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Physicochemical parameters of Bulgarian monofloral rape and lime honeys

E. Mladenova[1*], R. Balkanska[2], Ts. Voyslavov[1]

[1]*Sofia University "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, Department of Analytical Chemistry, 1, James Bourchier Blvd., 1164 Sofia, Bulgaria*
[2]*Institute of Animal Science, Department "Special Branches – Bees", Spirka "Pochivka", 2232 Kostinbrod, Agricultural Academy, Bulgaria*

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The quality of human life is mainly determined by the quality and safety of consumed food. The purpose of the present study was physicochemical characterization of Bulgarian monofloral bee honeys. A total of 40 honey samples (rape n=14 and lime n=26) were analysed. Botanical origin of the honey samples was determined by mellisopalynological analysis. Color (mm Pfund), water content (%), electrical conductivity ($mS.cm^{-1}$), specific rotation ($[\alpha]^{20}_D$), pH, diastase (Gothe units), invertase ($U.kg^{-1}$), proline ($mg.kg^{-1}$), hydroxymethylfurfural (HMF, $mg.kg^{-1}$) were assessed in order to provide physicochemical characterization. In the present study monofloral rape and lime honeys meet the quality standards of European legislation for the parameters studied. The results confirmed that physicochemical properties of honeys were closely related to their floral sources. The honeys produced in Bulgaria are of good quality in relation to the studied parameters, confirming the general image of products with high quality. Significant linear correlations were confirmed between diastase and invertase in rape honey and also between color and electrical conductivity in lime honey.

Keywords: food science, lime honey, monofloral honey characterization, rape honey

INTRODUCTION

Honey is the natural product processed by honeybees (*Apis mellifera* L.) from flower nectars or plant secretions, by aid of their own secretions [1]. As a natural food, honey can provide various human health benefits and it plays an important role in human diet. Honey is a substance with complex composition. It primarily consists of sugars but it also contains mineral elements, vitamins, enzymes, organic acids, polyphenols, and amino acids [2, 3]. Honey can be classified as monofloral produced from the nectar and pollen of one dominant plant. It may contain smaller amounts of other nectars and pollens as well. The polyfloral honey is produced from nectar and pollen from various plants, none of which is predominant [4]. The honey floral source largely determines its physicochemical properties and health benefits [5]. Many monofloral honey types are known around the world. In Bulgaria black locust (*Robinia pseudoacacia* L.), lime (*Tilia* spp.), rape (*Brassica spp.*), sunflower (*Helianthus annuus*) honeys are among the commonest monofloral honey types. These honey types also differ in their physicochemical parameters. Different types of polyfloral or monofloral honeys are usually found on the market. In the recent years an increasing commercial interest to produce monofloral kinds of honey is being observed. A lot of consumers appreciate the possibility to choose between different types of monofloral honeys. For this reason, identification of monofloral honey deserves greater attention. In this respect there are not many data of the physicochemical properties of monofloral honeys. This is particularly relevant because monofloral honeys are classified as high-quality products because of their desirable flavor and taste. The present study was undertaken to characterize the physicochemical parameters of monofloral honeys (lime and rape) produced in Bulgaria. Research including full physicochemical characterization of so many monofloral honey samples is provided for the first time in Bulgaria.

MATERIALS AND METHODS

Samples

Forty honey samples (rape n=14 and lime n=26) were collected from guaranteed beekeepers with good practices in 2018 from different regions of Bulgaria (Table 1) where no industrial anthropogenic pollution is observed. The honeys were stored at room temperature in closed glass containers prior to analysis.

