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THE SACCHARIDE PROFILE OF POLISH HONEYS DEPENDING ON THEIR BOTANICAL ORIGIN

Zielińska S. [1], *Wesołowska M.* [1], *Bilek M.* [2], *Kaniuczak J.* [2], *Dżugan M.* [1]

Address(es): Zielińska S.,
[1]Department of Chemistry and Food Toxicology, Faculty of Biology and Agriculture, University of Rzeszów, 2 Ćwiklińskiej St., 35-601 Rzeszów, Poland.
[2]Faculty's Laboratory, Faculty of Biology and Agriculture, University of Rzeszów, 2 Ćwiklińskiej St., 35-601 Rzeszów, Poland.

*Corresponding author: zielinskasy@gmail.com

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ABSTRACT

Thirty honey samples (18 monofloral, 6 multifloral and 6 honeydew) were collected directly from apiaries localized in the South-Eastern Poland. Monosaccharide profiles (glucose/fructose ratio) in honey were examined by HPLC method with ELSD-detection. The results were compared with other parameters used in honey sugar analysis, i.e. a level of reducing sugars measured by Lane-Eynon, sugar extract (refractometric), specific rotation angle and glucose content determined with Reflectoquant® test (Merck). Moreover, some physicochemical parameters such as: water content, free acids and HMF content by White method were tested.
The content of monosaccharides determined by HPLC (as sum of glucose and fructose) and Lane-Eynon methods were compared (r=0.83) and changed from 68% in rape honey to 78% on average in goldenrod honey. All studied honeys showed the ratio of fructose/glucose above 1.5. The measurement of the specific optical rotation allowed to distinguish nectar (-) and honeydew (+) honey, but due to the heterogeneity of the results, they could not be used for identification of the floral honeys. The results of reflectometric test for glucose level were positively correlated with values measured by HPLC method (r=0.73).

Keywords: Honey, monosaccharide, HPLC, optical activity, reflectometric test, botanical origin

INTRODUCTION

Honey is a complex natural product, containing more than 400 different substances, e.g. various carbohydrates, organic acids, proteins, amino acids, enzymes, aroma substances, mineral substances, pigments, waxes, etc (**Dimins *et al.*, 2008**).
Sugars (saccharides) represent the main components of honey. Besides the two main monosaccharide constituents (glucose and fructose), there are the minor components consisting of about 25 oligosaccharides (di-, tri-, tetrasaccharides) (**Anklam, 1998**). The general standard for a minimum content of the sum of fructose and glucose 60 g/100 g for a nectar honeys and 45 g/100 g for all honeydew honeys can be proposed (**Bogdanov *et al.*, 2000**). In Polish honeys, glucose and fructose compose about 70-80% of sugar fraction in nectar honeys and 55-65% in honeydew honeys (**Kowalski *et al.*, 2013**). The content of maltose and sucrose in honey reaches up to 9 and 8%, respectively (**Dimins *et al.*, 2008**).
The relative amount of the two monosaccharides fructose and glucose is useful for the classification of unifloral honey, as well as the fructose-glucose and glucose-water rations. The minor sugars have a relatively low diagnostic value for the determination of botanical origin (**Anklam,1998**). The fructose/glucose ratio is determined by the botanical origin and it influences the inclination to crystallize. Crystallization is prevented by fructose, but promoted by glucose (**Adriana *et al.*, 2012**).
Saccharides can be determined by a number of different methods based on the use of their physical characteristic or by chemical or enzymatic methods (**Anklam, 1998**). The apparent reducing sugars as well as the apparent sucrose content are measured by the Fehling method. These methods are very time consuming and although they have been used for almost 100 years, their precision has not been tested collaboratively. They do not satisfactorily characterize honey quality and origin and for this reason have been replaced by specific chromatographic methods. HPLC with silica-based amino columns and refractometric detection or with ion-exchange columns and pulsed amperometric detection is simpler and more suitable for routine analysis comparable to gas chromatography (**Bogdanov *et al.*, 2000**). Many of authors using HPLC method to determination of honeys sugar content (**Rybak-Chmielewska, 2007; Dimins *et al.*, 2008; Primorac *et al.*, 2009; Primorac *et al.*, 2011; Adriana *et al.*, 2012; Kowalski *et al.*, 2013; Rybak-Chmielewska *et al.*, 2013**).
Honey has the property to rotate the polarized light. This depends largely on types and relative proportions of sugars in honey. Each sugar has a specific effect, and the total optical rotation is dependent on its concentration. This is a consequence of the normal predominance of fructose in honey, which has a negative specific rotation ($(\alpha)_D^{20}$= - 92.4°) over glucose ($(\alpha)_D^{20}$= + 52.7°) (**Dinkov, 2003**). Optical rotation is a parameter that is discussed in relation to determination of botanical origin and adulteration of honey. In some countries the rotation is applied to differentiation of honey groups – nectar, honeydew and compound honeys but the limit values have not been harmonized so far (**Pridal and Vorlova, 2002**).
The aim of this study was to establish the relationship between content of reducing sugar of honey and some physicochemical parameters and possibilities of using these criteria for identification botanical origin of Polish honeys.

