Honey authentication using rheological and physicochemical properties

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Abstract The aim of this study was to evaluate the influence of honey botanical origins on rheological parameters. In order to achieve the correlation, fifty-one honey samples, of different botanical origins (acacia, polyfloral, sunflower, honeydew, and tilia), were investigated. The honey samples were analysed from physicochemical (moisture content, fructose, glucose and sucrose content) and rheological point of view (dynamic viscosity—loss modulus G'' , elastic modulus G' , complex viscosity η^* , shear storage compliance— J' and shear loss compliance J''). The rheological properties were predicted using the Artificial Neural Networks based on moisture content, glucose, fructose and sucrose. The models which predict better the rheological parameters in function of fructose, glucose, sucrose and moisture content are: MLP-1 hidden layer is predicting the G'' , η^* and J'' , respectively, MLP-2 hidden layers the J' , while MLP-3 hidden layers the G' , respectively. The physicochemical and rheological parameters were submitted to statistical analysis as follows: Principal component analysis (PCA), Linear discriminant analysis (LDA) and Artificial neural network (ANN) in order to evaluate the usefulness of the parameters studied for honey authentication. The LDA was found the suitable method for honey botanical authentication, reaching a correct cross validation of 94.12% of the samples.

Keywords Rheology · Authentication · PCA · LDA · ANN

Introduction

Honey is a natural product, with a high viscosity, sweet and with a specific aroma, and it is known as the oldest sweetener by humans. The composition, color, aroma, and flavor of honey depend firstly on flowers, climate, geographical regions and honey bee species involved in its production (Kadri et al. 2017).

The botanical origin of honey is influenced mainly by the frequency of the pollen types of nectariferous species (Belay et al. 2017). Honey is classified as a monofloral one, if the dominant pollen grains reach an established threshold and show the typical flowing property of the corresponding type (Arrigoni et al. 2014). Various unifloral honeys exhibit usual sensory, physicochemical and melissopalynological properties. However, the analyses of these properties are protracted and involve dedicated experts (Siddiqui et al. 2017). Over the last years, the authentication of honey has been based on the evaluation of sensory and physicochemical parameters (Oroian et al. 2017; Popek et al. 2017; Manzanares et al. 2014; Isla et al. 2011), phenolic compounds (Biluca et al. 2017; Oroian and Ropciuc 2017), volatile profile (Oroian et al. 2015a; Escriche et al. 2011), mineral content (Czipa et al. 2017; Oroian et al. 2015b, Kaygusuz et al. 2016), sugar profile (de Sousa et al. 2016), proton transfer reaction mass spectrometry (PTR-MS) (Kuš and van Ruth 2015), nuclear magnetic resonance (Boffo et al. 2012), antioxidants (Lachman et al. (2010), DNA metabarcoding (Prosser and Hebert 2017), and FT-IR spectrum (Osés et al. 2017; Mehaya et al. 2015; Gok et al. 2015).

Honey rheology is one of the most important characteristic of honey because it affects the quality and is useful for the processing equipment design (Anupama et al. 2003). The rheological properties, e.g. viscosity, depend on

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several factors: temperature, moisture content, chemical composition (Oroian 2012, 2015). The viscosity is considered an important parameter in all stages of honey production, starting from the extraction of honey from combs to straining, pumping, processing, as well as packing (Nayik et al. 2016).

Considering the importance of the honey rheological properties and their implications on the quality and process control of food and on the consumers organoleptic perception, the aim of this study was to characterize honey from the physicochemical and rheological point of view, and to evaluate the usefulness of these parameters for honey authentication.

Materials and methods

Materials

For this study 10 samples of acacia honey, 8 samples of tilia honey, 11 samples of sunflower honey, 10 samples of honeydew honey, and 12 samples of polyfloral honey were analysed. All samples were purchased from beekeepers located into Suceava County, Romania. To prevent the influence of crystals and air bubbles (Oroian 2012) on rheological properties, the samples were heated at 55 °C to dissolve any crystals, prior to the analyses.