* To whom all correspondence should be sent:
E-mail: *elimladenova@chem.uni-sofia.bg*

Table 1. Sampling regions of Bulgarian rape and lime honey samples

Region	District	Locality	Monofloral honey
Southwestern	Sofia	Kostinbrod city	lime (n = 3)
Northeastern	Dobrich	Chestimensko village	lime (n = 3)
	Razgrad	Tochilari village	rape (n = 2)
		Ostrovo village	lime (n = 5)
		Medovene village	lime (n = 1)
		Brestovene village	lime (n = 3)
		Zavet city	lime (n = 1)
		Isperih city	lime (n = 1)
	Shumen	Kyolevcha	lime (n = 1)
	Silistra	Yordanovo village	rape (n = 1)
		Professor Ishirkovo village	rape (n = 1)
North Central	Veliko	Strazhica city	lime (n = 1)
	Tarnovo	Gorna Oryahovitsa city	lime (n = 1)
		Krusheto village	lime (n = 1)
	Gabrovo	Gradnitsa village	lime (n = 1)
	Ruse	Smirnenski village	rape (n = 6)
		Brestovitsa village	rape (n = 2)
		Senovo city	rape (n = 2)
		Nikolovo village	lime (n = 1)
		Pisanets village	lime (n = 1)
		Krivnya village	lime (n = 1)
Northwestern	Vidin	Belogradchik city	lime (n = 1)

Methods

Melissopalynological analysis was done according to the method established by the Bulgarian Institute for Standardization [6, 7].

Honey color was determined with Lovibond™ Honey ColorPod Handheld Photometer. Honey samples were measured in 1 cm path length. The results are presented in Pfund scale (mm Pfund).

Physicochemical parameters (water content, electrical conductivity, specific rotation, pH, diastase and invertase activity, proline and hydroxymethyl furfural (HMF) content were determined according to Bogdanov *et al.* [8] without any modifications. They are briefly presented as follows.

Water content was determined by the refractometric method using the Abbé refractometer and expressed in %.

Electrical conductivity of a honey solution at 20g/100g (dry matter) was determined using the conductometric method on Milwaukee Mi170 Autoranging. Electrical conductivity was expressed in $mS.cm^{-1}$.

Specific rotation was measured as approximately 12 g of honey sample (corresponding to about 10 g of dry matter) was dissolved in high-purity water. The solution was clarified with Carrez I and II solutions, filtered and read in a polarimeter (Disc Polarimeter with 589 nm sodium lamp).

The honeys' acidity (pH) was measured in 10% aqueous honey solution using a Mi150 pH meter.

Diastase activity was determined following the spectroscopic method of Shade at 660 nm. Diastase activity was expressed in Gothe units.

Invertase was determined by using *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) as a substrate. Five grams of honey was used for the analysis. The absorbance was measured at 400 nm (PG Instruments T60 UV/VIS spectrophotometer). The values were expressed as $U.kg^{-1}$.

Proline content in $mg.kg^{-1}$ was measured using a spectrometric assay with a spectrophotometer (PG Instruments T60 UV/VIS spectrophotometer) at 510 nm using 1 cm cuvettes.

Determination of the hydroxylmethyl-furfural (HMF) was based on the White method after clarification with Carrez I and II solutions. The absorbance was measured at 284 and 336 nm (PG Instruments T60 UV/VIS spectrophotometer).

The reagents needed for standard solutions preparation were of high analytical grade. All aqueous solutions were prepared in high-purity water (Millipore Corp., Milford, MA, USA).

Statistical analysis

All statistical analysis was performed by means of the SPSS software (IBM Corp.) version 23 for Windows. Pearson correlation analysis was applied to establish relationship among the physico-chemical parameters. The Pearson correlation coefficient is a statistical measure that quantifies the strength of a linear relationship between two variables.

RESULTS AND DISCUSSION

To determine the origin of honey, melissopalynological analysis was done before determining the physicochemical parameters of honey samples. Table 2 shows the basic statistics for the dominant pollen type in the honey samples. All of the honeys declared as lime contained from 30% to 78% of *Tilia spp.* pollen. The pollen content in rape honey is even higher (from 46% to 93%). Similarly, Kasprzyk *et al.* [9] found from 68% to 96% of rape pollen in monofloral honey samples. Tilia pollen in lime honey samples ranged from 20.04 – 41.4% (average 28.1%) according to Was *et a.l* [10].

