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Physicochemical and rheological characterization of honey from Mozambique

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ABSTRACT

Obtaining information about honey from Mozambique is the first step towards the economic and nutritional exploitation of this natural resource. The aim of this study was to evaluate physicochemical (moisture, hydroxymethylfurfural “HMF”, electrical conductivity, Pfund colour, CIE L*a*b* colour and sugars) and rheological parameters elastic modulus G′, loss modulus G″ and complex viscosity η*) obtained at different temperatures (from 10 to 40 °C). All the physicochemical parameters were in agreement with the international regulations. Most of the honey samples were classed as honeydew honey since they were dark and had conductivity values above 0.800 mS/cm. The moduli G′, G″ and η* decreased with increasing temperature. G ′ and G″ were strongly influenced by the applied frequency, whereas η* did not depend on this parameter, demonstrating Newtonian behaviour. An artificial neural network (ANN) was applied to predict the rheological parameters as a function of temperature, frequency and chemical composition. A multilayer perceptron (MLP) was found to be the best model for G″ and η*($r^2 > 0.950$), while probabilistic neural network (PNN) was the best for G′($r^2 = 0.758$). Sensitivity testing showed that in the case of G″ and G′ frequency and moisture were the most important factors whereas for η* they were moisture and temperature.

1. Introduction

Mozambique, located on the east coast of Africa, is a developing country with great potential in terms of the availability of agro-ecological resources. It has a great diversity of climate, vegetation and geographic regions. This results in a variety of melliferous flora that can be exploited throughout the year by transhumance (Alcobia, 1995). Mozambique produces around 600 tonnes of honey a year (FAOSTAT, 2014) increasing by 100 tonnes in the last five years. However, given the availability of agro-ecological resources, Joosten and Smith (2004, pp. 2–3) state that there is a potential to produce 3.600 tonnes a year.

Apicultural development is a valuable human activity that plays an important role in the preservation of biodiversity due to its involvement in the pollination of both wild and cultivated plants. In Mozambique, 78% of the territory is suitable for carrying out this activity; however, the contribution of beekeeping to agricultural is non-existent (Zandamela, 2008). Therefore, it would be of great interest to implement policies to develop beekeeping in this country. This would meet the needs of the domestic market and avoid dependence on imports. All of this would reduce the price of honey and encourage the population to increase the consumption of this nutritious food. Better exploitation of this resource by rural people would mean a significant source of income and therefore a decrease in poverty. For Mozambique, moreover, it would contribute to the improvement of its economy, with the indirect benefit of protecting the environment and biodiversity.

In other developing African countries such as Burkina Faso, beekeeping activities have increased in recent years thanks in part to beekeeping promotion centres installed by beekeeper organizations (Nombré, Schweitzer, Boussim, & Rasolodimby, 2010). These activities are aiding in the production of honey and are playing an important role by creating sustainable livelihoods. Current development in Burkina honey is reflected in the number of scientific papers published in recent years. For example, those focused on the impact of storage conditions on the physicochemical characteristics of Burkina Faso honey (Nombré et al., 2010; Schweitzer, Nombré, Aidoo,& Boussim, 2013a); the impact of

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climatic changes on nectar considering honey production by honeybee colonies (Schweitzer, Nombré, Aidoo, & Boussim, 2013b); the rheological properties of Honey Burkina Faso (Escriche, Oroian, Visquert, Gras, & Vidal, 2016). However, there is an almost total lack of both scientific and non-scientific information concerning the honey from Mozambique. Due to the importance of the rheological properties of honey as a consequence of their implications in organoleptic perception by the consumer and the quality control of raw material and process control, the aim of the present study is to evaluate the physicochemical parameters and the rheological behaviour of Mozambique honey.

2. Materials and methods

2.1. Collection and preparation of samples

Thirty honey samples harvested in 2014 from the three provinces in Mozambique with the highest production of honey were used in this study: 10 from Nampula, 10 from Sofala and 10 from Zambezia. Honey samples were obtained from traditional beehives built with local materials (hollow trunks, bark cylinders, or interwoven twigs) and placed in trees or other places beyond the reach of predators. The honey was extracted by hand from these hives by pressure, hand-pressed or with wooden presses. Approximately 1 kg of each honey sample was purchased directly from the collectors to carry out the present study.

2.2. Physicochemical analyses

The harmonised methods of the international honey commission were followed to analyse the physicochemical parameters (hydroxymethylfurfural "HMF", moisture and electrical conductivity), and colour Pfund (Bogdanov, 2002). In addition, colour CIE L*a*b* (parameters of the *Commission Internationale de ÍEclairage*) and water activity (a_w) were determined.