Physicochemical analysis

The moisture content, glucose, fructose and sucrose were determined according to the Harmonised methods (Bogdanov et al. 2002).

The sugars in honey samples were carried out by a HPLC model ADVP-SHIMADZU, with RI-detector, according to a method described by Bogdanov et al. (2002). For the separation of the sugars a 250 × 4.6 mm i.d. and particle size 5 µm amino column was used. HPLC conditions were: flow rate of 1.3 mL/min, mobile phase A acetonitrile and mobile phase B water at a 80:20 ratio (v/v). All the sugars were expressed as g/100 g. All the analyses were performed in triplicate.

Dynamic rheology

A Mars 40 rheometer (Thermo Haake, Germany) was used for the determination of dynamical rheological properties of honey with a parallel plate system (Ø 40 mm) at a gap of 1000 µm. Prior to the determination, all the samples were placed between the plates and maintained for 5 min to recover itself and to reach the desired temperature. The measurements were made at

20 °C. For determining the linear viscoelastic region, a stress sweep test was performed at 1 Hz. The tests were made at a frequency range of 0.1–10 Hz at 1 Pa stress (which was in the linear region). The rheological parameters used were: loss modulus G'' (Pa), elastic modulus G' (Pa), complex viscosity η^* (Pa·s), shear storage compliance J' (1/Pa), and shear loss compliance J'' (1/Pa). A Rheowin Job software (v. 4.63, Haake) was used to obtain the experimental data and to calculate the complex viscosity (η^*). All the samples were analysed in triplicate.

Statistical analysis

The Unscrambler X 10.1 (Camo, Norway) was used for the statistical analysis (analysis of variance (ANOVA), Principal component analysis (PCA) and Linear discriminant analysis (LDA)). The Neurosolution 7.0 trial version (IBM, USA) was used for the artificial neural networks—ANN—(multilayer perceptron (MLP), probabilistic neural network (PNN) and modular neural network (MNN)).

Results and discussions

Physicochemical properties

The physicochemical parameters (moisture content, fructose, glucose and sucrose) of honey according to botanical origin are presented in Table 1. The moisture content of the samples ranged between 14.5 and 19.8%, being lower than the maximum allowable level of 20% established by the Codex Alimentarius (2001). A moisture content higher than 20% may accelerate the fermentation processes during storage (Oroian 2012). The moisture content is influenced by the origin ($P < 0.05$); the honeydew honeys had the lowest concentration, while the sunflower showed the highest concentrations. Our moisture contents are similar to those reported in literature (Bath and Singh 1999; Manzanares et al. 2014; Escriche et al. 2014, 2017; Popek et al. 2017; Atanassova et al. 2016; Oroian and Ropciuc 2017). Singh and Bath (1997) reported similar moisture content for

Trifolium and *Eucalyptus lanceolatus* honeys, but the higher moisture content for *Brassica juncea* honey.

The honey sugar composition is influenced by the botanical origin (Kaškonienė et al. 2010), thus the level of different sugars in honey may indicate the honey authenticity (Oddo et al. 2004). Acacia honey had the highest concentration of fructose, as it was observed in the case of Spanish and Czech acacia honeys (Juan-Borras et al. 2014), while sunflower honeys had the lowest level of fructose. In the case of glucose, the highest levels were observed for the honeydew honeys, while the acacia honeys displayed

