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PHYSICAL PROPERTIES AND INFRARED CHARACTERIZATION OF HONEYS FROM WESTERN MACEDONIA

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Abstract

The principal physical characteristics and behavior of honey are due to its sugars, but the minor constituents – such as flavoring materials, pigments, acids, and minerals – are largely responsible for the differences among individual honey types. Some physical properties (surface tension, density, water content, refractive index, color, viscosity, and conductivity) were determined and analyzed for fourteen honey samples from North-Western Macedonia to evaluate their global behavior and comparison with other honey samples. The water content and Brix degree varied within the range of (12.75–18.59%) respectively (80.52-85.33). Water content in the honey samples was also determined by FT-IR analysis using a thin-film technique between two KBr pallet mirrors at 3490-2750cm^{-1}. Also, the deformation band of the water molecule is assigned at 1638 cm^{-1} and the very week but characteristic combination band at 2111cm^{-1}. The stretching vibrational modes of the OH groups are very broad and shifted towards lower frequencies as a consequence of many hydrogen bonds. Stretching vibrations (both, antisymmetric and symmetric) of the backbone of the saccharides (consisting of CH and CH2 groups) can be noticed at 2936 and 2850 cm^{-1}. Furthermore, a very characteristic fingerprint of honeys is registered in the region from 1417cm-1 till 900 cm^{-1} with a high intensive maximum in 1057 cm^{-1}.

***Keywords**: honey, FTIR, physical properties.*

1. Introduction

According to the Official Journal of the European Communities, 2001 and Codex Alimentarius Commission Standards, 2001, "honey stipulates a pure product that does not allow for the addition of any other substance". Honey is a sweet, viscous food substance produced by bees (the genus *Apis*) from the sugary secretions of plants - floral nectar or from secretions of other insects such as honeydew. The different types of honey depend on the nectar collected by the bees from flower and regional climatic conditions and gave the different composition of carbohydrates. Carbohydrates constitute about 95 to 97% of the dry weight of honey. Fructose and glucose are the most predominant sugars present at ratio 1.2:1.0 and they are responsible for most of the

physical and nutritional characteristics of honey. Relative percentages of each sugar in pure honey are: Fructose (33 – 43%), Glucose (25 – 35%) and Sucrose (1 – 2%). Honey is essentially concentrated aqueous solution of inverted sugars, but it also contains a very complex mixture of other saccharides, proteins, enzymes, amino acids, organic acids, polyphenols, vitamins and minerals [1].

While other methods are well known and used for the detection of adulteration in honey, we followed the possibility to separate honey by type and to detect possible adulteration with the use of simple physical methods (density, viscosity, conductivity, color) and FTIR spectroscopy [2].

Physical properties of honey are very important because they are influenced by crucial factors such as: the type of flowers, way of processing and most of all area of origin [3]. Their values are reflected in the high diversity of types of honey analyzed [4]. Using the different models, a correlation is found between some of the physical parameters [5]. The influence of temperature and water content on the rheological properties of honeys is studied [6,7,8]. Physicochemical characteristics and mineral contents are of great interest because they reflect the quality of different kinds of honey [9,10]. Infrared characterization of honey samples is used for the authentication botanical origin, quality control and determination of their geographical origin [11,12,13].

This work hypothesizes that some of the measured physical parameters can be used to classify honeys by type, and possibly to detect any adulteration. Among other methods for honey adulteration detection [2], we see that FTIR spectra of adulterated honey samples, because they are significantly different from the spectra of pure honey, allow easy identification of adulterated honey [14].

2. Materials and methods

2.1. Honeys are delivered as pure filtered honey directly from bee hive from 14 points in north/western Macedonia. Locations are chosen in order to cover broader region from manufacturers who guaranteed pure honey. All samples are collected in the same period in sterile glass holders, and are furthermore separated in smaller sterile tubing. For all honeys, manufacturers declared as best to their knowledge the harvesting region of the bees, or the type of the honey they gave us. In this way honeys are separated in three groups: flower, mixed and pine, as harvesting region, flower plains, pine forests, or mixed harvesting region. All honeys are held at temperature of 20 ℃ during the experimental time. Water used in preparation of honey solutions is miliQ water with conductivity less than 1 μS.