Moisture content was analysed by refractrometry (Abbe-type model T1 Atago, Bellevue, Washington, USA) and the Chataway table. HPLC-UV chromatographic methodology using water-methanol (in a proportion of 90 mL of water per 10 mL of methanol) as the mobile phase for this analysis was chosen to quantify the HMF level. The column used was a ZORBAX (Eclipse Plus C18, 4.6 × 150 mm, 5 μm particle size), from Agilent (Agilent Technologies, Santa Clara, California, USA) and the detector was set to 285 nm (Escriche et al., 2016).

Electrical conductivity was determined by conductimetry (Crison Instrument, Barcelona, Spain, model C830). Colour Pfund was obtained with a millimeter Pfund scale C 221 Honey Colour Analyzer (Hanna Instruments, Eibar, Spain).

Colour CIE L*a*b* was obtained using a spectrocolorimeter Minolta CM-3600d (Minolta, Osaka, Japan). The samples were placed in 20-mm-thick holders and measured against a black-and-white background. Translucency was determined by applying the Kubelka–Munk theory for multiple scattering of the reflection spectra. Colour coordinates CIE L* a* b* were obtained from R_∞ between 400 and 700 nm for D65 illuminant and 2° observer (CIE, 1986; Visquert, Vargas, & Escriche, 2014).

Water activity was measured using an electronic dew-point water activity meter (25 °C ± 0.2 °C), Aqualab Series 4 model TE (Decagon Devices, Pullman, Washington, USA), with a temperature-controlled system (Chirife, Zamora, & Motto, 2006). All analyses were performed in triplicate.

Sugar content (glucose, fructose, and sucrose) was analysed in a Compact LC, model 1120 (Agilent Technologies, Ratigen, Germany), coupled to an Evaporative Light Scattering detector (Agilent Technologies model 1200 Series, Ratigen, Germany) and using EZ Chrom Elite software. A Waters Carbohydrate 4.6 × 250 mm, 4 μm chromatographic column was used. The mobile phase was water/acetonitrile (25:75) in isocratic mode at a flow of 0.8 mL/min. Quantification of sugars was realized using external standards constructing the corresponding calibration curves.

All analyses were performed in triplicate.

2.3. Dynamic rheological properties

The dynamic rheological properties of honey samples were obtained with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) at different temperatures (10, 15, 20, 25, 30, 35 and 40 °C), using a parallel plate system (Ø 60 mm) with a gap of 500 μm (Oroian, Amariei, Escriche, & Gutt, 2013a,b; Oroian, 2015; Escriche et al., 2016). Measurements were made in triplicate for each sample and condition. After loading the sample, a waiting period of 5 min was used to allow the sample to reach the desired temperature. In order to determine the linear viscoelastic region, stress sweeps were run at 1 Hz first. Then, the frequency sweeps were performed over the range f = 0.1–10 Hz at 1 Pa stress. The 1 Pa stress was in the linear viscoelastic region. Rheowin Job software (v.2.93, Haake) was used to obtain the experimental data and to calculate storage (or elastic) modulus (G′), loss (viscous) modulus (G″), and complex viscosity (η*). The complex viscosity η^* represents the total resistance of the material to flow (Marangoni & Wesdorp, 2012) and is defined as the ratio of the maximum resulting stress amplitude (τ^*) over the maximum applied strain amplitude (γ^*) times the angular velocity (ω), as follows:

$$\eta^* = \frac{\tau^*}{\omega \cdot \gamma^*}$$

2.4. Statistical analysis

An analysis of variance (ANOVA) (using Statgraphics Centurion for Windows, Warrenton, Virginia, USA) was carried out to study the influence of the province of origin on the physicochemical and colour parameters (Juan-Borrás, Escriche, Hellebrandova, & Domenech, 2014). The method used for multiple comparisons was the LSD test (least significant difference) with a significance level $\alpha = 0.05$.

The ANNs (artificial neural networks) were developed using the Neurosolutions 6 trial version (NeuroDimension Inc., Gainsville, USA). The system is composed of five inputs (temperature, frequency, moisture content, fructose and glucose content) and three outputs (complex viscosity, loss modulus and elastic modulus). Each model applied to predict the viscoelastic parameters of the samples was checked to discern its suitability using the mean squared error (MSE) and mean absolute error (MAE). The viscoelastic data (complex viscosity, loss modulus and storage modulus) were divided into three groups: one group for training (33.3% of the data), one group for cross-validation (33.3% of the data) and the last one for testing (33.4 per cent of the data) (Ramzi, Kashaninejad, Salehi, Mahoonak, & Mohamma, 2015; Oroian, 2015).

3. Results

3.1. Physicochemical and colour characterization

Table 1 shows the average (and standard deviation), minimum and maximum values of the moisture, HMF, electrical conductivity, a_w, colour (CIE L*a*b* and Pfund) and sugar content (glucose, fructose and sucrose) of the honey samples from the three

Table 1
Mean (and standard deviation), minimum and maximum values of the moisture, HMF, electrical conductivity, a_w, colour (CIEL*a*b* and mm Pfund) and sugars of the honey samples from three provinces of Mozambique (Nampula, Sofala and Zambezia). ANOVA results (F-ratio and significant differences) obtained for the factor "province" for each variable.

