Chemometric Analysis of Selected Honey Samples from Western Part of Romania

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Romania plays an important part in the European and world honey production and trade. Chestnut (Castanea sativa) and Linden (Tilia cordata) are very important types of honey in Romania, because there are quite large areas where these trees grow. Honeydew is collected by honey bees, which transform it into a strongly flavoured dark colour honey less sweet than floral honey. It is quite common in a number of countries from Europe, including Romania. The aim of this study was to determine the colour, pH, total acidity, ash, electrical conductivity and water content of selected honey samples from western part of Romania and performed chemometric analysis for honey type discrimination. Univariate analysis explores each parameter in the data set, for all honey types, and multivariate statistic gives us the opportunity to determine which variables can discriminate the three honey types as clusters.

Keywords: honey, physico-chemical analysis, PCA, HCA

Natural honey is a sweet, flavourful liquid food of high nutritional value and immense health benefits [1]. The composition of honey is mainly sugars and water, but in addition, it also contains several vitamins and minerals. The other constituents of honey are amino acids, antibiotic-rich inhibine, proteins, phenol antioxidants, and micro-nutrients [2].

The substitution of refined carbohydrates by honey has been shown to be beneficial [3]. Honey contains quickly absorbing simple sugars, this way it can provide energy immediately. It is an important element in the modern dietetics so it is subject to many studies all around the World, [4-8].

With a very long tradition of beekeeping, Romania contributes to the European market with significant amount of unifloral and polyfloral honey of remarkably good quality [9,10]. A deep control of the honey quality is necessary and it is very important to verify the compliance with the quality specifications of the European Union [11].

Chestnut honeys has generally a touch bitter in taste, dark colour, high electrical conductivity, high fructose content, high value of fructose/glucose, which leads to a lower level of crystallization [12].

Linden honey it is of a greenish colour, when fresh, but in time, its colour becomes clear to amber with a yellow tone. The aroma is of a low acidity, medium sweetness and, sometimes, a light bitterness. The electrical conductivity is lower than 0.7 mS/cm; *p*H<5; close value for fructose and glucose determined a medium to fast rate of crystallization with fine to medium sized crystals [12, 13].

Honey dew is collected by honey bees, which transform it into a strongly flavoured dark colour honey less sweet than floral honey. Generally honeydew honeys have common physical-chemical characteristics like: high electrical conductivity, high ash content, free acidity and *p*H, positive values of specific rotation, low values of fructose, glucose and higher rates of oligosaccharides, while organoleptic characteristics are more variable [12,14].

Currently the measurement of electrical conductivity is the most useful quality parameter for honey classification which can be determined by relatively inexpensive instrumentation. This has been confirmed by Persano Oddo and Piro [12].

Colour perception is one of the fundamental ways by which humans recognise, associate and understand about the things around them. The colour measurement techniques (instrumental and visual) should be precise, simple and fast, so that they can help both, producer and customer, to control the quality and to achieve a better selection.

The aim of our study was to realise a physical-chemical characterization of some Chestnut, Linden and Honeydew honey from western part of Romania and with these results to performed chemometric analysis for honey type discrimination.

Experimental part

Material and methods

The study was carried on 20 honey samples from western part of Romania, in 2013 harvest year. Experimental data was sampled as follow: chestnut honey (CH) in six samples, linden honey (LH) in nine samples and honeydew honey in five samples, each of all with six replications. Honey samples were heated in a water bath at temperature below 45°C, degrees until a fluid consistency.

Physico-chemical parameters

Ash, Water, *p*H, Total acidity (TotAcid.), Electrical conductivity (ElCond.) were analysed according to the Romanian Standard Analysis Methods [15] and Harmonised methods of the IHC [16].

The colour of honey samples, were analysed using specific CIE-1976 (L*a*b) and CIE-(L*C*h*) methods [17], a spectrophotometric measurement of the transmitance of 50% honey solution (w/v), with Shimadtzu UV mini 1240 spectrophotometer, in the visible range of 380-720 nm with

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a 10 nm intervals. [17,18]. This method implies spectral analysis.

L* value indicates degree of lightness, positive a* indicates red, negative a* green component, positive b* indicates yellow, and negative b* blue component, C* indicate chroma and h* indicate hue [19].

Statistical analysis

All honey samples were subjected to unbalanced one-way analysis of variance ANOVA (P = 0.05) and the means differences multicomparison Tukey test (P = 0.05); the software used was GraphPad Prism 5.03 [20].