MATERIAL AND METHODS

Samples

Honey samples (n=30) were collected directly from beekeepers localized in the South-Eastern Poland (Podkarpacie region) and were produced in the 2012-2013 season. Honey was stored at a laboratory temperature (20° C) until the time of analysis. Lime honey (n=6), honeydew (n=6), multifloral (n=6), rape (n=4), buckwheat (n=4), goldenrod (n=4) were tested.

Physicochemical properties

The basic physicochemical parameters of tested honeys were determined with refractometer (water, sugar extract) and free acids by titration method (**Bogdanov, 2009**).
Determination of sugars profile (glucose and fructose) in honeys was performed by high-performance liquid chromatography system (Varian Star 800). The HPLC separation of sugars was achieved in Grace Prevail Carbohydrate chromatographic column packed with 5µm shell particles (250 mm x 4.6 mm) (Altima Amino 100A 5u), using acetonitrile (POCH Poland)/water (80:20) as mobile phase, at a flow rate of 1.0 cm^3/min and an ELSD detector (385 LC). Before injection samples were filtered with MCE syringe filters (0.45µm). Sample volume was 25 µl. The separated carbohydrates were identified on the basis of their retention times, and quantification was performed by external calibration. Carbohydrate standards (within the range of 0.5 to 30 $mg \cdot ml^{-1}$) of anhydrous glucose, fructose, sucrose were purchased from Sigma (St. Louis, USA).

The content of reducing sugars was measured according to Lane-Eynon (**Bogdanov, 2009**). The glucose concentration was examined with Reflectoquant® Glucose Test (No. 116720, Merck Germany) using reflectometer RQflex® according to manufacturer instruction.
A rotation angle for 1% honey extract, after deproteinization with Carrez fluid, was determined by polarimetric method and specific rotation $(\alpha)_D^{20}$ was calculated with Biot equation (**Bogdanov, 2009**).
Honey samples were tested on HMF content according to White method (**Bogdanov, 2009**).

Statistical analysis

Means, standard deviations, and coefficients of variation for obtained parameters were calculated. The differences between varietal honeys were examined by Kruskall-Wallis or U Mann-Whitney tests. The relations between parameters were evaluated using the Spearman correlation coefficient. For the data analysis Statistica 9.0 (Statsoft Inc.) was used.

RESULTS AND DISCUSSION

By analyzing the basic parameters as: water content, sugar extract, acidity and HMF content in the all investigated honeys (Table 1), it was found that the results fit in to mandatory requirements (PN-88A-77626, 1988). The highest water content (above 20%) for same samples of lime, goldenrod, buckwheat and honeydew honeys were observed which is in agreement with results obtained by Majewska *et al.* (**2012**). A free acids level of analyzed honey samples was from 14.7 to 45.0 mval/kg. The highest acidity was found for a buckwheat honey and was statistically significant ($p<0.05$), while the rape honey was characterized by the lowest content of this parameter (Table 1). Similarly, Kowalski *et al.* (**2013**) observed the highest acidity in buckwheat honey, whereas acacia and rape - the lowest.
Characteristic compound of honey, produced from the acid-catalyzed degradation of sugars (mainly fructose), is HMF (5-hydroxymethylfurfural). The level of this compound increases with honey age (**Jasicka-Misiak and Kafarski, 2011**). The content of HMF found in tested honeys was ranged from 4.2 mg/kg (honeydew) to 13.1 mg/kg (lime). The level of HMF depends on honey variety and was from 0 to 9.2 mg/kg for a buckwheat honeys (**Majewska *et al.*, 2012**), 0.5-13.1 mg/kg for a rape honeys (**Szczęsna *et al.*, 2011**), 4.8-66 mg/kg for multifloral honeys (**Majewska *et al.*, 2010**), and for honeydew honeys below 0.5 mg/kg (**Rybak-Chmielewska *et al.*, 2013**) and 0.2-0.8 mg/kg (**Primorac *et al.*, 2009**).
All samples of analyzed nectar honey had negative values of specific rotation (Table 1). The most negative value of specific rotation was observed in the case of goldenrod honey (-10.6°), and the lowest in multifloral honey (-6.3°). Inversely, honeydew honeys were characterized by positive values of specific rotation (5.3°). The similar values of specific rotation were found by other authors (**Dimins *et al.*, 2008; Pridal and Vorlova, 2002; Dinkov, 2003**).

