biological Potential for the Botanical and Geographical Discrimination of Brazilian Bee Pollen. Food Bioci. 2018, 25, 91–110. [CrossRef]

31. Commission, E. Commission Decision 2002/657/EC Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results. Off. J. Eur. Union 2002, L221, 8–36. [CrossRef]

32. Saxena, S.; Gautam, S.; Sharma, A. Microbial Decontamination of Honey of Indian Origin Using Gamma Radiation and Its Biochemical and Organoleptic Properties. J. Food Sci. 2010, 73, M19–M27. [CrossRef]

33. Chirsanova, A.; Capcanari, T.; Boistean, A.; Simiunic, R. Physico-Chemical Profile of Four Types of Honey from the South of the Republic of Moldova. Food Nutr. Sci. 2021, 12, 874–888. [CrossRef]

34. Aloygizou, A.; Grigorakis, S.; Gotsiou, P.; Loupassaki, S.; Calokerinos, A.C. Quantification of Hydrogen Peroxide in Cretan Honey and Correlation with Physicochemical Parameters. J. Anal. Methods Chem. 2021, 2021, 5554305. [CrossRef] [PubMed]

35. Mohamed, M.; Sirajudeen, K.N.S.; Swamy, N.S.; Sulaiman, S.A. Studies on the Antioxidant Properties of Tualang Honey of Malaysia. Afr. J. Tradit. Complement. Altern. Med. 2010, 7, 59–63. [CrossRef] [PubMed]

36. Castiglioni, S.; Astolfi, P.; Conti, C.; Monaci, E.; Stefano, M.; Carlioni, P. Morphological, Physicochemical and FTIR Spectroscopic Properties of Bee Pollen Loads from Different Botanical Origin. Molecules 2019, 24, 3974. [CrossRef] [PubMed]

37. Can, Z.; Yildiz, O.; Sahin, H.; Akuz Turumtay, E.; Silici, S.; Kolayli, S. An Investigation of Turkish Honeys: Their Physico-Chemical Properties, Antioxidant Capacities and Phenolic Profiles. Food Chem. 2015, 180, 133–141. [CrossRef] [PubMed]

38. Tornuk, F.; Karaman, S.; Ozturk, I.; Toker, O.S.; Tastemur, B.; Sagdic, O.; Dogan, M.; Kayacier, A. Quality Characterization of Artisanal and Retail Turkish Blossom Honeys: Determination of Physicochemical, Microbiological, Bioactive Properties and Aroma Profile. Ind. Crops Prod. 2013, 46, 124–131. [CrossRef]

39. Demir Kanbur, E.; Yuksel, T.; Atamov, V.; Ozcelen, A.E. A Comparison of the Physicochemical Properties of Chestnut and Highland Honey: The Case of Senoz Valley in the Rize Province of Turkey. Food Chem. 2021, 345, 128864. [CrossRef]

40. Manzanares, A.B.; Garcia, Z.H.; Galdon, B.R.; Rodriguez, E.R.; Romero, C.D. Physicochemical Characteristics of Minor Monofloral Honeys from Tenerife, Spain. LWT-Food Sci. Technol. 2014, 55, 572–578. [CrossRef]

41. Nozai, M.J.; Bernal, J.L.; Toribio, L.; Jimenez, J.J.; Martin, M.T. High-Performance Liquid Chromatographic Determination of Methyl Anthranilate, Hydroxymethylfurfural and Related Compounds in Honey. J. Chromatogr. A 2001, 917, 95–103. [CrossRef] [PubMed]

42. Belitz, H.-D.; Grosch, W. Sugars, Sugar Alcohols and Honey. In Food Chemistry; Springer: Berlin/Heidelberg, Germany, 1999; pp. 801–828. [CrossRef]

43. Shapla, U.M.; Solayman, M.; Alam, N.; Khalil, M.I.; Gan, S.H. 5-Hydroxymethylfurfural (HMF) Levels in Honey and Other Food Products: Effects on Bees and Human Health. Chem. Cent. J. 2018, 12, 35. [CrossRef]

44. Bath, P.K.; Singh, N. A Comparison between Helianthus Annuus and Eucalyptus Lanceolatus Honey. Food Chem. 1999, 67, 389–397. [CrossRef]