Correlations were obtained by Pearson's correlation coefficient (R). Bivariate linear regressions were performed, in order to analyse the functional dependence between the variables Ash, ElCond and the other studied variables. Results with $p < 0.05$ confidence level were considered statistically significant. The multivariate analysis comprised of principal component analysis (PCA) and hierarchical cluster analysis (HCA). For all the latter analyses it was used the PAST version 3.07 software [21].

Results and discussions

Physico-chemical analysis

The average values and standard deviation obtained for the different physicochemical parameters evaluated: Ash, ElCond, TotAcid, *p*H, Water, Colour CIE L*a*b* and CIE L*C*h*, are shown in table 1. These values were compared with the limits established for honey in the International Regulatory Standards by the Council Directive 2001/110/ES.

The values for Ash don't exceed the limit of 1.2 mS/cm for CH (0.57-1.1%) and HD (0.71-0.91%), or 0.6 mS/cm for LH (0.09-0.31%).

Electrical conductivity values, range between 0.86 and 1.64 mS/cm, for CH and HD, are ≥ 0.8 mS/cm, and for LH range between 0.29 and 0.64 mS/cm, are ≤ 0.8 mS/cm, in accordance with International regulations.

Total acidity values are between 11.1 and 44.9 meq/kg, and they don't exceed the limit of 50 meq/kg permitted by EU directive.

Results in the same range, for previously named parameters, were noticed by [6, 13, 22].

The values of water content range from 17.2 to 19.8% are below 20%, which is the maximum value stated by the Council Directive 2001/110/ES. The moisture content of honey has a special significance and can give a primary honey quality assessment. High moisture content in honey may be due a direct or indirect adulteration [23].

*p*H values, CIE L*a*b* and CIE L*C*h* colour are not regulated parameters.

LH has the highest value for L*, that indicates a light colour honey in comparison with CH and HD, both of them being dark colour honey.

CH has the greatest red (a*) and yellow (b*) component, followed by HD and LH with the lowest values of these components (table 1, fig.1).

Statistical analysis

Parameter Ash is strongly positive correlated and statistically significant with ElCond and with a*, b*, C* components (table 2). The correlations between Ash and ElCond will be discussed from the bivariate regressions point of view. h* is strongly positive correlated with b*, and other components like a*, b* and C* are strongly correlated ($R>0.7$) and statistically significant ($p<0.0001$). L* is strongly negative correlated with a*, b*, C* and with Ash and ELCond (table 2). When the content of Ash and ElCond increase the lightness (L*) of honey samples decrease.

Associations of analysed honey parameters were investigated by bivariate linear regressions (table 3). Ash is strongly positive correlated ($R=0.86$) and statistically significant ($p<0.0001$) with ELCond. This fact is due to salts contained by honey which generates high ELCond. Statistical significant ($p<0.0001$), and strongly positive correlations ($R>0.7$) are register between Ash and a*, b*, C* and moderate correlation with h* (R = 0.695). Another strong, but negative correlation is observed between Ash and L* (R= -0.843).

Table 1

PHYSICO-CHEMICAL AND COLORIMETRIC PARAMETERS OF CHESTNUT (CH), LINDEN (LH) AND HONEYDEW (HD)

Parameters	CH (N = 36)	LH (N = 54)	HD (N = 30)
Ash %	0.710b ±0.096	0.186c ±0.062	0.824a ±0.062
ELCond mS/cm	1.304a ±0.129	0.486c ±0.108	0.946b ±0.077
TotAcid meq/kg	14.798c ±3.364	23.390b ±2.276	31.454a ±7.333
pH	4.403a ±0.449	4.425a ±0.355	3.236b ±0.642
Water %	18.074b ±0.404	18.437a ±0.720	18.602a ±0.480
L*	57.043b ±4.668	79.545a ±4.893	58.454b ±2.929
a*	18.869a ±1.165	11.139c ±1.674	14.983b ±0.672
b*	60.857a ±2.312	28.873c ±4.615	48.827b ±1.099
C*	63.735a ±2.013	31.003c ±4.532	51.078b ±1.117
h*	72.740a ±1.458	68.648b ±3.289	72.939a ±0.711

Honeys – values are expressed as mean ± standard deviation; different letters along rows describe the statistical significant differences between means values calculated in post-hoc Tukey test of one-way ANOVA