Before we started with experimental measurements all active researchers and several students were asked to give their first impressions on the honey samples (color, type, and smell) according to the separation groups established, flower, mixed or pine honey. This data and the given data from manufacturers are supplied in table 1 at the end of the document.

For the preparation of dissolutions, honeys are previously heated to 45 ℃ for 5 min and then cooled to room temperature after which the required w/w solution is made by slow stirring with a magnetic stirrer for a period of 30 minutes. Stirrer speed is increased in the last 10 min to achieve total homogeneity of the solution. The speed must be low enough so no bubbles are being introduced in the sample. After stirring, the solution is left to sit for 20 min prior to measurements.

2.2. Characterization methods

Physical characterization of the honeys is done using standard characterization methods described in *Harmonized methods of the International Honey Commission*, 2009 [15] and direct measurement methods as described in the following text. Prior to all measurements, honeys are heated up to 45℃ in order to melt any crystallization and then are cooled to 25 ℃ for measurements. The measurements for all methods are in multiples and deviations are represented in the results.

2.2.1. The density of honey is measured using DCAT 11 tensiometer and SCAT software with a density measuring immersion probe. The density of the honeys is determined in pure honey without any dissolution. Prior to density measurement samples are left to sit for 10 minutes to minimize any perturbation. All samples are measured in triplets to achieve consistency. With this method, the high viscosity of the honey does not introduce errors or problems in the measurement procedure.

2.2.2. Viscosity measurements are gathered using a digital Brookfield viscometer with a cylindrical probe. All measurements are done at a controlled temperature using a thermal bath at$25℃ \pm 0.5℃$. To achieve good results after immersion of instrument probe all samples are left to sit for 10 min prior to measurement.

2.2.3. Surface tension via, the surface contact angle of the honey is measured using a Wilhelmy plate method with DCAT 11 tensiometer. The surface tension is measured with diluted honey from 60 to 10% w/w honey to Milli-Q water. All samples are left to sit for 10 min prior to measurement. The Wilhelmy plate used is platinum plated with a wetting length of 19.2mm (per side), a thickness of 0.2 mm, and a height of 10 mm. prior to each measurement the plate is thoroughly cleaned and dried.

2.2.4. Refractive index measurements, water contend and Brix angle are measured and derived using the Abbe refractometer. Refractive indexed is measured at 25 ℃ and correction for temperature is made as $Ri(T) = Ri(20C) + 0.00023(T - 20)$. Water content is determined using the following equation $Wc = \frac{1.73190 - \log(R.I. - 1)}{0.002243}$ [16, 17]. Brix angle is determined as a refractometric dry substance as $\text{Brix} = -2512 + 3121.12 * R.I. + 927.13 * R.I^2$ equivalent to sucrose content in water [18].

2.2.5. Conductivity is measured using a conductivity probe working at 4kHz and a resolution of 1 µS. Prior/Before to measurement conductivity probe is calibrated using KCl solutions at different concentrations. The conductivity of honey was measured for several samples for pure honey and diluted to 10% w/w with Milli Q water. It was observed that for all trials conductivity at 100% and 10% honey was the same so for further measurements conductivity was measured at 10% w/w honey samples.

2.2.6. Optical measurements are done using Ocean Optics diffraction grating spectrophotometer. Color measurements are attained in transmission in a cuvette with a 10 mm optical path. The light source is a halogen lamp and color is measured with the Spectra Suit software of the spectrophotometer by integration in the optical region 450-750nm. Tricolor maps and data are gathered for all samples but we choose to represent data via CIE values L*, a*, b* way. Here L scale represents: light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light; a* scale represents: red vs. green where a positive number indicates red and a

negative number indicates green and b* scale represents: yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.

2.2.7. Infrared spectra were obtained by using a Fourier Transform Infrared (FTIR) spectrometer, Nicolet 6700 Spectroscopy System, a Thermo Scientific production, with high energy resolution. An MCT (mercury cadmium telluride) detector was used to obtain the absorption spectra in transmission geometry. For recording the spectra at the interval of 4000 – 500 cm^{-1}, we used the thin-film technique between two KBr pallet mirrors. The evaluation of the spectra was carried out with the OMNIC program, divided the whole spectrum into three intervals: (3700 – 2700 cm^{-1}), (2700 – 1700 cm^{-1}) and (1700 – 600 cm^{-1}) – the fingerprint region.

