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General linear models based on physicochemical parameters of monofloral and multifloral bee honey: Part 2

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Abstract

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The objective of the present study is to establish regression models defining the relations between physicochemical parameters of bee honey and the type of honey. 89 samples of bee honey (acacia honey, coriander honey, limetree honey, rapeseed honey, sunflower honey, and multifloral honey) collected from different regions of Bulgaria were used in the study. The following parameters have been defined: water content (WC); content of fructose (F), glucose (G), electrical conductivity (EC), refractive index (RI), and pH. Moderate to strong negative correlation between WC and RI has been found for all studied types of bee honey. In the samples from lime tree, coriander, and multifloral honey, positive but different in strength correlation has been observed – from weak to moderate correlation between pH and EC. The analysis of the samples with acacia honey showed differences in strength and direction of: lime tree and multifloral honey in terms of the correlations between pH and total F + G content; of lime tree, rapeseed, and sunflower honey in the correlations between F+G and RI and between WC and F + G. General Linear Models (1-5) have been compiled based on the connections between the considered physicochemical parameters and the different types of honey, which would allow subsequent estimation of the studied parameters.

Keywords: honey, physicochemical parameters, correlation analysis, regression analysis

Introduction

In order to establish the quality and suitability of bee honey for food and other purposes, a number of physicochemical tests are performed: organoleptic; determination of water content, glucose, fructose, and sucrose content; electrical conductivity, active acidity, diastasis activity; the amount of hydroxymethylfurfural (HMF); pollen analysis.

The methods for determining the quality indicators of honey, which are used in Bulgaria, are specified in BSS 3050-80 and Ordinance No. 48/2003. The Bulgarian Ministry of Health, adopted by a Council of Ministers Decree No. 196, describes the requirements to the composition and characteristics of different types of honey intended for human consumption in an Ordinance. The main method for determining the botanical origin of honey is pollen analysis (Bogdanov, 1997; Mateo & Bosch-Reig, 1998).

According to data provided by Shkenderov & Ivanov (1983) and Ivanov (2000, 2006), the active acidity (pH) of honey varies from 3.2 to 6.5. In their studies, Piro et al. (2002) found high fructose, glucose content, and low electrical conductivity in acacia honey.

Hydroxymethylfurfural (HMF) is an indicator that is monitored in determining the quality of untreated and processed bee honey (White, 1978; White & Doner, 1978; Thrasyvoulou et al., 1982; Thrasyvoulou & Blandenopoulou, 1984). Its content in honey is indicative of the way it has been processed and stored but does not provide information on the botanical origin of honey (Krauze & Zalewski, 1991; Devillers et al., 2004).

Krauze & Zalewski (1991), Kivrak et al. (2017) and Krishnan et al. (2021) indicated electrical conductivity, pH, proline content, and active acidity as the most important chemical indicators for determining the type of bee honey. The authors distinguished monofloral from multifloral honey by applying a chemometric assessment of the physicochemical parameters of honey, through Principal component analysis (PCA) and managed to distinguish honey from rapeseed, acacia, and honeydew. Iglesias et al. (2004) and Soria et al. (2005) found that the content of monosaccharides and the concentration of glutamic acid are the main criteria for the differentiation of nectar and honeydew honey.

Scientific studies have described methods for proving the type of bee honey by multifactor analysis of the physicochemical parameters – pH, ash content, and monosaccharides in nectar and honeydew honey (Kirkwood et al., 1960). Correlation dependences of various degrees and direction have been established between some of the physicochemical indicators of bee honey. Tsigouri & Passaloglou-Katrali (2000) determined significant correlations between electrical conductivity and invertase activity of honey, ($r = 0.761$; $p <0.01$), and between diastase and invertase activity ($r = 0.484$; $p = 0.03$), respectively. Ratiu et al. (2019) found a low correlation between honey acidity and antioxidant activity ($r = 0.38$, $p = 0.05$), as well as a moderately negative correlation of antioxidant activity to sucrose levels. The same authors also report differences in the correlation coefficients in the analysis of the physicochemical indicators of honey depending on the duration of storage and the year of its production.

The information from the available literature regarding the correlations between the individual physicochemical parameters of bee honey is scarce, which is why we believe that it is justified to expand research in this direction.

The objective of the present paper is to compile regression models defining the relations between physicochemical parameters and the type of honey as a part of the quality examination of different types of Bulgarian honey.

Material and Methods

Physicochemical analysis of honey samples of varied origin

To fulfil the objective of the study during the beekeeping season of 2019, samples of bee honey from apiaries located on the territory of the Republic of Bulgaria were collected. The samples have a different botanical origin. Prior to the physicochemical analyses, the honey had been stored in the dark, under room conditions.