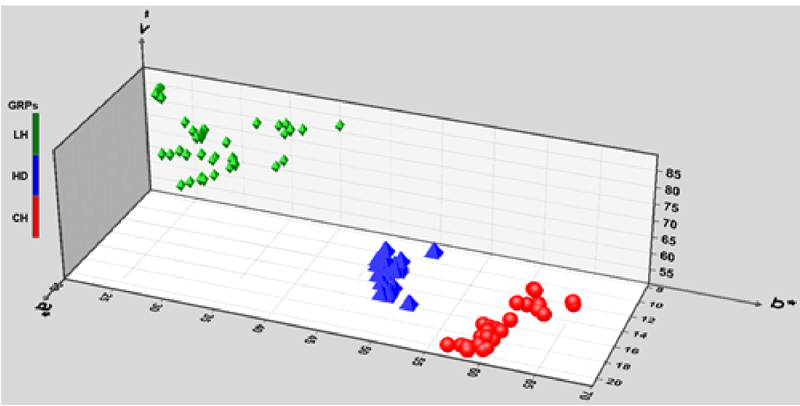

Fig. 1. Chestnut (CH with red dots), linden (LH with green diamonds) and honeydew (HD with blue pyramids) honeys colorimetric properties in CIE L*a*b* chromatic space

Table 2
CORRELATION MATRIX OF PHYSICO-CHEMICAL AND COLORIMETRIC PARAMETERS OF CHESTNUT (CH), LINDEN (LH) AND HONEYDEW (HD) HONEYS

R \ *p*	Ash	ELCond	TotAcid	pH	Water	L*	a*	b*	C*	h*
Ash		**<0.0001**	0.9771	**<0.0001**	0.2467	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
ELCond	**0.860**		**<0.0001**	0.1865	**0.0051**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
TotAcid	-0.003	-0.415		**<0.0001**	**0.0002**	0.8814	**0.0008**	**0.0002**	**0.0002**	0.0736
pH	-0.439	-0.121	-0.375		0.0685	**<0.0001**	0.0716	0.0990	0.0953	0.1966
Water	-0.107	-0.254	0.337	-0.167		0.5643	0.0657	**0.0158**	**0.0175**	**0.0178**
L*	**-0.843**	**-0.790**	0.014	0.399	0.053		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
a*	**0.716**	**0.863**	-0.302	-0.165	-0.169	**-0.895**		**<0.0001**	**<0.0001**	**<0.0001**
b*	**0.853**	**0.937**	-0.335	-0.151	-0.220	**-0.871**	**0.901**		**<0.0001**	**<0.0001**
C*	**0.847**	**0.938**	-0.336	-0.153	-0.217	**-0.879**	**0.916**	**0.999**		<0.0001
h*	0.695	0.613	-0.164	-0.119	-0.216	-0.499	0.364	**0.720**	0.696	

– Pearson coefficient (R) values are presented in lower diagonal part and statistical significance (p) is presented in the upper diagonal part

Table 3
BIVARIATE LINEAR REGRESSION STATISTICAL RESULTS FOR ASH AND ALL OTHER PARAMETERS

	Slope a	Intercept b	*R*	R^2	*p*
Ash-ELCond	1.061	0.314	**0.860**	**0.739**	**0.0001**
Ash-TotAcid	-0.067	22.862	-0.003	0.000	0.9784
Ash-pH	-1.014	4.630	-0.439	0.192	**0.0001**
Ash-Water	-0.219	18.479	-0.107	0.011	0.2430
Ash-L*	-33.136	84.172	**-0.843**	**0.710**	**0.0001**
Ash-a*	8.519	10.138	**0.716**	0.512	**0.0001**
Ash-b*	40.915	22.897	**0.853**	**0.727**	**0.0001**
Ash-C*	41.406	25.035	**0.847**	**0.718**	**0.0001**
Ash-h*	7.312	67.273	0.695	0.483	**0.0001**

Table 4
BIVARIATE LINEAR REGRESSION STATISTICAL RESULTS FOR ELECTRIC CONDUCTIVITY (ELCOND) AND ALL OTHER PARAMETERS

	Slope a	Intercept b	*R*	R^2	*p*
ELCond-TotAcid	-8.4844	30.01	-0.41483	0.17208	**0.0001**
ELCond-pH	-0.22738	4.3132	-0.12142	0.014743	0.1777
ELCond-Water	-0.42217	18.726	-0.25403	0.064531	**0.0047**
ELCond-L*	-25.172	88.828	**-0.78995**	0.62402	**0.0001**
ELCond-a*	8.3245	7.3729	**0.86303**	**0.74482**	**0.0001**
ELCond-b*	36.44	12.613	**0.93678**	**0.87756**	**0.0001**
ELCond-C*	37.141	14.404	**0.9377**	**0.87928**	**0.0001**
ELCond-h*	5.2306	66.52	0.61324	0.37607	**0.0001**