Table 2. Pollen content in Bulgarian rape and lime honey samples

Honey type	Rape	Lime
n	14	26
Min	46	30
Max	93	78
Mean	66	58
SD	16	16

As can be seen from Table 2 rape and lime honey samples have high average content of specific pollen. The percentage of pollen was higher than what is considered to be minimal according to Bulgarian regulation documents [8] – 40% and 30% for rape and lime, respectively. Melissopalynological assay of honey pollen remains to be the fundamental tool for pollen analysis in honey.

The physicochemical properties of honeys are affected by their floral sources. In Table 3 are presented the obtained average values ± standard deviation for the following physicochemical parameters in Bulgarian monofloral rape and lime honeys – color, water content, electrical conductivity, specific rotation, pH, diastase, invertase, proline and hydroxymethyl furfural.

In general, rape and lime honey are bright honeys. Colour varied between 1 – 17 mm Pfund for rape honey and between 3 – 35 mm Pfund for lime honey. For all analysed samples the color of honey varied in short ranges. According to Persano Oddo and Piro [4] the color of rape honey expressed in mm of Pfund scale ranged from 20.0 to 34.3. Szczęsna *et al.* [11] presented a great variability for honey color (8 – 59 mm Pfund) for the same honey type.

Table 3. Physicochemical properties of Bulgarian rape and lime honey samples[a]

Parameter	Rape (n=14)	Lime (n=26)
Color [mm Pfund]	5.36 ± 4.58	17.81 ± 8.87
Water content [%]	17.38 ± 1.07	18.04 ± 0.94
Electrical conductivity [$mS.cm^{-1}$]	0.135 ± 0.024	0.646 ± 0.117
Specific rotation $[\alpha]^{20}_{D}$	-21.00 ± 3.21	-14.33 ± 2.89
pH	3.64 ± 0.19	4.30 ± 0.28
Diastase [Gothe units]	23.22 ± 3.39	17.06 ± 2.89
Invertase [$U.kg^{-1}$]	86.17 ± 28.04	116.39 ± 24.35
Proline [$mg.kg^{-1}$]	212.53 ± 55.39	343.66 ± 84.36
Hydroxymethyl furfural [$mg.kg^{-1}$]	4.95 ± 0.43	5.53 ± 0.49

[a]Average ± SD

The water content of the samples varied in the range of 15.8 to 19.4% for rape honey and from 16.2 to 19.8% for lime honey. In all collected honey samples water content was lower than the cut-off value at ≤ 20% for unfermented product [12]. The data obtained for water content of rape and lime monofloral honeys were in reasonable agreement with those obtained in previously published reports [13].

Electrical conductivity is an important criterion in determining the botanical origin of honey [14]. The results obtained in the honey samples showed a range of 0.106 – 0.201 $mS.cm^{-1}$ for rape honey and 0.295 – 0.786 $mS.cm^{-1}$ for lime honey. The values were below the maximum value of 0.8 $mS \cdot cm^{-1}$ provided for nectar honey [12]. According to European Directive [11] the values higher than 0.8 $mS.cm^{-1}$ can indicate honeydew honey. Electrical conductivities for rape and lime honeys were within similar ranges to those reported by other authors [11, 15].

In general, there is a strong positive correlation between electrical conductivity and the color of honey. This indicates that as one property increases, the other tends to do so as well. This is in agreement with the study of Chirsanova *et al.* [16]. They showed a correlation between electrical conductivity and color ($r=0.999$, $p<0.05$) in honey samples from different origins. In the present study a significant positive linear correlation ($r=0.427$; $p<0.05$) was found between electrical conductivity and color in lime honey (Fig. 1). These results are similar to those reported by Patrignani *et al.* [17]. Their study showed a correlation between honey color and electrical conductivity ($r=0.69$, $p<0.05$). The investigation was conducted with 25 honey samples not exactly monofloral. The moderate Pearson correlation coefficient observed in our study, while indicating a relationship between the variables, may be influenced by the possible mixing of lime honey with honeydew honey. Lime honey, known for its distinctive characteristics, may occasionally be mixed with honeydew honey during the collection process. Such mixing could introduce variability that would weaken the strength of the observed correlation. The results obtained contribute to our understanding of the relationship between color and electrical conductivity by highlighting key patterns and potential mechanisms. This suggests that while there may be some relationship between these parameters, other factors are likely to contribute to the observed variance in these parameters. Further investigation is required to explore these factors.