3. Results

Average values of results for all measured physical parameters are given in table 2 at the end of the document. Here, where possible we proposed values for separation of honey by type from the values of the measured physical parameters.

Density is a parameter that is easy to measure and gives great separation of honeys by type, we concluded that a natural separation value from flower to mixed is around 1.44 g/cm^3 while the separation value from mixed to pine is around 1.49 g/cm^3. This can be seen from the data in table 1 and figure 1. The discrepancy can be seen in samples 6 and 11, where the manufacturer stated mixed and flowers accordingly, while our measurements put sample 6 as flower and sample 11 as mixed.

Conductivity is another parameter that is very easy to measure and can be used for direct separation criteria for type of honey. Conductivity varied in range from 100 to 800 μS. We concluded that the natural separation value from flower to mixed is around conductivity of 250 μS, while the separation value from mixed to pine is around 500 μS. This can be seen from the data in table 1 and figure 2. Here the only discrepancy is sample 10, that the manufacturer declared as flower, while our measurement puts it as mixed.

The color measurements shown in figure 3 didn't give clearly visible separation of honey by type, but they gave qualitative pointers of what can be expected and observed. Flower honeys have a blue and green hue (negative values of b* and a*), with high L* value, while pine honeys have a yellow and red hue (positive b* and a*) with low L*, whilst mixed honeys are in between. The L* value indicating light and dark corresponds very well with the first impressions of the researchers about the honey.