Pollen analysis had previously been made on all honey samples at the Scientific Laboratory (Central Research Laboratory) of Trakia University, Stara Zagora, Bulgaria, according to BSS 3050/80 to determine its botanical origin.

The following groups have been formed:

– Monofloral honey – 59 pcs. (acacia 12, coriander 12, limetree 12, rapeseed 11, sunflower 12).
– Multifloral honey – 30 pcs.

The physicochemical study was performed at the Central Research Laboratory at Trakia University. Liquid chromatography was used with "Thermo Scientific Surveyor Plus" system following the harmonised methods of the European honey commission (Bogdanov et al., 1997) and the methods described in BSS 3050-80 and Ordinance No. 48/2003.

The following parameters have been studied:

– glucose (%), fructose (%), and sucrose (%) – by high-efficiency liquid chromatography.
– the refractive and water content index (%) by ABBE refractometer;
– electrical conductivity (μS/cm) by Conductivity meter with electrical conductivity cell;
– active acidity (pH) by pH-meter;

Based on the obtained results for glucose and fructose content in honey samples the Glucose + Fructose amount was calculated, according to the Annex to Art. 7 of the Ordinance on the requirements to bee honey for human consumption (Council of Ministers Decree No. 196, Bulgaria).

Statistical data analysis

Statistical data processing covers obtaining the main statistics (population mean values and Standard Deviations (SD), and correlation coefficients between the observed physicochemical parameters (Active acidity (pH); Electrical conductivity, μS/sm; Water content, %; Refractive index; Fructose + Glucose (F+G) content, %), a normality distribution verification of the datasets via Kolmogorov-Smirnov test, and multivariate data analysis at p-value < 0.05 to develop General Linear Models (GLM) about the influence of the type of honey on examined physicochemical traits. The overall look of the models is . In the equations are the predicted values of the physicochemical parameters, are the population mean values, G is the fixed factor (the type of honey), and are the residual errors of the models. The data analysis was performed using SPSS Statistics 26.0.0.1 package.

Results and Discussion

Basic statistics

The basic statistics (population means and Standard Deviations) of the studied physicochemical parameters are presented in Table 1. The authors in a preprint article named have discussed statistically significant differences among the various types of honey, based on the physicochemical parameters: Physicochemical characteristics of Bulgarian bee honey: Part 1. Here the data about the mean values of the physicochemical parameters are given due to their involvement in the models.

Correlation analysis

Table 2 presents the results from the Bivariate correlation method about all studied parameters for acacia honey (pH, Electrical conductivity, Water content, Refractive index, Fructose, and Glucose content). The correlation coefficients marked with the symbol (*) are significant at level $p \leq 0.05$. As is evident from the table, the relation between the parameters pH and F+G (r = 0.881), as well as between Water content and F+G (r = 0.836) is strong and positive, i.e. the increase of the pH level in acacia honey increases sugar content as well. Moderately positive is the dependence between EC and WC (r = 0.693). Strong to moderate, but negative is the relation among the parameters Water content, Electrical conductivity, F+G, and Refractive index (r = -0.996; -0.758; -0.781), respectively. The negative dependence suggests that the increase of one parameter leads decreasing of the others.

The calculated correlations for the studied indicators for lime tree honey are presented in Table 3. The Active acidity is in strong to moderate positive correlation with the indicators EC and WC (r = 0.977; 0.600), but in moderate negative correlation with the parameters RI and F+G (r = -0.611; -0.667). Strong positive correlation is observed between the RI and F+G (r = 0.997). Concerning the WC, a very strong but negative correlation is registered with the indicators RI and F+G (r = -1.000; -0.996), which suggests that with the increase of these indicators water content in lime tree honey decreases.

A similar negative correlation between the WC and the parameters RI and F+G (r = -0.689; -0.606) has been detect-

Table 1. Basic statistics of observed physicochemical parameters of different types of honey

Type of honey	μ ± SD					
	N	Active acidity (pH)	Electrical conductivity, μS/cm	Water content, %	Refractive index	Fructose and glucose content, %
Acacia	12	3.61±0.28	376.00±172.89	17.17±0.47	1.49±0.001	56.89±11.46
Coriander	12	1.93±0.05	486.67±80.37	16.67±0.43	1.49±0.003	66.36±2.85
Lime tree	12	4.12±0.51	639.67±163.42	19.07±0.86	1.49±0.002	61.90±17.53
Rapeseed	11	3.47±0.19	243.09±114.32	18.72±1.37	1.49±0.004	65.55±13.54
Sunflower	12	3.20±0.09	453.50±54.36	17.58±1.23	1.49±0.003	76.20±2.72
Multifloral	30	3.70±0.46	669.47±512.61	17.03±1.49	1.49±0.004	66.90±7.75