Table 1 Results of studied honeys physicochemical analysis

Parameter/ kind of honey	Water content %		Free acidity mval/kg		HMF content mg/kg		Specific rotation °($[\alpha]_D^{20}$)	
	Mean ± SD	min-max	Mean ± SD	min-max	Mean ± SD	min-max	Mean ± SD	min-max
Lime n=6	19.2±1.78	17-21.7	30.7±17.4	10.0-50.8	13.1±12.2	2.5-28.0	-7.9±2.2	(-11.8)-(-5.3)
Rape n=4	18.6±1.0	17.2-19.3	14.7±4.6	9.0-20.0	7.4±3.8	2.1-10.2	-6.7±5.5	(-11.8)-(-0.9)
Goldenrod n=4	19.2±1.7	17.0-20.7	29.8±11.8	15.1-42.9	12.8±10.0	4.3-26.0	-10.6±1.5*	(-12.3)-(-8.8)
Buckwheat n=4	19.1±2.1	16.5-20.8	45.0±6.4*	37.8-50.8	11.3±3.9	6.4-16.0	-7.6±3.5	(-12.7)-(-5.3)
Multifloral n=6	17.7±1.2	15.7-19.0	15.4±6.6*	11.9-28.7	6.5±5.6	0.5-13.9	-6.3±3.5	(-11.0)-(-2.2)
Honeydew n=6	19.3±1.0	18.4-20.7	35.6±8.5	27.0-51.0	4.2±3.4	Nd-8.2	5.3±2.7*	2.2-9.6

* significant differences $p<0.05$

Negative specific rotation of honey is the result of carbohydrates ability to rotate linear polarized light. Negative specific rotation of nectar honeys results from the predominance of fructose which has a high negative specific rotation (α_D^{20}=-92.3°), while honeydew honeys have positive values due to the lower content of fructose and higher contents of di- and oligosaccharides that have positive specific rotation. In addition, specific rotation can be a useful parameter for unifloral honeys differentiation even though a notable overlapping occurs with different honey types (**Primorac *et al.*, 2011**). However, the results obtained by Dimins *et al.* (**2008**) indicate that the specific rotation of light cannot be used for identification of honey kinds.

The content of monosaccharides in samples of honey, expressed as a sum of fructose and glucose for all types of honey was from 66.9% (rape honey) to 78.8% (goldenrod honey) and 69.0% (rape honey) to 77.6% (multifloral honey), respectively by HPLC and Lane-Eynon method (Table 2). The content of reducing sugars determined by both methods did not vary significantly ($p>0.05$) as well as the results were significantly correlated (Figure 1). However, results of refractometric measurements of sugar extract were not correlated with sugar concentrations: HPLC – refractometry ($r=-0.22$) and Lane-Eynon – refractometry ($r=-0.20$) and exhibited higher values (by 10%).

Table 2 Results of honeys sugars analysis.