45. Kozłowicz, K.; Różyło, R.; Gladyszewska, B.; Matwijczuk, A.; Gladyszewski, G.; Chocyk, D.; Samborska, K.; Piekut, J.; Smolewska, M. Identification of Sugars and Phenolic Compounds in Honey Powders with the Use of GC–MS, FTIR Spectroscopy, and X-Ray Diffraction. Sci. Rep. 2020, 10, 16269. [CrossRef]

46. Anjos, O.; Campos, M.G.; Ruiz, P.C.; Antunes, P. Application of FTIR-ATR Spectroscopy to the Quantification of Sugar in Honey. Food Chem. 2015, 169, 218–223. [CrossRef]

47. Paulić, D.; Ciursa, P.; Ropciuc, S.; Dranca, F.; Oriora, M. Physicochemical Parameters Prediction and Authentication of Different Monofloral Honeys Based on FTIR Spectra. J. Food Compos. Anal. 2021, 102, 104021. [CrossRef]

48. Anguebes, F.; Pat, L.; Ali, B.; Guerrero, A.; Córdova, A.V.; Abatal, M.; Garduza, J.P. Application of Multivariable Analysis and FTIR-ATR Spectroscopy to the Prediction of Properties in Campeche Honey. J. Anal. Methods Chem. 2016, 2016, 5427526. [CrossRef]

49. Gok, S.; Severcan, M.; Goormaghtigh, E.; Kandemir, I.; Severcan, F. Differentiation of Anatolian Honey Samples from Different Botanical Origins by ATR-FTIR Spectroscopy Using Multivariate Analysis. Food Chem. 2015, 170, 234–240. [CrossRef]

50. Horvatinec, J.; Svečnjak, I. Infrared (FTIR) Spectral Features of Honey Bee (Apis mellifera L.) Hemolymph. J. Cent. Eur. Agric. 2020, 21, 37–41. [CrossRef]

51. Ciursa, P.; Oriora, M. Rheological Behavior of Honey Adulterated with Agave, Maple, Corn, Rice and Inverted Sugar Syrups. Sci. Rep. 2020, 11, 23408. [CrossRef]

52. Nguyen, H.T.L.; Panoyoyai, N.; Paramita, V.D.; Mantri, N.; Kasapis, S. Physicochemical and Viscoelastic Properties of Honey from Medicinal Plants. Food Chem. 2018, 241, 143–149. [CrossRef] [PubMed]

53. Sterling, L.H. Introduction to Physical Polymer Science, 4th ed.; John Wiley & Sons: Hoboken, NJ, USA, 2005.

54. Roos, Y.H. Glass Transition Temperature and Its Relevance in Food Processing. Annu. Rev. Food Sci. Technol. 2010, 1, 469–496. [CrossRef]
55. Cordella, C.; Antinelli, J.F.; Aurieres, C.; Faucon, J.P.; Cabrol-Bass, D.; Sbirrazzuoli, N. Use of Differential Scanning Calorimetry (DSC) as a New Technique for Detection of Adulteration in Honeys. 1. Study of Adulteration Effect on Honey Thermal Behavior. *J. Agric. Food Chem.* **2002**, *50*, 203–208. [CrossRef]

56. Ouchemoukh, S.; Schweitzer, P.; Bachir Bey, M.; Djoudad-Kadji, H.; Louailche, H. HPLC Sugar Profiles of Algerian Honeys. *Food Chem.* **2010**, *121*, 561–568. [CrossRef]

57. Jouppila, K.; Roos, Y.H. Glass Transitions and Crystallization in Milk Powders. *J. Dairy Sci.* **1994**, *77*, 2907–2915. [CrossRef]

58. Genin, N.; René, F. Analyse Du Rôle de La Transition Vitreuse Dans Les Procédés de Conservation Agro-Alimentaires. *J. Food Eng.* **1995**, *26*, 391–408. [CrossRef]

59. Kim, M.J.; Yoo, B. Glass Transition Temperature of Honey Using Modulated Differential Scanning Calorimetry (MDSC): Effect of Moisture Content. *J. Food Sci. Nutr.* **2010**, *15*, 356–359. [CrossRef]

60. Roos, Y.; Karel, M. Applying State Diagrams to Food Processing and Development. *Food Technol.* **1991**, *45*, 66–68.