Table 2. Crosstab correlation between examined physicochemical parameters of acacia honey

Acacia honey n = 12	Active acidity (pH)	Electrical conductivity, μS/cm	Water content, %	Refractive index	(F + G), %
Active acidity (pH)	1	-0.304	0.476	-0.392	0.881*
Electrical conductivity, μS/sm		1	0.693*	-0.758*	0.184
Water content, %			1	-0.996*	0.836*
Refractive index				1	-0.781*
Fructose and Glucose content, %					1

Table 3. Crosstab correlation between examined physicochemical parameters of lime tree honey

Lime tree honey n = 12	Active acidity (pH)	Electrical conductivity, μS/cm	Water content, %	Refractive index	(F + G), %
Active acidity (pH)	1	0.977*	0.600*	-0.611*	-0.667*
Electrical conductivity, μS/sm		1	0.417	-0.430	-0.495
Water content, %			1	-1.000*	-0.996*
Refractive index				1	0.997*
Fructose and glucose content, %					1

Table 4. Crosstab correlation between examined physicochemical parameters of rapeseed honey

Rapeseed honey n = 11	Active acidity (pH)	Electrical conductivity, µS/cm	Water content, %	Refractive index	(F + G), %
Active acidity (pH)	1	-0.377	-0.180	0.585	0.265
Electrical conductivity, µS/sm		1	-0.017	-0.086	-0.064
Water content, %			1	-0.689*	-0.606*
Refractive index				1	0.706*
Fructose and Glucose content, %					1

Table 5. Crosstab correlation between examined physicochemical parameters of sunflower honey

Sunflower honey, n = 12	Active acidity (pH)	Electrical conductivity, µS/cm	Water content, %	Refractive index	(F + G), %
Active acidity (pH)	1	0.045	-0.518	0.621*	0.558
Electrical conductivity, µS/sm		1	0.066	0.009	-0.345
Water content, %			1	-0.990*	-0.954*
Refractive index				1	0.936*
Fructose and glucose content, %					1

ed both in rapeseed honey (Table 4) and in sunflower honey (r = -0.990; -0.954) (Table 5). Just like lime tree honey, rapeseed honey (Table 4) and sunflower honey (Table 5) are characterized by a positive but moderate to strong correlation between the RI and F+G (r = 0.706 и r = 0.936).

The correlation coefficients of the studied parameters for coriander honey are presented in Table 6.

The results show moderate positive correlation between pH and EC (r = 0.796) and strong negative correlation between pH and WC (r = -0.918).

Weak positive, but statistically significant correlation for multifloral honey (Table 7) has been registered between pH and EC (r = 0.374). On the other hand, the dependence between F+G and pH (r = -0.570), as well as between F+G and EC (r = -0.670) is moderately negative. A very strong negative correlation has been found between WC and RI (r = -0.999).

General Linear Models

The multivariate data analysis was applied to create predictive models defining the correlations between the studied physicochemical parameters and the type of honey. The results are presented in Table 8, and the obtained linear equations are statistically significant at $p < 0.05$.

The relation between the parameter Active acidity (pH) and the type of honey is the following:

$$\ddot{Y} = 35.863X + 875.965 + e, \qquad (1)$$

where X is the population mean of pH value for the particular type of honey. The data given in Table 8 show that the value of the coefficient of determination is $R^2 = 0.778$, i.e. about 77.8% of the variations in parameter pH are dependent on the effect of the type of honey.

Table 6. Crosstab correlation between examined physicochemical parameters of coriander honey

Coriander honey, n = 12	Active acidity (pH)	Electrical conductivity, µS/cm	Water content, %	(F + G), %
Active acidity (pH)	1	0.796*	-0.918*	0.198
Electrical conductivity, µS/sm		1	-0.490	-0.435
Water content, %			1	-0.572
Fructose and glucose content, %				1

Table 7. Crosstab correlation between examined physicochemical parameters of multifloral honey

Multifloral honey, n = 30	Active acidity (pH)	Electrical conductivity, µS/cm	Water content, %	Refractive index	(F + G), %
Active acidity (pH)	1	0.374*	-0.167	0.166	-0.570*
Electrical conductivity, µS/sm		1	0.001	0.006	-0.670*
Water content, %			1	-0.999*	0.013
Refractive index				1	-0.006
Fructose and glucose content, %					1

The relation between the Electrical conductivity and the type of honey can be defined with the following equation:

$$\hat{Y} = 2.000E6X + 1.798E7 + e, \quad (2)$$

where X is the mean value of the EC for the particular type of honey.