Table 1 Physicochemical properties of honey according to their botanical origin

Physicochemical parameters	Honey type—mean (SD)					<i>F</i> -ratio
	Acacia	Tilia	Polyfloral	Honeydew	Sunflower	
Moisture content (g/100 g)	17.0(1.3)abc	17.8(1.5)ab	17.1(1.1)bc	16.3(0.7)c	18.2(1.6)a	2.95*
Fructose (g/100 g)	42.21(2.56)a	38.90(1.53)b	34.71(1.18)c	35.51(1.51)c	33.72(1.29)c	50.76***
Glucose (g/100 g)	27.91(2.80)c	31.62(1.83)b	31.78(1.10)b	34.91(1.31)a	31.28(1.25)b	14.12***
Sucrose (g/100 g)	1.30(0.32)a	1.41(0.15)a	1.76(0.51)a	0(0)b	1.41(0.24)a	11.20***

the lowest level. The low content of sucrose indicates that honey samples were properly matured before harvesting (Juan-Borras et al. 2014), and the values of sucrose are in agreement with those reported for the Mozambique honeys (Escriche et al. 2017) and European honeys (Odoó et al. 2004). The honeydew samples did not contain sucrose.

Honey rheology

In Fig. 1 is presented a typical honey rheogram observed at 20 °C. The honey sample exhibited Newtonian behaviour (the honey complex viscosity is not influenced by the frequency generating a viscosity plateau); regarding the G' and G'' , it can be observed that $G'' \gg G'$ due to the viscous nature of honey (Oroian 2012). The J' and J'' are strongly negatively correlated with the frequency. The elastic modulus and loss modulus are strongly influenced by the frequency applied. The Newtonian behaviour of honey has been reported for other honeys from different countries, as follows: Burkina Faso (Escriche et al. 2016), Mozambique (Escriche et al. 2017), Poland (Juszczak and Fortuna 2006), Israel (Cohen and Weihs 2010) and Spain (Oroian et al. 2013a, b). Regarding the influence of moisture content, it was observed that the rheological parameters magnitude are decreasing strongly with the increasing of the moisture

content because water is reducing the molecular friction and hydrodynamic forces into the matrix (Al-Mahasneh et al. 2014).

Honey rheological parameters prediction using artificial neural networks

The study was conducted using the artificial neural networks prediction of the rheological parameters as output parameters (loss modulus G'' (Pa), elastic modulus G' (Pa), complex viscosity η^* (Pa·s), shear storage compliance— J' (1/Pa) and shear loss compliance— J'' (1/Pa)) using the physicochemical parameters as input parameters (fructose, glucose, sucrose and moisture content). The multilayer perceptron (MLP), modular neural network (MLP) and probabilistic neural network (PNN) were used for predicting the rheological parameters. The statistical parameters used for checking the suitability of the model were: mean squared error (MSE), regression coefficients (R^2) and mean absolute error (MAE). The input data was divided into three categories: training (40% of the experimental data), cross validation (30% of the experimental data) and testing (30% of the experimental data), and for each model it was used a different number of hidden layers (intermediate layer between the input and output layer) ranging from one to three. Each model was based on 51 experiments and the values of the rheological parameters were taken from the frequency of 1 Hz. In Table 2 are presented the statistical parameters for each model analysed; the suitability of the model was not improved by the increasing of the number of hidden layers. Taking into account the statistical parameters, the suitable model for elastic modulus prediction is MLP-3 hidden layers, for loss modulus, complex viscosity and shear storage compliance is MLP-1 hidden layer, and for shear loss compliance is MLP-2 hidden layers, respectively. The lower values of regression coefficients for G' are because it is hard to achieve a proper evolution of this parameter due to the fact that honey has a low elastic part ($G'' \gg G'$) and the presence of any crystals can interfere in the determination (Oroian 2015).

There are other studies related to the artificial neural network prediction of rheological parameters based on

Fig. 1 Typical rheogram for honey: rhombus— G'' , triangle— η^* , square— G' , cross— J' , multiplication— J''