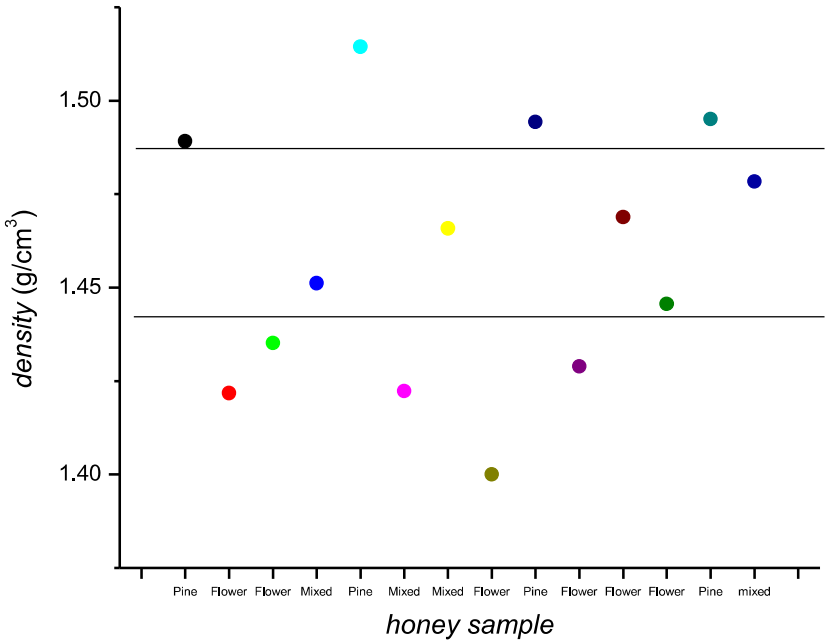

Figure 1. Density separation of honey by type

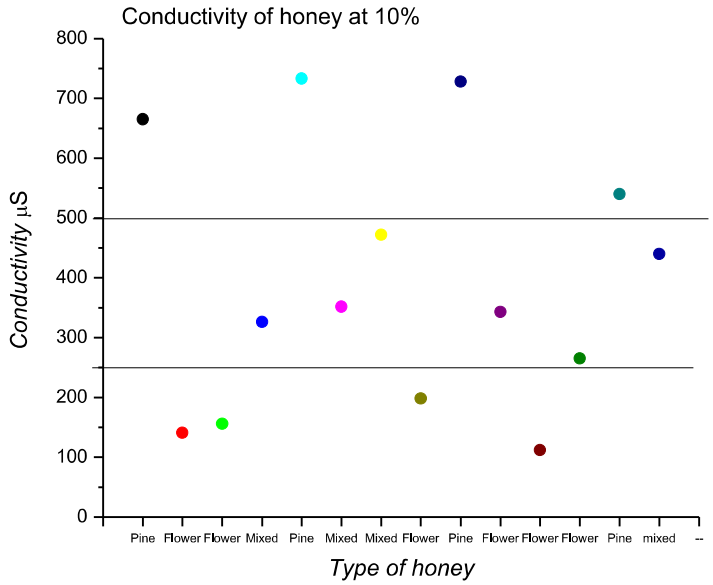

Figure 2. Conductivity separation of honey by type

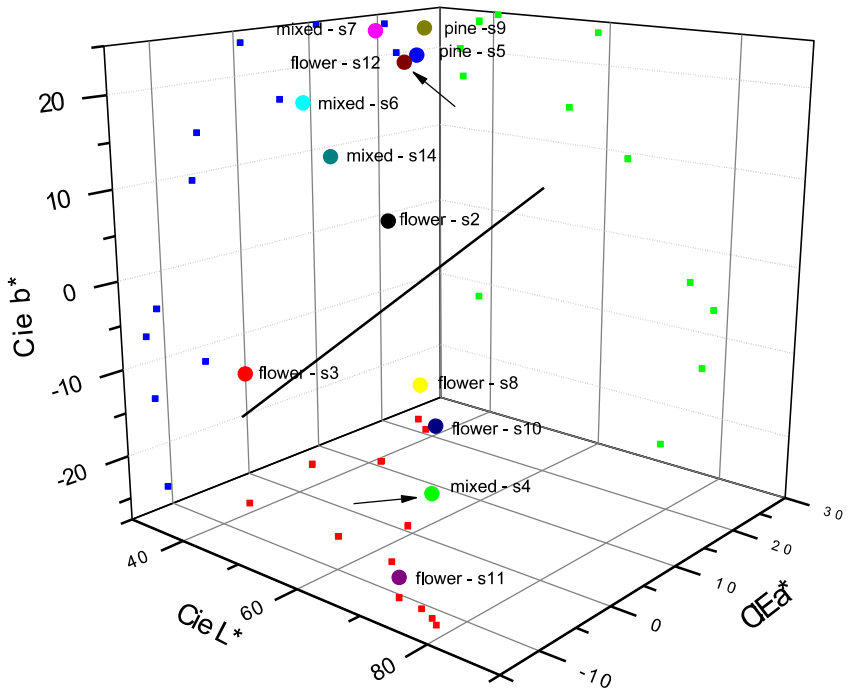

Figure 3. CIE (L*, a*, b*) color map of honey samples. Flower honeys have high L, while pine honeys have low L

The results of the viscosity measurement shown in comparison to honey sample and water content in figure 4 cannot be used for the classification of honey, but they show that viscosity of honey has a linear trend with water content in the region from 14-18% water content.

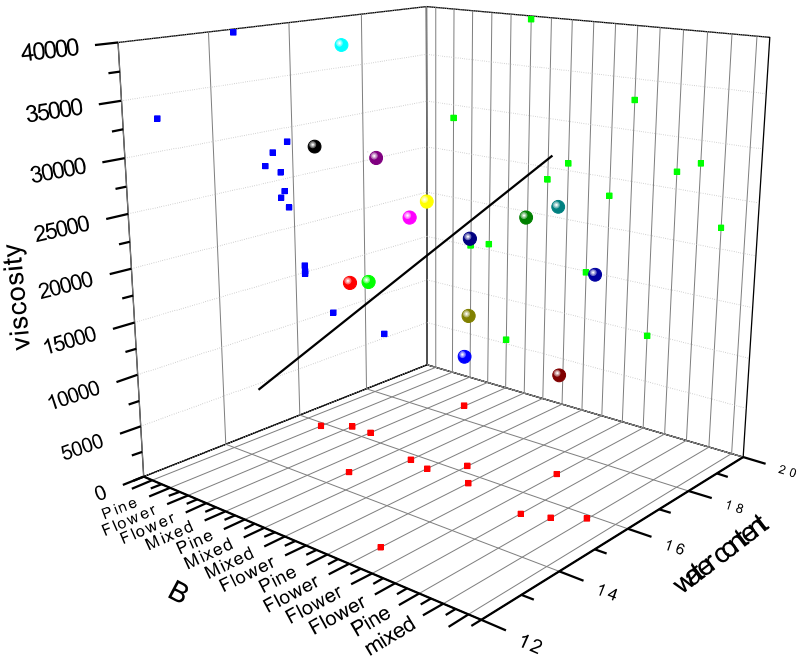

Figure 4. Viscosity and water content correlation for honey type. Viscosity has a linear dependence of water content in the range 14-18% water content