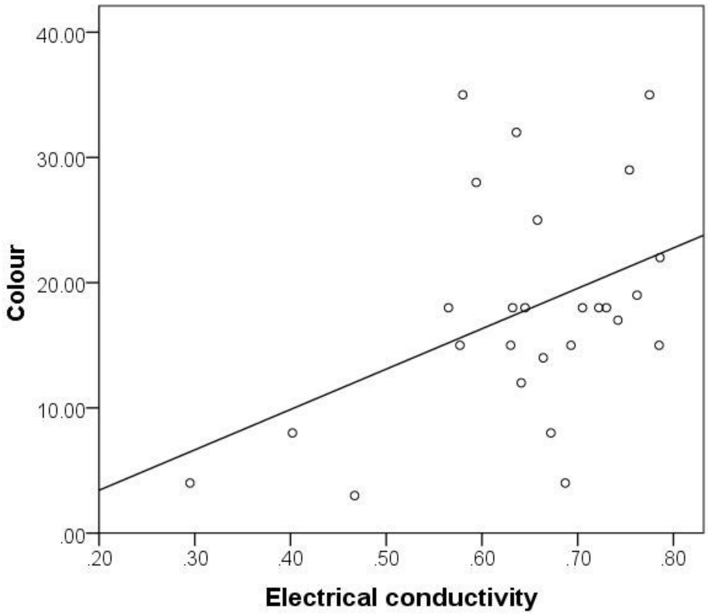

Figure 1. Linear correlation between color and electrical conductivity in Bulgarian lime honey.

Specific rotation $[\alpha]^{20}_D$ depends on the ratio between carbohydrates that rotate polarised light to the right or to the left. If the carbohydrates that rotate polarised light to the left prevail, the specific rotation of the honey sample is negative. In all analysed honey types specific rotation was negative. This also confirms that honey was of nectar origin. Negative specific rotation of nectar honeys results from the predominance of fructose which has a strongly negative specific rotation [18]. For the rape and lime honey samples specific rotation ranges from -27.50 to -17.50 $[\alpha]^{20}_D$ and from -20.00 to -10.50 $[\alpha]^{20}_D$, respectively. Zielińska *et al.* [18] found lower minimal and maximal values for specific rotation in lime and rape honey. This might be due to different percentage of sugars in the honey samples.

Results showed that color, electrical conductivity, and specific rotation are useful parameters which can be used to classify some monofloral honeys. Furthermore, very often they are used in routine honey quality control.

Although the pH limit has not yet been described by European Directive [12] it has an important role in honey's shelf life and authenticity. Honey is characteristically acidic with pH between 3.2 and 4.5 [3]. Natural acidity of the honey inhibits the growth of microorganisms [19]. A raise in the percentage of the Brazilian honey adulterated with high fructose corn syrup resulted in significantly increased pH values [20]. The pH values ranged from 3.20 to 4.70 for all analysed honey samples. As can be seen from Table 3, lime honey has slightly higher average pH value compared to pH of rape honey samples. Truzzi *et al.* [21] also reported higher pH value for lime honey (average pH 4.70).

Enzymatic activities in honey can decrease when the honey is old or heated [22]. According to the results obtained, diastase varied between 18.90 and 29.86 Gothe units for rape honey samples and between 10.16 and 24.55 Gothe units for lime honey. Diastase activity in lime honey determined in the present study was similar to that indicated by Persano Oddo and Piro [4].