Parameter/ kinds of honey	Reducing sugars by HPLC %						Reducing sugars by Lane-Eynon %		Sugar extract (refractometric) %	
	Fructose		Glucose		F+G		F+G			
	mean±SD	min-max	mean±SD	min-max	mean±SD	min-max	mean±SD	min-max	mean±SD	min-max
Lime n=6	43.2±9.1	28.3-50.6	27.4±5.5	17.0-32.2	70.6±14.6	52.8-81.1	69.8±8.5	58.6-76.3	79.3±1.7	77.0-81.5
Rape n=4	40.8±8.4	31.8-49.7	26.1±4.9	20.1-31.1	66.9±13.3	51.9-78.6	69.0±10.2	57.9-79.5	79.8±0.7	79.3-80.8
Goldenrod n=4	49.9±8.6	38.1-56.9	28.8±2.8	26.4-31.4	78.8±11.4	64.5-86.8	72.9±9.8	58.4-79.8	79.4±1.6	77.9-81.6
Buckwheat n=4	47.8±6.1	39.3-53.8	27.0±3.5	24.0-31.1	74.8±9.6	63.4-80.1	71.0±6.6	61.4-75.8	79.4±2.0	77.8-82.0
Multifloral n=6	46.6±5.3	37.0-52.0	29.0±6.7	19.0-36.3	75.5±12.0	56.0-84.1	77.6±7.0	67.6-86.6	80.8±1.3	79.5-82.8
Honeydew n=6	44.3±2.6	40.3-48.3	26.4±1.4	24.1-27.7	70.6±3.9	67.8-72.4	69.4±7.7	59.2-78.6	79.1±1.2	77.3-80.1

$p>0.05$ significant differences

According to Bogdanov *et al.* (**2000**), the Fehling method does not satisfactorily characterize honey quality and origin and for this reason recently it has been replaced by specific chromatographic methods. The content of reducing sugars is differentiated in quality standards of nectar and honeydew honey, according to which the sum of glucose and fructose cannot be less than 60 g/100 g for a nectar honey and 40 g/100 g for a honeydew honey (**Codex Alimentarius, 2001**). It was

established that multiplicity of tested honeys complied with requirements of Polish and EU quality standards, except of 3 samples of nectar honeys. Moreover, obtained results correspond to data found by other authors (**Dimins *et al.*, 2008; Majewska *et al.*, 2010; Szczęsna *et al.*, 2011; Kowalski *et al.* 2013**).

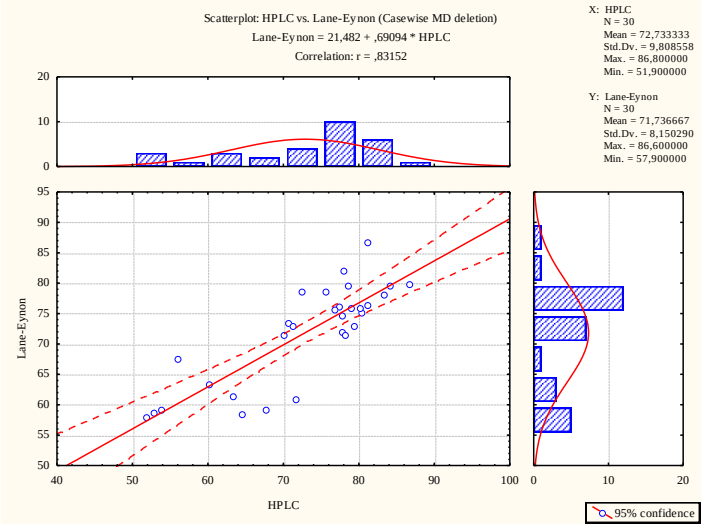

Figure 1 Correlation between HPLC and Lane-Eynon methods

The content of sugar type is correlated to the specific rotation, which is useful for differentiation of blossom (laevorotatory, negative specific rotation) and honeydew honeys, which mostly have positive values (dextrorotatory). Except that, specific rotation is useful additional parameter for botanical identification, but there are no clear bounds between different honey types (**Primorac *et al.*, 2009; Bogdanov *et al.*, 2004**). In our study the correlations between specific rotation and other studied parameters were not significant: HPLC – optical rotation (r=-0.22) and Lane-Eynon – rotation (r=-0.14). Significant correlations between glucose content and specific rotation was observed only for the same kind of honey - lime (r = 0.75, p<0.05) and multifloral (r = -0.72, p<0.05) honeys.

The HPLC analysis of fructose in honeys has shown the average content between 40.8 – 49.9 % (Table 2), with the highest content in goldenrod and lowest in rape honeys (p>0.05). Glucose concentrations in all types of honey were similar (p>0.05), from 26.1 (rape) to 29% (multifloral). **Kowalski *et al.* (2013)** observed in nectar honeys higher level of fructose as well as glucose, from 46.17 to 53.6% and 32.39 – 47.02%, respectively.