Table 2 ANN statistical parameters for rheological parameters

No	Model name*	Hidden layers	Training			Cross validation			Testing		
			MSE	R ²	MAE	MSE	R ²	MAE	MSE	R ²	MAE
Elastic modulus—G'											
1	MLP	1	3.782	0.613	1.146	14.95	0.012	1.888	1.823	0.038	0.919
2	MLP	2	2.09	0.809	0.773	15.37	0.061	1.979	2.803	0.104	0.932
3	MLP	3	4.85	0.768	1.278	8.99	0.678	1.854	2.237	0.608	1.047
4	PNN	1	5.72	0.124	1.093	15.19	0.459	1.731	0.570	0.515	0.659
5	PNN	2	4.81	0.439	1.203	15.71	0.203	1.962	1.454	0.120	0.986
6	PNN	3	3.74	0.630	0.972	13.53	0.170	1.906	1.486	0.248	0.788
7	MNN	1	2.91	0.718	1.050	15.28	0.050	1.949	2.005	0.045	1.004
8	MNN	2	2.83	0.732	0.950	13.89	0.160	1.899	1.707	0.113	0.938
9	MNN	3	5.58	0.251	1.160	14.32	0.230	1.790	0.671	0.006	0.780
Loss modulus—G''											
1	MLP	1	4027.2	0.935	35.39	6127.8	0.911	54.21	1371.88	0.965	27.668
2	MLP	2	2109.4	0.975	27.64	8016.45	0.895	63.37	6815.21	0.897	52.536
3	MLP	3	8738.8	0.855	73.17	8596.8	0.908	75.79	4312.4	0.894	52.36
4	PNN	1	10723.5	0.790	62.15	15602.4	0.738	87.07	8427.2	0.787	75.15
5	PNN	2	4982.0	0.917	48.27	12643.6	0.798	88.29	3429.3	0.924	49.37
6	PNN	3	13471.9	0.809	76.35	20194.8	0.660	106.71	10227.2	0.780	88.70
7	MNN	1	3763.7	0.939	37.96	5592.4	0.919	56.57	3753.3	0.903	45.49
8	MNN	2	3896.0	0.937	31.58	5923.7	0.912	48.07	1235.2	0.971	17.91
9	MNN	3	19373.3	0.798	100.07	24815.0	0.770	108.9	13455.5	0.819	97.89
Complex viscosity—η*											
1	MLP	1	94.99	0.951	5.22	134.76	0.929	7.618	38.11	0.967	4.597
2	MLP	2	42.33	0.981	3.24	188.90	0.902	63.37	146.89	0.912	7.462
3	MLP	3	58.28	0.980	6.42	97.20	0.954	7.600	100.92	0.953	6.995
4	PNN	1	261.3	0.800	10.15	400.27	0.736	14.44	189.15	0.820	11.71
5	PNN	2	128.9	0.945	6.17	278.81	0.837	12.95	115.7	0.899	9.48
6	PNN	3	186.54	0.889	9.72	419.78	0.726	16.89	184.8	0.818	11.66
7	MNN	1	109.71	0.930	6.84	153.16	0.912	9.41	114.2	0.884	7.99
8	MNN	2	117.99	0.918	5.86	145.24	0.919	8.11	38.96	0.964	4.14
9	MNN	3	654.01	0.630	17.68	889.64	0.766	21.45	508.5	0.734	18.49
Shear storage compliance—J'											
1	MLP	1	8.7×10^{-8}	0.715	0.0001	1.9×10^{-8}	0.688	0.0001	5.2×10^{-7}	0.263	0.0003
2	MLP	2	8.5×10^{-9}	0.976	0.0001	6.3×10^{-8}	0.742	0.0001	6.2×10^{-7}	0.702	0.0004
3	MLP	3	2.2×10^{-8}	0.972	0.0001	4.6×10^{-8}	0.703	0.0001	5.8×10^{-7}	0.020	0.0004
4	PNN	1	1.1×10^{-7}	0.496	0.0002	2.4×10^{-8}	0.597	0.0001	5.4×10^{-7}	0.300	0.0003
5	PNN	2	7.4×10^{-8}	0.833	0.0001	2.0×10^{-8}	0.669	0.0001	5.2×10^{-7}	0.256	0.0003
6	PNN	3	1.5×10^{-8}	0.940	0.0001	6.8×10^{-8}	0.597	0.0001	6.6×10^{-7}	0.207	0.0004
7	MNN	1	1.8×10^{-8}	0.925	0.0001	4.7×10^{-8}	0.710	0.0001	5.4×10^{-7}	0.225	0.0004
8	MNN	2	1.6×10^{-8}	0.952	0.0001	5.8×10^{-8}	0.676	0.0001	6.2×10^{-7}	0.004	0.0004
9	MNN	3	6.6×10^{-8}	0.937	0.0001	2.9×10^{-8}	0.658	0.0001	5.3×10^{-7}	0.103	0.0004
Shear loss compliance—J''											
1	MLP	1	4.6×10^{-5}	0.954	0.003	5.4×10^{-5}	0.934	0.005	6.5×10^{-5}	0.879	0.005
2	MLP	2	5.3×10^{-5}	0.944	0.003	0.0001	0.722	0.005	0.0002	0.397	0.011
3	MLP	3	0.0001	0.849	0.008	0.0002	0.546	0.011	0.0004	0.005	0.016
4	PNN	1	0.0003	0.653	0.009	6.8×10^{-5}	0.742	0.007	0.0002	0.345	0.011
5	PNN	2	0.0003	0.609	0.009	0.0001	0.470	0.009	0.0002	0.227	0.012
6	PNN	3	0.0002	0.730	0.008	0.0001	0.187	0.001	0.0004	0.274	0.013