	NAMPULA			SOFALA			ZAMBEZIA			ANOVA
Physicochemical Parameters	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max	F-ratio
Moisture (g/100 g)	22.1(0.3)[a]	21.7	23.3	17.7(1.2)[b]	16.6	19.2	20.5(0.2)[c]	20.30	20.60	47.06***
HMF (mg/kg)	15.5(5.6)[b]	8.1	24.5	37.0(9.9)[a]	26.1	47.2	28.4(4.2)[a]	25.33	31.28	15.22***
Electrical conductivity (mS/cm)	1.300(0.020)[a]	1.351	1.402	0.871(0.621)[b]	0.391	1.372	1.281(0.017)[a]	1.212	1.315	4.77*
a_w	0.660(0.010)[b]	0.650	0.680	0.599(0.030)[a]	0.560	0.620	0.612(0.010)[a]	0.610	0.620	29.54***
Colour CIEL* a*b*										
L	32.4(7.4)[a]	25.2	44.6	29.5(0.9)[a]	28.8	30.0	25.6(2.6)[a]	23.9	26.6	1.16 ns
a*	6.0(3.9)[a]	1.1	10.7	4.5(0.2)[a]	4.2	4.6	1.4(0.1)[a]	1.3	1.5	1.82 ns
b*	10.7(8.9)[a]	1.5	24.8	6.7(0.4)[a]	6.4	7.1	2.2(0.4)[a]	1.9	2.3	1.26 ns
Colour (mm Pfund scale)	140 (1)[a]	137	142	104.(22)[b]	84.0	125	141(2)[a]	140	143	17.89***
Sugars (g/100g)										
Glucose	30.4(1.4)[a]	27.8	31.9	30.0(0.7)[a]	29.4	30.9	30.8 (0.4)[a]	30.6	31.1	0.23ns
Fructose	40.8(1.5)[a]	38.3	42.7	41.0 (0.6)[a]	40.4	41.7	40.1(0.1)[a]	40.1	40.2	0.36ns
Sucrose	<1.0			<1.0			<1.0			
Fructose/Glucose	1.34(0.10)[a]	1.24	1.52	1.35(0.03)[a]	1.32	1.39	1.25(0.02)[a]	1.28	1.31	0.35ns

*p < 0.05; **p < 0.01; ***p < 0.001. For each factor, different letters in the rows indicate homogeneous groups (significant differences at 95% confidence level as obtained by the LSD test).

provinces of Mozambique: Nampula, Sofala and Zambezia. In addition, the ANOVA result (F-ratio and significant differences) obtained for the factor "province" for each the variable analysed is shown. Bearing in mind that the higher the F-ratio, the greater the effect that a factor has on a variable, moisture was the parameter most affected by the origin followed by a_w, whereas the sugars and CIE L*a*b* coordinates were the least affected.

All the physicochemical parameters analysed showed significant differences between groups. Nampula honey samples presented the highest average moisture level of 22.1 g/100g (between 21.7 and 23.3 g/100g) and the lowest average HMF content of 15.5 mg/kg (ranged between 8.1 and 24.5 mg/kg). The opposite behaviour was found in Sofala honeys for both parameters, showing the lowest average moisture level of 17.7 g/100g (between 16.6 and 19.2 g/100g) and the highest average HMF content of 37.0 mg/kg (between 26.1 and 47.2 mg/kg). Zambezia honeys had an intermediate level of these parameters with means values of 20.5 g/100g and 28.4 mg/kg, respectively.

The moisture content is an important quality factor of honey, not only because it influences the organoleptic characteristics (viscosity, palatability and taste), but also because it determines shelf-life (Bogdanov, 2002). Moisture content above 20 g/100g facilitates the growth of osmophilic yeasts, while moisture content less than 14 g/100g makes honey extraction difficult due to the high viscosity. According to the criteria of the Council Directive (2002), (maximum permitted limit of 20 g/100g), only the Sofala samples fulfilled this criteria since all the honey samples from Nampula and Zambezia exceeded this value. High moisture values may be associated with inadequate extraction and storage conditions of honey, as most producers do not have appropriate training. However, they may also be related to the humid climate of some subtropical areas of Mozambique. The moisture values obtained in the present study are similar to those found in South African honey: from 15.3 to 21.7 g/100g (Serem & Bester, 2012; Zandamela, 2008) and in North African honey (from 14.6 to 21.8 g/100g) (Malika, Mohamed, & Chakib, 2005; Ouchemoukh, Louaileche, & Schweitzer, 2007; Saxena, Gautam, & Sharma, 2010). In general, in European honey the average moisture values are comparable to those of the present study, exceeding the limit of 20 g/100 g in very few occasions (Escriche, Visquert, Juan-Borras, & &Fito, 2009; Kadar, Escriche, Juan-Borras, Carot, & Domenech, 2011; Juan-Borrás, Domenech, Conchado, & Escriche, 2015).