The levels of fructose and glucose found in studied honeydew honeys were 44.3% and 26.4%, respectively. The similar level of glucose in this kind of honey was marked by **Rybak-Chmielewska *et al.* (2013)**. In opposite, these authors observed the lowest content of fructose - 34.2% on average.

The specific property of tested varietal honeys was the higher content of fructose than glucose. The calculated fructose/glucose proportion (F/G ratio) were from 1.56 to 1.77 (Figure 2). Similar F/G ratio was observed in study of Romanian acacia and rape honeys (1.4 on average) (**Adriana *et al.*, 2012**). Moreover, they discovered that significant correlations between F/G ratio and origin from the same region. Numerous studies have shown opposite relationship, glucose content higher than fructose, and then fructose/glucose ratio was about 1 (**Dimins *et al.*, 2008; Primorac *et al.*, 2009; Szczęsna *et al.*, 2011**). The high glucose content causes fast crystallization of the honey, usually within one week from extraction (**Szczęsna *et al.*, 2011**).

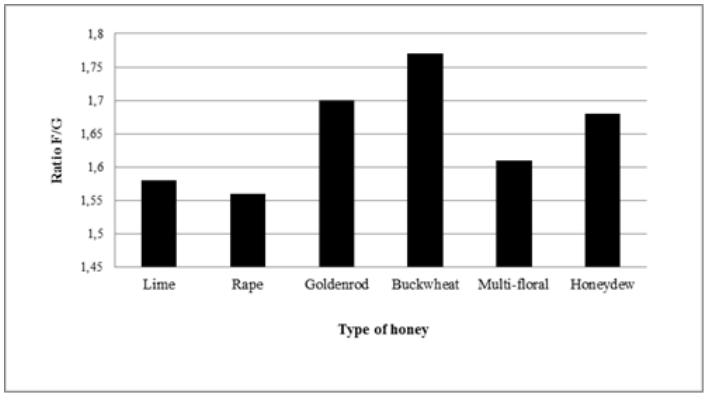

Figure 2 The ratio F/G of honeys determined by HPLC

It was found that glucose content obtained by reflectometric method was comparable with results of HPLC (Figure 3), the differences were by 2-4% (p>0.05). It is promising because this test is simple and economic, moreover when additive Reflectoquant Total Sugar Test ® (Merck) was used, simultaneous determination of glucose and fructose would be possible. Results of Merck test were positively correlated with HPLC method (r=0.72).

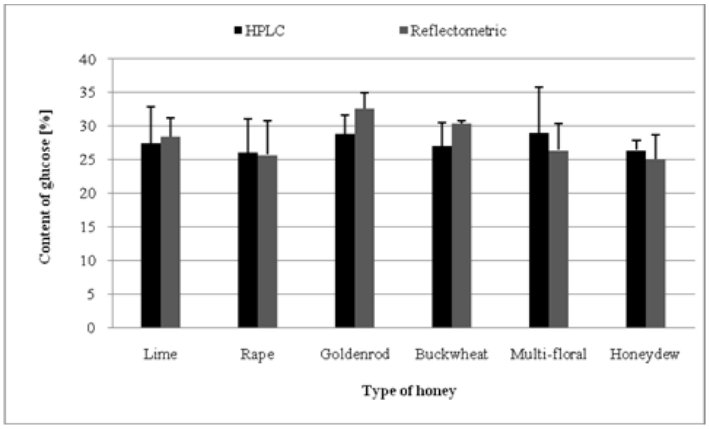

Figure 3 The content of glucose determined by HPLC and reflectometric methods. p>0.05 significant differences

CONCLUSION

Physicochemical parameters of tested honeys i.e. water content, free acids and HMF did not exceed the limits of Polish as well as European regulations, but they do not lead any information about their botanical origin whereas comparison the content of main sugars and specific rotation can be useful for indentifying of honey type.

The highest sugar content in goldenrod and multifloral honeys whereas the lowest in rape honeys were observed (by HPLC and Lane-Eynon method). All studied honeys showed the ratio of fructose/glucose above 1.5. Optical rotation of honeys allowed to distinguish honeydew (+) from floral (-) honeys. However, due to the heterogeneity of the results polarimetric analysis could not be used for identification of the nectar honeys.

The HPLC is more accurate and precise method for determining the profile of sugar in honeys and was positively correlated with Lane-Eynon method (r=0.83). There was no significant correlation between polarimetric examination and other methods used for honey variety identification.

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