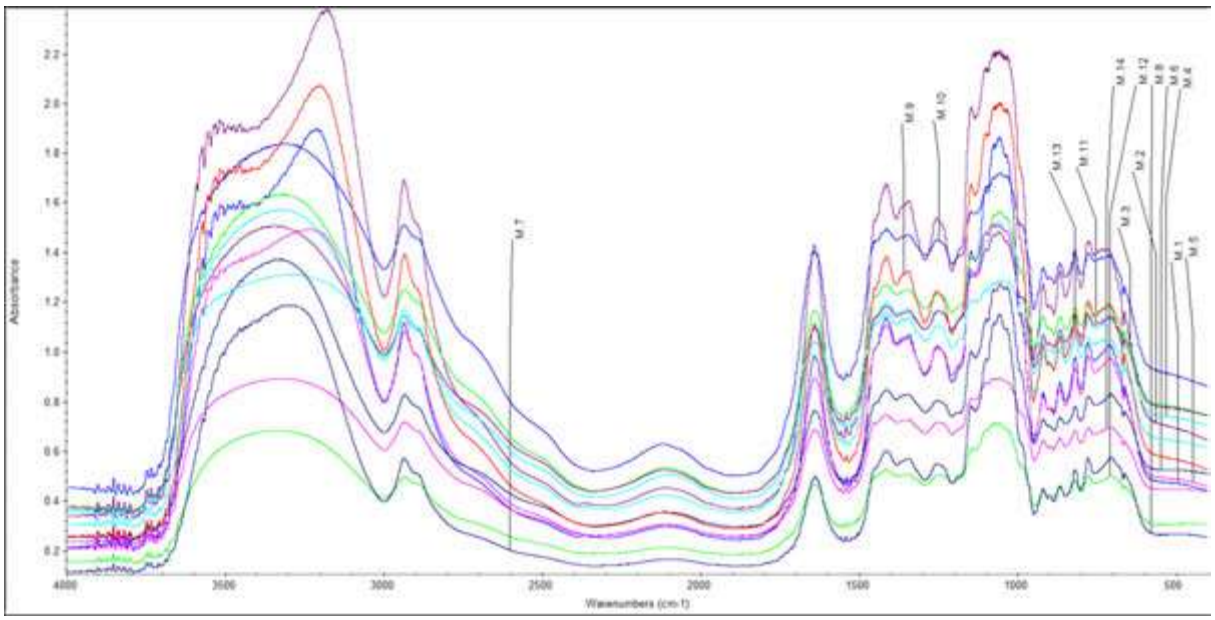

Figure 5. FTIR spectra of 14 pure honey samples from north western part of Macedonia

The various spectral features occur in different regions of the honey spectrum and the chemical structure data belong essentially to two different spectral intervals, (3700 – 2700 cm^{-1}) and (1700 – 600 cm^{-1}) – the fingerprint region. The FTIR spectra of the 14 samples were recorded on the

same day at room temperature (20 °C) without prior sample processing and all FTIR spectra are shown in Figure 5.

The absorption bands in the first zone 3700 and 3000 cm^{-1} are due to stretching vibrations of the functional group -OH from carbohydrates and water presents in the honey samples. Because honey is mostly consisting of saccharides the stretching vibrational mode of the OH groups is very broad and shifted towards lower frequencies as a consequence of many hydrogen bonds. The absorption band at 3000–2700 cm^{-1} corresponds to the stretching vibration of bonds C-H that constitutes the chemical structure of sugars and this band is represented as an asymmetric doublet. Also, stretching vibrations (both, antisymmetric and symmetric) of the backbone of the saccharides (consisted of CH and CH_2 groups) can be observed at 2936 and 2850 cm^{-1}.