HMF is an important quality parameter whose speed of formation is favoured by time and temperature of storage and/or heating (Escriche et al., 2009). Some of the samples had values of HMF higher than the maximum limit of 40 mg/kg permitted by European standards (Council Directive, 2002), however, none of them exceeded 80 mg/kg, the acceptable limit for honey from regions with a Tropical climate, as is the case in Mozambique (Codex Standard for Honey, 2001).

In honey from central and southern regions of Mozambique and from Burkina Faso similar values of HMF to those in the present study were reported, between 2.84 and 44.83 mg/kg and 1.02–35.60 mg/kg, respectively (Escriche et al., 2016; Zandamela, 2008).

Honey is a hygroscopic substance due to its low water activity (a_w), which is usually found below 0.630. Some authors consider this value as a limit for good quality honey (Gleiter, Horn, & Isengard, 2006). Most of the samples of the present study showed higher values than this level, not exceeding in any case the value of 0.700 considered the limit of acceptance (Mossel, Bhandari, D'Arcy, & Caffin, 2003). The highest a_w average values of 0.666 were found in Nampula honey (ranged from 0.650 to 0.680) whereas non-significant differences were found for this parameter between Sofala (0.560–0.620) and Zambezia (0.610–0.620). This indicates that the honeys of this region have less water in the free state and therefore are more stable in the development of microorganisms, enzymatic and chemical reactions dependent on water. These values were similar to those reported in other African honeys (Escriche et al., 2016).

Non-significant differences were found between provinces for the CIE L*a*b* colour parameters. Higher values and a greater range of variability for luminosity were found in Nampula samples (25.2–44.6) than Sofala (28.8–30.0) and Zambezia (23.9–26.6). Positive values of both a* and b* coordinates indicate that all samples had shades of colour between red and yellow (first quadrant of CIEL a*b* space). In general, the low values of a* and b* reflect the low colour purity of the samples, especially in Zambezia honey.

Regarding the colour measured by the Pfund scale, it is worth noting the existence of statistically significant differences between geographical areas ($p < 0.001$). The Pfund colour values were similar in Nampula (137–142 mm) and Zambezia (140–143 mm). The Sofala honey values were lower (84–125 mm) than the before mention regions. This result is consistent with the observed differences between regions for conductivity values: the lowest conductivity level was shown in Sofala honeys (average: 0.871 mS/cm, and rage: 0.391 to 1.372) and the highest in Nampula and Zambezia, with average values of 1.300 and 1.281 mS/cm, respectively. The conductivity values were in the same range as those reported by other authors in Burkina Faso honey (Nombré et al., 2010; Schweitzeret al., 2013a; Escriche et al., 2016). In general, colour and conductivity are parameters that are inter-correlated and also with the mineral content and the botanical and geographic origin of honey. The darker the honey, the higher the mineral content and the conductivity (Visquert et al., 2014; Juan-Borrás et al., 2014). The colour of the honey is related to certain pigments such as carotenes, and xanthophylls, as well as the mineral content found in the nectar of flowers or secretions of plants. According to the European Directive about the quality of honey, conductivity values above 0.800 mS/cm are required to consider a honey as honeydew-honey. Considering this criterion, in the present study, 87% analysed samples could be considered as such.

The sugar values were as expected for pure honey. The levels of glucose (from 27.8 to 31.9 g/100g), fructose (38.3 and 42.7 g/100g) and sucrose (less than 1 g/100g) did not vary significantly between regions. The low content of sucrose indicates that these honeys were properly matured before harvesting (Juan-Borrás et al., 2014). Values of sucrose between 1 and 2 g/100 g were previously reported in African (Escriche et al., 2016) and European honey (Persano-Oddo & Piro, 2004). All samples exhibited a F/G ratio higher than 1.20, which reflects their low possibility of crystallization. Other authors such as Venir, Spaziani, and Maltini (2010) or Nayik, Dar, and Nanda (2016) stated that an F/G ratio of 1.14 or less would indicate fast crystallization, while values over 1.58 are associated with no tendency to crystallize.

3.2. Rheology characterization

Since there were no significant differences between regions in relation to the physicochemical parameters, the rheological study of Mozambiquean honey was carried out without differentiating the samples by origin. Fig. 1 shows a typical rheogram for this type of honey. It can be observed that the magnitudes of elastic modulus (G'), loss modulus (G") and complex viscosity (η^*) decrease temperature increases. The decrease in the magnitude of the rheological parameters is due to a decrease in the molecular friction and hydrodynamic forces (Patil & Muskan, 2009; Al-Mahasneh, Rababah, Amer, & Al-Omoush, 2014).