Table 2 continued

No	Model name*	Hidden layers	Training			Cross validation			Testing		
			MSE	R ²	MAE	MSE	R ²	MAE	MSE	R ²	MAE
7	MNN	1	4.4×10^{-5}	0.944	0.004	8.3×10^{-5}	0.848	0.005	0.0001	0.658	0.010
8	MNN	2	4.8×10^{-5}	0.937	0.004	0.0001	0.780	0.006	0.0002	0.589	0.009
9	MNN	3	0.0001	0.884	0.008	7.0×10^{-5}	0.711	0.007	0.0002	0.335	0.012

*MLP multilayer perceptron, PNN probabilistic neural network, MNN modular neural network

water content, temperature and shear rate (Ramzi et al. 2015), temperature, frequency and moisture content (Oroian 2015), or chemical composition, temperature and frequency (Escriche et al. 2017). In these studies it was observed that the frequency and temperature has a higher impact on the rheological parameters (Oroian 2015; Escriche et al. 2017), and for this reason in this work were predicted only the parameters on chemical composition (moisture content, fructose, glucose and sucrose content) to see which compound influences more the rheological parameters.

Honey authentication

The statistical methods (PCA, LDA and ANN) were used for achieving the authentication of honey using the physicochemical and rheological parameters.

Principal component analysis (PCA)

The principal component analysis evaluated the total effect of botanical origin of honey on rheological and physico-chemical parameters from a graphical representation (Fig. 2). The first two principal components (PC1 + PC2) are representing 99% of the total variance: PC1 represents 98% of the variance, while PC2 represents 1% of the variance, respectively. In Fig. 2 is presented the principal component analysis scores and it can be observed that the honey samples are grouped into five different groups, each one corresponding to a botanical origin. The acacia, tilia and honeydew are clearly separated as groups, while sunflower and polyfloral honeys are mixed. The mixing of the samples between sunflower and polyfloral may be because of the high variability of polyfloral honeys. Regarding the influence of the parameters, it can be observed that G'' , fructose and glucose content influence significantly the

Fig. 2 Principal component analysis scores of the honey samples: A—acacia, H—honeydew, P—polyfloral, T—tilia, S—sunflower

projection, while J' , J'' , η^* , sucrose and moisture content show no significant influence. The G'' is strongly correlated with the PC1, and in consequence with the honeydew honeys; the honeydew honeys which have the lowest moisture content have also the highest G'' values. The acacia honey projection is influenced by the fructose content; the acacia honeys have the highest concentration of fructose.