The mid-zone shows the absence of triple bonds. On the other side, it can be noticed in all spectra very broad bands with very low intensity in the region from 2250-2100 cm^{-1} which is due to the combinational vibrations of the water molecules. The bands in the zone 1700–1600 cm^{-1} are due to the bending vibrations of -OH from water (1642–1638 cm^{-1}) and stretching vibrations of functional groups C=O of fructose and aldehyde CH=O of glucose. In the fingerprint region, multiple absorbance bands are present; the bands between 1600 and 600 cm^{-1} are due to the stretching vibrations of bonds C-O, C-C, and C-H and the bending vibrations of C-H present in the chemical structure of carbohydrates. A very characteristic fingerprint of honeys is registered in the region from 1417cm^{-1} till 900 cm^{-1} with a high intensive maximum at 1057 cm^{-1}, which probably belongs to fructose.

From the evaluation of FTIR spectra, it turns out that three of them can be grouped based on the relative intensity characteristics of the spectral lines: samples M1, M5, and M9 are shown in Figure 6. The spectra of the samples M1 and M5 after 3000 cm^{-1} are almost superimposed with each other in frequency and intensity. The main difference of this group is that in the 2940 cm^{-1} to 2850 cm^{-1} region there are two spectral lines presented as an asymmetric doublet, where the first line has higher intensity. Also, doublets appeared in the 1420 cm^{-1} to 1340 cm^{-1} zone, where we observed again an asymmetric doublet. In the interval between 1150 – 1000 cm^{-1} we observed a very large band (which belongs to Fructose and Glucose) where samples M9 and M1 are the most intensive, maybe to a higher percent of Fructose and Glucose.

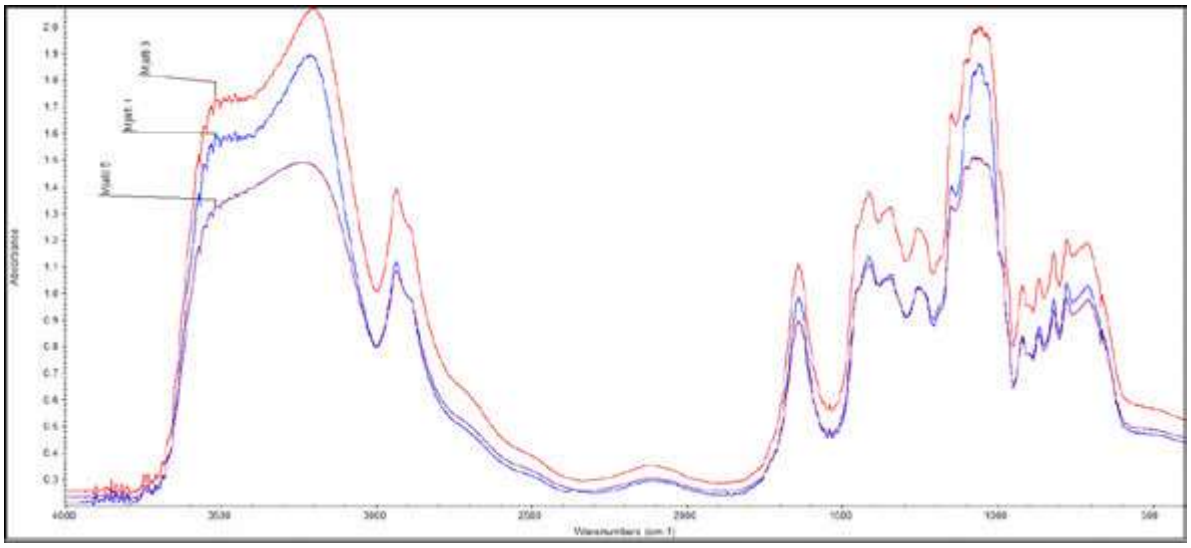

Figure 6. FTIR spectra of pure honey samples M1, M5 and M9

4. Discussion

After data analysis, it was found that some of the physical parameters: density, conductivity, and color, are very easy to measure and give good separation of honey buy type. On the other hand, viscosity and refractive index have medium measurement difficulty; they give applicable results but not well for honey classification. The surface tension of the honey is hard to measure and for our need, it gives no applicable results. Furthermore, creating a much larger database of points for different honey types is necessary to confirm the proposed separation criteria for the honey type from the measured physical property.