The elastic modulus and loss modulus are strongly influenced by the frequency applied, while the complex viscosity is not influenced by this parameter. Complex viscosity can be used for the characterization of the honey as a Newtonian or non-Newtonian fluid (Oroian et al., 2013a). According to Fig. 1, complex viscosity has the same magnitude at a certain temperature irrespective of the frequency applied; this behaviour is normal for a Newtonian honey. The Newtonian behaviour of honey has been observed in the case of honey from other African countries such as Burkina Faso (Escriche et al., 2016) and Ethiopia (Belay et al., 2017); European countries such as Spain (Oroian et al., 2013a), Romania (Oroian, 2012) and Poland (Juszczak & Fortuna, 2006) and Middle Eastern countries such as Israel (Cohen & Weihs, 2010) or Turkey (Karaman, Yilmaz, & Kayacier, 2011).

3.3. Artificial neural network prediction of rheological parameters

In order to predict the rheological parameters, an ANN was used in this study. Therefore, three output parameters (elastic modulus, loss modulus and complex viscosity) and five input parameters (temperature, moisture, frequency, fructose, and glucose) were considered. To achieve the best ANN for the prediction of the

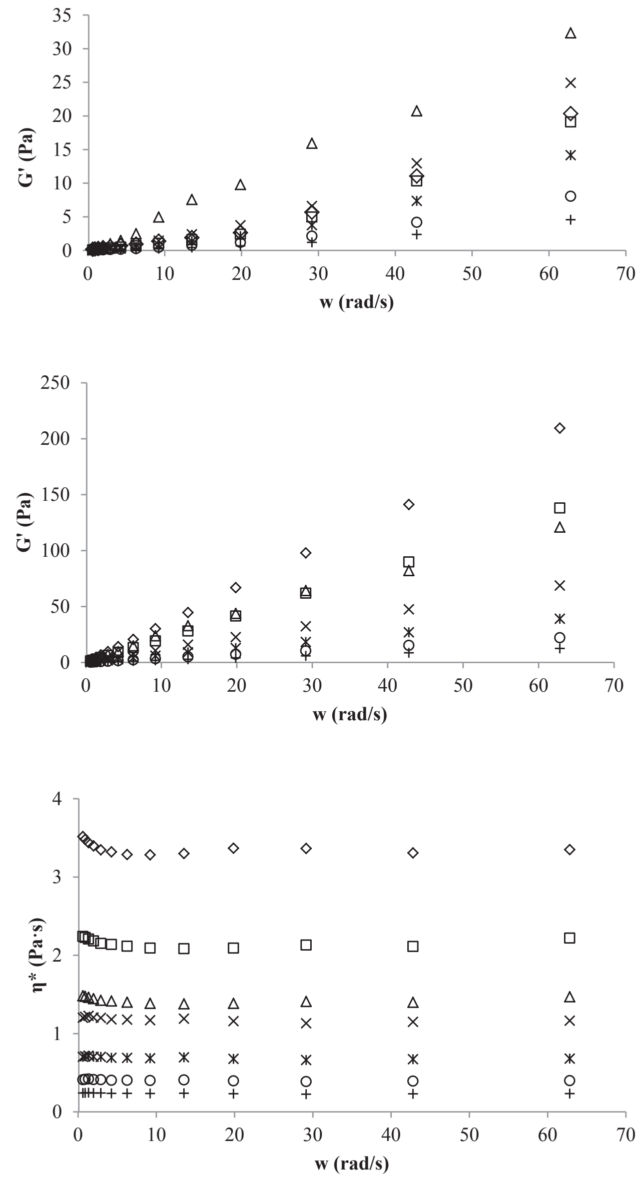

Fig. 1. Typical rheograms for honey from Mozambique: G' (elastic modulus); G" (loss modulus); η^* (complex viscosity) at different temperatures: rhombus (10 °C); square (15 °C); triangle (20 °C); cross (25 °C); star (30 °C); circle (35 °C); plus (40 °C).

rheological parameters four methodologies were used: multilayer perceptron (MLP), probabilistic neural network (PNN), recurrent neural network (RNN) and modular neural network (MNN). The suitability of the model was checked using statistical parameters such as: MSE, MAE and coefficient of regression (R^2). The best model must have the lowest values for MSE and MAE and maximum R^2. The data for each model was analysed as follows: training (33.3% of the experimental data), cross validation (33.3% of the experimental data) and testing (33.4% of the experimental data).

In order to enhance the capabilities of their neural networks, different numbers (from 1 to 3) of hidden layers (intermediate layer between the input and output layer) were used, for each model. There were a total number of 364 experimental data. In Tables 2–4 the MAE, MSE and R^2 values for each model are presented. Even if the number of the experimental data were equal for training,

Table 2
ANN statistical parameters for elastic modulus G'.