High values of correlation coefficients ($R>0.7$), from bivariate linear regressions (table 4), and statistical significant ($p<0.0001$) are displayed between ELCond and a*, b*, C* colour component, and statistical significant ($p<0.0001$) but strongly negative correlation with L* (R= -0.78995). These results for Ash-ELCond correlations comply with those reported by [22, 24-26].

In order to assess the clusters of the studied honey samples were performed principal component analysis (PCA) and hierarchical cluster analysis (HCA). Principal component analysis results are presented in table 5 and figure 2; all variables from table 1 were used as input for PCA. Table 5 presents the numerical values for the eigenvalues and their percentage explained variance. First principal component (Component 1) explains 61.984% from total variance and the second principal (Component 2) explains 17.871% of total variance, so the first two principal components explain 79.855% of total variance that assures accuracy for further statistic results. Eigenvalues of the first two principal components are higher than unit, meaning that one can select only these two principal components to accurately analyse the multivariate behaviour of the honey samples.

Table 5
PRINCIPAL COMPONENT ANALYSIS (PCA) RESULTS FOR THE EIGENVALUES

PC	Eigenvalue	% variance
1	**6.18935**	**61.894**
2	**1.78705**	**17.871**
3	0.753828	7.5383
4	0.616807	6.1681
5	0.461302	4.613
6	0.151443	1.5144
7	0.027858	0.27858
8	0.010957	0.10957
9	0.001403	0.014025
10	2.46E-06	2.46E-05

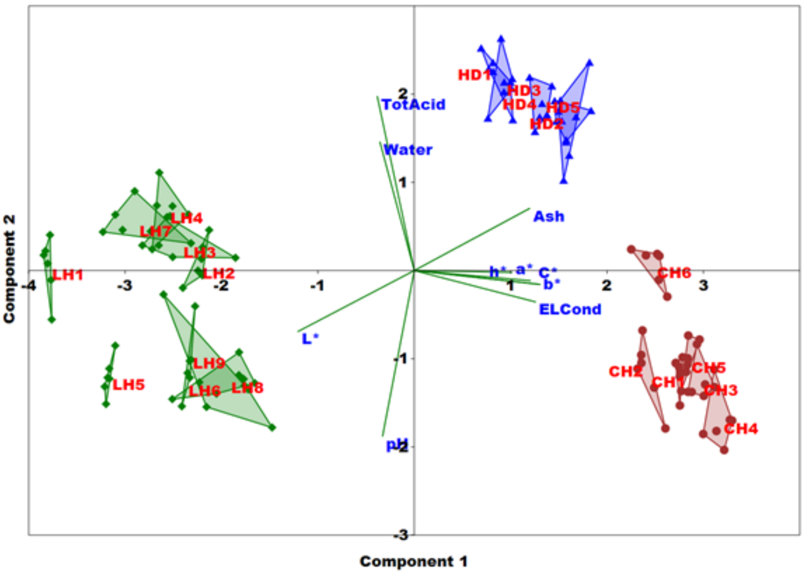

Fig. 2. Principal component analysis (PCA) biplot for the physico-chemical and colorimetric parameters of chestnut (CH), linden (LH) and honeydew (HD) honeys

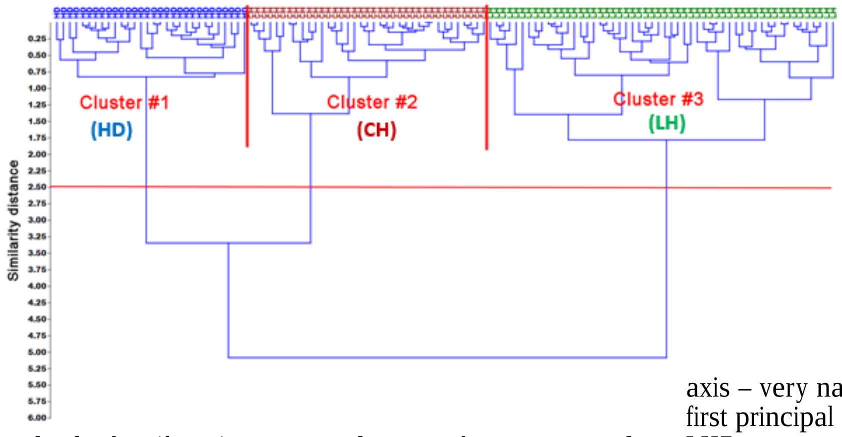

Fig. 3. Dendrogram of hierarchical cluster analysis (HCA) of chestnut (CH), linden (LH) and honeydew (HD) honeys samples