From the evaluation of FTIR spectra it turns out that three of them can be grouped obased on the basis of the relative intensity characteristics of the spectral lines: samples M1, M2, M5 and M9 are shown in Figure 6. The spectra of the samples M1 and M5 after 3000 cm^{-1} are almost superimposed with each other in frequency and intensity. The main difference of this group is that in the 2940 cm^{-1} to 2850 cm^{-1} region there are two spectral lines presented as an asymmetric doublet, where the first line has higher intensity. Also, doublets appeared in the 1420 cm^{-1} to 1340 cm^{-1} zone, where we observed again an asymmetric doublet. In the interval between 1150 – 950 cm^{-1} we observed very large band (which belong to Fructose and Glucose) where samples M9 and M1 have the most intensive band, may be to higher percent of Fructose and Glucose. In all spectra we observe relative small peak around 980 – 990 cm^{-1}, which represents the characteristic infrared line of Sucrose.

From the collection area we could conclude that the group of four Honey samples M1 (Restan, Kicevo), M5 (Dupjan, Kicevo) and M9 (Zajaz, Kicevo) belong to mountain - pine type.

Table 1. Regions where different types of honey are collected, including color and smell.

No.	Region	Type	Color	Smell
M1	Restan, Kicevo, Macedonia	Pine	Very Dark	Pine
M2	Restan, Kicevo, Macedonia	Flower	Very Light	Flower
M3	Restan, Kicevo, Macedonia	Flower	Light	Flower
M4	Trapcindoll, Kicevo, Macedonia	Mixed	Semi Dark	Flower
M5	Dupjan, Kicevo, Macedonia	Pine	Dark	Pine
M6	Prespa, Macedonia	Mixed	Semi Dark	Flower
M7	Makedonski Brod, Macedonia	Mixed	Semi Dark	Pine
M8	Mavrovo, Macedonia	Flower	Semi Light	Flower
M9	Zajaz, Kicevo, Macedonia	Pine	Very Dark	Pine
M10	Mount Sar, Tetovo, Macedonia	Flower	Light	Flower
M11	Trapcindoll, Kicevo, Macedonia	Flower	Very Light	Flower
M12	Bllaca, Skopje, Macedonia	Flower	Semi Light	Flower
M13	Zajaz, Kicevo, Macedonia	Pine	Dark	Pine
M14	Kumanovo, Macedonia	mixed	Semi Light	Pine

Table 2. Physical Properties of different types of Honeys

Honey Sample	Density g/cm³	Viscosity	Surface tension mN/m	Refractive index	Brix	Water Cont. %	Cond µS	Optical parameters		
								L	a	b
M1	1.4891	28600	45.875	1.497	882.62	115.87	665	--	--	--
M2	1.4218	15075	45.231	1.496	882.28	116.26	141	63.5	-5.72308	9.26154
M3	1.4352	15650	47.905	1.496	882.28	116.26	156	37.64	-5.1	-11.93
M4	1.4512	5450	49.489	1.491	880.52	118.59	326	76.56	-11.42	-14.4
M5	1.5144	39675.5	45.368	1.502	884.33	114.6	733	33.85	22.875	20.0625
M6	1.4223	23750	45.561	1.4975	882.79	115.77	352	34.38889	5.81111	16.63333
M7	1.4658	25725	49.355	1.498	882.97	115.67	472	40.87143	11.08571	24.68571
M8	1.4000	14825	45.476	1.4965	882.45	116.26	198	74.1375	-10.725	-4.2
M9	1.4943	23190	42.699	1.4975	882.79	115.67	728	37.4375	21.0875	23.7625
M10	1.4289	32950	46.515	1.505	885.33	112.75	343	77.95	-12.11667	-7.03333
M11	1.4688	9750	47.389	1.494	881.58	117.04	112	70.45	-10.46667	-25.28333
M12	1.4456	26650	43.578	1.4985	883.14	115.28	265	58.13333	0.9	23.66667
M13	1.4951	27790.5	44.319	1.498	882.97	115.48	540	--	--	--
M14	1.4784	22050	43.22	1.497	882.62	115.87	440	53.5875	-4.975	14.45

Conclusion

Our analysis concludes that some easily measurable physical properties and FTIR analysis can be used to separate honey by type of harvesting region. Some discrepancies are detected. They can be easily checked and a better classification can be derived if the number of samples is much higher. Increasing the number of samples is the future goal of our department. In this research according to its physical parameters only one honey, the M4 sample shows significant differences that can point out to adulteration of the honey.

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