No	Model Name*	Hidden layers	Training			Cross Validation			Testing		
			MSE	R^2	MAE	MSE	R^2	MAE	MSE	R^2	MAE
1	MLP	1	82.050	0.730	3.690	143.291	0.600	4.141	70.427	0.757	3.598
2	MLP	2	87.611	0.708	4.204	144.092	0.599	4.623	73.420	0.718	4.115
3	MLP	3	101.792	0.669	5.510	163.713	0.534	5.887	88.20	0.671	5.592
4	PNN	1	77.588	0.747	3.678	124.124	0.667	4.026	64.354	0.758	3.593
5	PNN	2	142.471	0.444	6.640	195.192	0.362	6.786	118.550	0.473	6.447
6	PNN	3	176.162	0.053	7.298	224.242	0.011	7.344	150.386	0.095	6.940
7	RNN	1	116.911	0.634	5.617	117.690	0.707	5.538	95.340	0.677	5.335
8	RNN	2	125.242	0.564	5.714	181.991	0.469	6.076	102.21	0.596	5.689
9	RNN	3	125.424	0.575	6.329	190.750	0.435	6.715	98.877	0.624	6.157
10	MNN	1	93.573	0.689	3.941	104.598	0.766	1.687	72.890	0.720	3.855
11	MNN	2	93.285	0.690	3.988	124.600	0.668	4.130	93.285	0.690	3.988
12	MNN	3	145.506	0.601	8.204	193.368	0.523	8.314	119.98	0.660	7.930

*MLP (multilayer perceptron), PNN (probabilistic neural network), RNN (recurrent neural network), MNN (modular neural network).

Table 3
ANN statistical parameters for loss modulus G''.

No	Model Name*	Hidden layers	Training			Cross Validation			Testing		
			MSE	R^2	MAE	MSE	R^2	MAE	MSE	R^2	MAE
1	MLP	1	3805.021	0.963	40.764	7018.581	0.953	49.594	6400.079	0.961	47.201
2	MLP	2	3754.833	0.964	39.660	8535.952	0.943	48.662	6547.131	0.948	46.042
3	MLP	3	12365.674	0.929	74.451	22979.894	0.883	81.955	18127.852	0.900	1.6251
4	PNN	1	4766.861	0.954	43.411	10558.402	0.924	51.459	7530.804	0.938	48.804
5	PNN	2	22323.752	0.766	100.350	30111.400	0.767	103.063	27586.721	0.794	102.284
6	PNN	3	53095.026	0.116	153.583	69119.294	0.121	158.734	61810.667	0.125	155.192
7	RNN	1	14554.271	0.881	85.448	16015.331	0.882	87.611	14702.995	0.883	87.575
8	RNN	2	9652.978	0.912	5.714	12841.700	0.904	63.237	10659.961	0.911	64.096
9	RNN	3	12463.684	0.889	82.569	177728.716	0.871	90.958	16265.986	0.867	2.870
10	MNN	1	6980.765	0.934	58.067	11156.754	0.921	64.429	8971.344	0.927	64.087
11	MNN	2	4948.064	0.953	48.154	9497.002	0.932	55.461	4948.068	0.953	48.154
12	MNN	3	33642.771	0.710	124.073	44517.051	0.726	128.858	37984.631	0.749	125.640

*MLP (multilayer perceptron), PNN (probabilistic neural network), RNN (recurrent neural network), MNN (modular neural network).

Table 4
ANN statistical parameters for complex viscosity η*.

No	Model Name*	Hidden layers	Training			Cross Validation			Testing		
			MSE	R^2	MAE	MSE	R^2	MAE	MSE	R^2	MAE
1	MLP	1	1.248	0.990	0.764	1.191	0.991	49.594	1.307	0.990	0.796
2	MLP	2	0.024	0.988	0.907	1.335	0.990	0.863	1.481	0.989	0.880
3	MLP	3	4.404	0.974	1.567	4.933	0.973	1.661	4.667	0.975	1.625
4	PNN	1	1.971	0.984	0.876	0.863	0.985	0.863	1.977	0.985	0.857
5	PNN	2	7.723	0.938	1.863	7.451	0.943	1.833	7.457	0.943	1.864
6	PNN	3	63.658	0.204	5.978	66.421	0.212	6.044	66.689	0.200	6.073
7	RNN	1	11.344	0.910	2.264	10.826	0.918	2.303	10.487	0.922	2.316
8	RNN	2	11.160	0.915	2.287	11.008	0.920	2.285	10.684	0.924	2.252
9	RNN	3	16.770	0.914	2.840	16.884	0.921	2.880	17.457	0.914	2.870
10	MNN	1	1.639	0.965	1.639	4.598	0.966	1.687	4.535	0.967	1.668
11	MNN	2	2.933	0.977	1.212	2.658	0.981	1.192	2.933	0.977	1.212
12	MNN	3	43.436	0.771	4.874	45.838	0.756	4.937	45.507	0.764	4.966