Linear discriminant analysis

The linear discriminant analysis was used for the authentication of honey using the physicochemical and rheological parameters. In the LDA projection (Fig. 3) there are five different groups. LDA reached a good classification of 98.04% of the samples (original validation) and 94.12% of the samples (cross validation), respectively. In the case of the cross validation, the function 1 explains 58.51% of the total variance, while function 2 explains 36.268%. In the case of cross validation, an acacia honey was classified as tilia, while in the case of two polyfloral it was observed a

Fig. 3 Linear discriminant analysis of honeys using physicochemical and rheological parameters: rhombus—acacia, square—tilia, triangle—polyfloral, cross—honeydew, multiplication sign—sunflower

wrong classification (one as acacia and one as sunflower). In the LDA projection (Fig. 3) there can be observed five different groups, each one corresponding to a different botanical origin.

The first discriminate function was dominated by fructose ($F1 = 0.741$, $F2 = -0.581$) and sucrose ($F1 = 0.511$, $F2 = 0.846$), respectively. The second discriminate function was dominated by sucrose ($F1 = 0.511$, $F2 = 0.846$), J'' ($F1 = -0.080$, $F2 = 0.280$) and J' ($F1 = -0.173$, $F2 = 0.209$).

Artificial neural networks

The honey samples have been classified according to their botanical origin using the physicochemical and rheological parameters using the artificial neural networks (multilayer perceptron (MLP), probabilistic neural network (PNN), and modular neural network (MNN)). The experimental data have been divided into three main categories as: training (40% of the samples), cross-validation (30% of the samples) and testing ones (30% of the samples). Each artificial neural network was calculated based 1, 2 and 3 hidden layers, respectively. For achieving the suitable model three statistical parameters were calculated: mean squared error (MSE), mean absolute error (MAE) and regression coefficients (R^2). In Table 3 are presented the statistical parameters. The suitable model for predicting the botanical origin of the honey is the MLP with 1 hidden layer, based on the statistical parameters presented in Table 3.

Conclusion

Honey samples exhibited a Newtonian behaviour irrespective of their botanical origin; the rheological parameters were influenced negatively by the increasing of the moisture content. The predictions of the rheological

Table 3 Honey authentication using artificial neural networks (ANNs)

No	Model Name*	Hidden layers	Training			Cross Validation			Testing		
			MSE	R^2	MAE	MSE	R^2	MAE	MSE	R^2	MAE
1	MLP	1	0.076	0.980	0.188	0.456	0.942	0.470	0.680	0.827	0.727
2	MLP	2	0.389	0.929	0.481	0.380	0.958	0.380	1.009	0.769	0.852
3	MLP	3	0.370	0.935	0.479	0.188	0.947	0.358	0.845	0.827	0.797
4	PNN	1	0.379	0.906	0.418	0.536	0.856	0.492	1.291	0.749	0.931
5	PNN	2	0.326	0.922	0.408	0.247	0.945	0.403	1.318	0.771	0.929
6	PNN	3	0.387	0.928	0.458	0.246	0.938	0.399	1.175	0.745	0.899
7	MNN	1	0.554	0.861	0.582	0.367	0.919	0.551	1.337	0.619	1.018
8	MNN	2	0.291	0.934	0.419	0.208	0.954	0.415	1.241	0.647	0.953
9	MNN	3	0.545	0.936	0.618	0.299	0.965	0.438	1.068	0.821	0.914

*MLP multilayer perceptron, PNN probabilistic neural network, MNN modular neural network

parameters reached higher regression coefficients for loss modulus, complex viscosity and shear loss compliance, while for elastic modulus and shear storage compliance the regression coefficients were lower. From the four chemical parameters, the moisture content has the highest influence on the magnitude of the rheological parameters (the moisture content sensitivity was higher than 0.55). The LDA is the suitable method for honey botanical authentication, reaching a correct cross validation of 94.12% of the samples.

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