The biplot (fig. 2) presents the samples scores and variables loadings resulting from the PCA. The variables loadings are represented as vectors with the starting point in the origin of the coordinates system and the ending point is marked by the variables names [27, 28]. Also, these vectors are correlated with the principal components axes and to each other, by the angles between them – small angles mean stronger correlation and large angles mean weak correlation between the vectors and between axes and vectors. Furthermore, small angles between vectors mean that they consist in a group with strong correlation between them and statistically significant bivariate regression. From this point of view one can distinguish grouping variables as follows (fig. 2): variables ELCond, a*, b*, C* and h* compose the first group, variables TotAcid and Water are the second group and variables Ash, L* and *p*H is each a standalone group. First variables group and Ash are positive loaded and strong correlated with the first principal component axis and the second variables group is positively loaded and strong correlated with the second principal component axis. Variables L* and pH are negative loaded with both principal axes. Also, variable L* is strong correlated with the first principal axis and variable pH is strong correlated with the second principal axis.

In the same manner, the biplot presents the honey samples grouping. The three honey types, CH, LH and HD, consist in non-overlapping groups (fig. 2), but the individual samples are widely spread out. All five honeydew honey samples (HD1– HD5) are positive loaded with both principal axes. First five chestnut honey samples (CH1–CH5) are positive loaded with the first principal axis. Sample CH6 can be considered positive loaded with the first principal axis and null loaded with the second principal axis, due to extreme strong correlation with first principal axis – very narrow angles of the replications points with first principal axis. Linden honey samples LH3, LH4 and LH7 are negative loaded with first principal axis and positive loaded with second principal axis; linden honey samples LH5, LH6, LH8 and LH9 are negative loaded with both principal axis. Linden honey samples LH1 and LH2 are negative loaded with first principal axis and null loaded with the second principal axis.

Combining these two biplot properties (scores and loadings) and based on the previously explanations, it can be derived the fact that the honey type groups (CH, LH and HD) are discriminated by the variables groups (fig. 2). Furthermore, it can be stipulated the multivariate abundance of the variables in the honey groups.

The variables: Ash, ElCond, a*, b*, C* and h* are more abundant in the honedew honey and chestnut honey group than in the linden honey group. The Water and TotAcid variables are abundant in LH3, LH4, LH7 samples and honeydew honey group than the rest. The variable L* is abundant only in linden honey group than the other two honey type groups. The pH variable is abundant in linden honey samples LH5, LH6, LH8 and LH9 and chestnut honey samples CH1 – CH5.

Honey type grouping can be validated as clustering by hierarchical cluster analysis (HCA) with the first two principal coordinates of honey samples as input – HCA algorithm was unweighted pair group method with arithmetic mean (UPGMA) and with Euclidean distance as similarity index [28].

The HCA results are presented as dendrogram graphical representation (fig. 3). In the HCA dendrogram are also presented the clusters of honey types (CH, LH and HD) generated for a threshold similarity distance of 2.50 (dotted red line in fig. 3).

Results presented in figure 1 and figure 2 shows that the variables L*, a* and b* can discriminate alone between the honey types and generate the same cluster number and content as generated by all the variables.

Conclusions

The results obtained for the basic physical-chemical parameters analysed in our study show that all the tested honey samples present a good quality, which is consistent with the legislative requirements.

The HCA clustering method validates that the initial honey type groups (from PCA) consists as clusters, too. This means that the analysed variables, the physical-chemical, colorimetric ones and all together, have the discrimination potential (e.g. are discriminators for) of the CH, LH and HD honey types. The Cohen statistics, that measure the clustering accuracy, has the coefficient value 0.9419 that means the HCA result has high accuracy, as the unit value is the highest.

Ash, ELCond and colour, were the more affected parameters by the type of honey, so these parameters have an important influence in honey type discrimination.

Grouping of parameters and honey type clustering can be used in the future to monitor changes from one year to another, or from different geographic regions.

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Manuscript received: 30.07.2015