*MLP (multilayer perceptron), PNN (probabilistic neural network), RNN (recurrent neural network), MNN (modular neural network).

testing and cross validation, great differences between the statistical parameters can be observed. Increasing the number of hidden layers did not increase the suitability of the model. According to the data presented in Table 2, the best model for predicting the elastic modulus values was PNN with 1 hidden layer ($R^2 = 0.758$). The determination of the elastic modulus can be influenced by the presence of unmelted sugar crystals (Oroian, Amariei, Escriche, & Gutt, 2013b). In the case of loss modulus (Table 3) and complex viscosity (Table 4) higher values for the regression coefficients than in the case of elastic modulus can be observed, with R^2 values of 0.961 and 0.990, respectively. The suitable model for predicting the loss modulus and complex viscosity was MLP with 1 hidden layer.

Fig. 2 shows the evolution of experimental and predicted data for the suitable models for the three rheological parameters. A chaotic distribution can be observed in the case of the elastic modulus, while for the complex viscosity the points are placed on a straight line. A chaotic distribution can be observed in the case of the elastic modulus, while for the complex viscosity the points are placed on a straight line. With the aim of better explaining the suitability of the model proposed using the ANN, Fig. 3 shows the residual vs measured values for G′, G″ and η*. Considering the distribution of the points, a worse behaviour of the residual values is deduced in the case of G' compared to G ″ and η*. The parameter G′ cannot be modelled with high regression coefficients in function of different

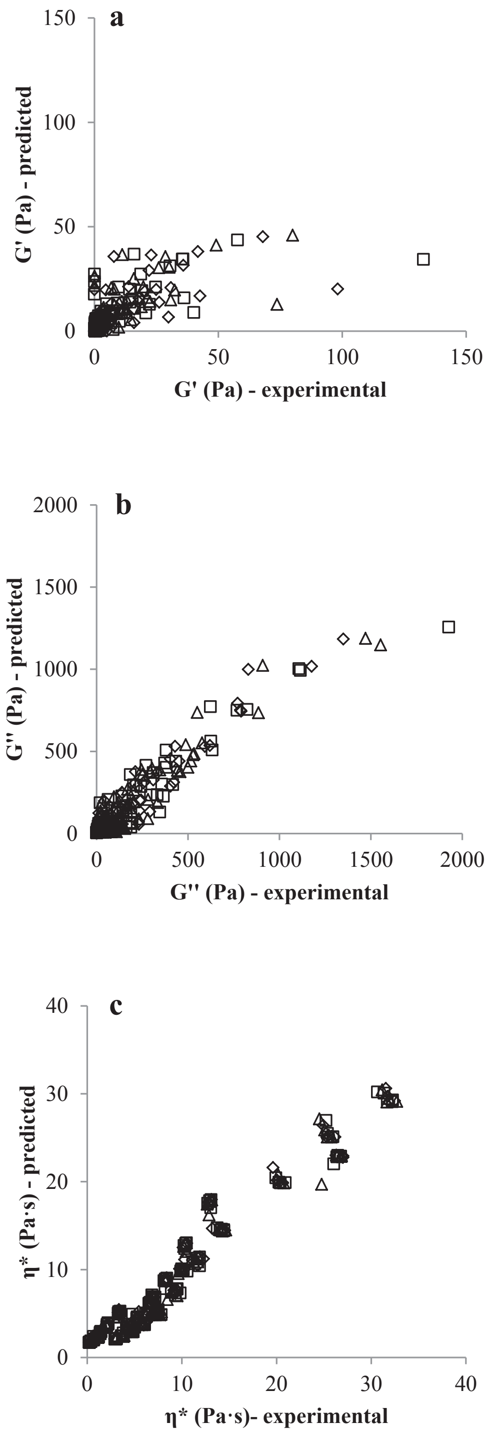

Fig. 2. Experimental data vs. predicted data using artificial neural network prediction: a [elastic modulus (G'), probabilistic neural network (PNN) with 1 hidden layer prediction]; b [loss modulus (G''), multilayer perceptron (MLP) with 1 hidden layer prediction]; c [complex viscosity (η^*), multilayer perceptron (MLP) with 1 hidden layer prediction]; rhombus (training), square (cross validation) and triangle (testing).