Invertase is ranging from 46.09 to 149.88 $U.kg^{-1}$ in rape honey and from 75.66 to 175.15 $U.kg^{-1}$ in lime honey. In this respect, invertase was within a similar range to those reported by other authors [23]. Lime honey exhibited higher average concentration of invertase compared to rape honey (Table 3). Makarewicz *et al.* [24] obtained lower levels of invertase in lime honey samples. According to the obtained results, the high invertase activity of honey showed freshness of all samples. It is well known that invertase is a very storage-sensitive enzyme. For this parameter the present study was found to be in agreement with the study of Lichtenberg-Kraag [25] for rape and lime samples. Furthermore, enzymatic activity in honey may vary. It depends on the age of the bees, the physiological status of the colony, the quantity of nectar flow. Indeed, high flow of nectar leads to a lower enzyme activity of honey [23]. A significant positive linear correlation was found between diastase and invertase in rape honey ($r=0.557$, $p<0.05$), which is presented on Fig. 2. This correlation is in agreement with the study of Vorlova and Celechovska [26]. Persano Oddo *et al.* [23] presented correlations in different honey types. Although the authors compared 499 honey samples, the correlation coefficient was $r=0.835$ ($p<0.001$). This indicated a strong positive relationship between the variables studied across the various honey types. In this respect, the moderate correlation coefficient in our study may be due to the small number of rape samples. Honey is a very complex natural food produced by honeybees under relatively uncontrolled factors. Various statistical tools can be used to better understand the relationships between physicochemical parameters.

Proline is the most abundant amino acid in honey. The average contents are presented in Table 3. Kropf *et al.* [15] presented similar proline content (about 300 $mg.kg^{-1}$) in lime honey. The proline content varied in large ranges in rape honey (153.76 – 333.70 $mg.kg^{-1}$) and in lime honey (216.38 – 532.22 $mg.kg^{-1}$). Szczęsna *et al.* [11] reported almost similar results for proline content and variation from 142 to 466 $mg.kg^{-1}$ for rape honey.

Hydroxymethylfurfural is an excellent indicator of honey freshness. The HMF for all evaluated honey samples presented a content below 40 $mg.kg^{-1}$ (maximum permissible limit established by regulatory organizations [12, 27], ranging from 1.05 to 7.18 $mg.kg^{-1}$ in rape honeys and 1.80 to 8.38 $mg.kg^{-1}$ in lime honeys. As can be seen, the observed values do not exceed the limits established by regulatory organizations.

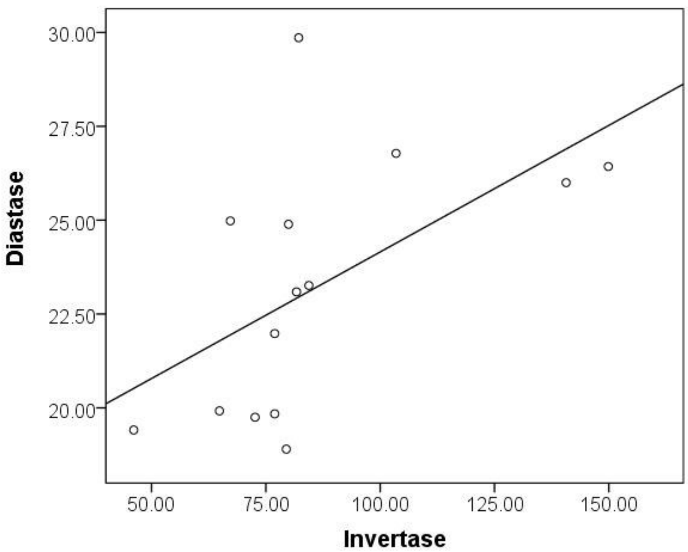

Figure 2. Linear correlation between diastase and invertase in Bulgarian rape honey.

CONCLUSIONS

In the present study a total of 14 monofloral rape and 26 lime honey samples meet the quality standards of European legislation for the parameters studied. Such full physicochemical characterization is provided for the first time for Bulgarian monofloral honeys. The results confirmed that physicochemical properties of honeys were closely related to their floral sources. The monofloral lime and rape honeys produced in Bulgaria are with good quality, established by physicochemical parameters characterisation. Significant linear correlations were found between diastase and invertase in rape honey and between color and electrical conductivity in lime honey.

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