The coefficient of determination $R^2 = 0.191$ (Table 8) shows that only about 19.1% of the variations in the parameter EC are dependent on the effect of the type of honey.

The general equation defining the dependence of the variable Water content on the type of honey is:

$$\hat{Y} = 61.857X + 24659.008 + e, \quad (3)$$

where X is the mean value of WC for the particular type of honey. As is evident from Table 8, about 35.5% of variances in the variable WC are due to the effect of the type of honey ($R^2 = 0.355$).

Equation (4) shows the relation between the parameters Refractive index and the type of honey, while Table 8 presents the data about the calculated General Linear Model.

$$\ddot{Y} = 0.0004X + 148.671 + e, \quad (4)$$

where X is the mean value of the RI for the particular type of honey.

The coefficient of determination $R^2 = 0.275$ reveals that only 27.5% of the variations in the variable RI are due to the effect of the type of honey (Table 8).

The results about the obtained General Linear Model showing the dependence of the overall parameter Fructose + Glucose on type of honey are presented in Table 8, and the equation of the model can be structured as:

$$\hat{Y} = 2472.522X + 338\,893.353 + e, \quad (5)$$

where X are the population means of the overall parameter F + G for the particular type of honey. The coefficient of determination is $R^2 = 0.224$ (Table 8), which shows that only 22.4% of the variations of the variable F + G could be accounted for by the effect of the type of honey.

Table 8. General Linear Models showing the dependence of physicochemical parameters on the type of honey

Regression Summary for Dependent Variable: Active acidity (pH) $R = 0.88204$; $R^2 = 0.778$; Adjusted $R^2 = 0.764$; $p < 0.00$ (Corrected Model)					
No. of cases: 89	Coef.	Std. error of estimate	Coef. of determination R^2	F (df = 88)	p-value
Intercept	875.965	10.259	0.988	58.030	0.000
Type of honey	35.863		0.778		0.000
Regression Summary for Dependent Variable: Electrical conductivity, μS/sm $R=0.43703$; $R^2 = 0.191$; Adjusted $R^2 = 0.142$; $p < 0.003$ (Corrected Model)					
No. of cases: 89	Coef.	Std. error of estimate	Coef. of determination R^2	F (df = 88)	p-value
Intercept	1.798E7	8477256.709	0.680	3.918	0.000
Type of honey	2.000E6		0.191		0.003
Regression Summary for Dependent Variable: Water content, % $R=0.59582$; $R^2 = 0.355$; Adjusted $R^2 = 0.316$; $p < 0.00$ (Corrected Model)					
No. of cases: 89	Coef.	Std. error of estimate	Coef. of determination R^2	F (df = 88)	p-value
Intercept	24659.008	112.302	0.995	9.143	0.000
Type of honey	61.857		0.355		0.000
Regression Summary for Dependent Variable: Refractive index $R = 0.52440$; $R^2 = 0.275$; Adjusted $R^2 = 0.234$; $p < 0.00$ (Corrected Model)					
No. of cases: 89	Coef.	Std. error of estimate	Coef. of determination R^2	F (df = 88)	p-value
Intercept	148.671	0.001	1.000	6.816	0.000
Type of honey	0.004		0.275		0.000
Regression Summary for Dependent Variable: Fructose + Glucose content, % $R = 0.47329$; $R^2 = 0.224$; Adjusted $R^2 = 0.177$; $p < 0.001$ (Corrected Model)					
No. of cases: 89	Coef.	Std. error of estimate	Coef. of determination R^2	F (df = 88)	p-value
Intercept	338893.353	8559.045	0.975	4.795	0.000
Type of honey	2472.522		0.224		0.001

Conclusion

The determined significant correlations between some physicochemical parameters of the investigated types of honey are different in strength and direction:

– The correlation between WC and RI is moderate to strong and negative for all studied types of honey, which confirms the information known from literature – by increasing WC the values of RI decrease;
– The correlation between pH and EC in lime tree, coriander and multifloral honey is positive, but different in strength – from weak to moderate;
– Acacia honey differs in strength and direction from: lime tree and multifloral honey in terms of correlations between pH and total F+G content; from lime tree, rapeseed and sunflower honey as regards the correlations between F+G and RI and between WC and F+G.

The obtained significant General Linear Models (1-5) would allow the determination of prognostic values of the physicochemical parameters discussed in the study based on different types of honey.

The linear nature of the relations between physicochemical parameters has not been proven, which suggests that studies could be continued by comparing various non-linear models. It is necessary to expand the studies in this aspect with a larger number and more types of samples.

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