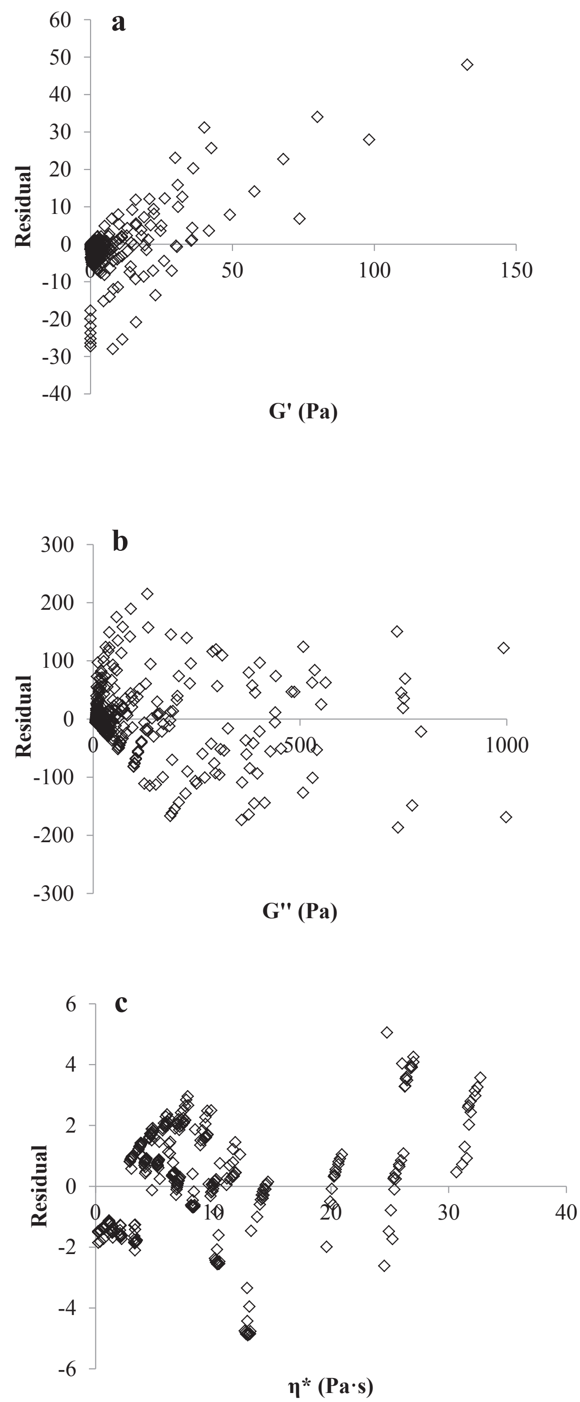

Fig. 3. Measured values of the rheological parameters versus residual values: a [elastic modulus (G'), probabilistic neural network (PNN) with 1 hidden layer prediction]; b [loss modulus (G''), multilayer perceptron (MLP) with 1 hidden layer prediction]; c [complex viscosity (η^*), multilayer perceptron (MLP) with 1 hidden layer prediction].

parameters (temperature, fructose, glucose, moisture content, frequency). This could be due to the fact that the elastic part of the honey (G′) is very sensitive to the presence of any particles in suspension (e.g. pollen grains, sugar, glucose crystals) which may interfere with the rheological testing. However, this is not a problem because in these types of honey the viscous part is more important than the elastic part (G'' >> G') (Oroian, 2015).

There are no other studies in the literature regarding the prediction of honey rheological parameters using ANN based on temperature, moisture, frequency, fructose, and glucose. To the authors knowledge there are some papers on the modelling of rheological behaviour using ANN based on water content, temperature and shear rate (Al-Mahasneh, Rababah, & Ma'Abreh, 2013;

Table 5
Sensitivity testing (percentage) of the input parameters (frequency, moisture, glucose content, fructose content, and temperature) on artificial neural networks (ANN) output models [probabilistic neural network (PNN)-1 hidden layer for G' and multilayer perceptron (MLP)-1 hidden layer for G'' and η^*] to predict the rheological parameters.

Input parameters	G′			G″			η*		
	Training	Testing	Cross validation	Training	Testing	Cross validation	Training	Testing	Cross validation
Frequency	47.51	48.19	47.83	41.28	40.64	40.66	1.28	1.18	1.19
Moisture	22.29	21.99	22.16	31.47	31.78	31.82	48.22	48.08	48.11
Glucose	14.25	13.90	14.13	7.49	7.69	7.71	6.17	6.61	6.63
Fructose	10.81	10.84	10.74	8.74	8.81	8.83	1.29	1.11	1.13
Temperature	5.13	5.08	5.14	11.02	11.07	11.09	43.04	43.03	43.07

Ramzi, Kashaninejad, Salehi, Mahoonak,& Mohamma, 2015) and the modelling of rheological parameters using ANN based on temperature, frequency and moisture content (Oroian, 2015). In both cases, higher regression coefficients for predicting the dynamic viscosity ($R^2 = 0.999$) using genetic algorithm–artificial neural network (Ramzi et al., 2015), and viscoelastic parameters ($R^2 > 0.998$) using the MLP were observed (Oroian, 2015).

Each input variable was analysed to estimate the weighting in the model design. This step can be useful before designing the model and will serve as a screening tool to omit unimportant inputs in order to reduce model complexity. This can be of special importance in the presence of highly colinear inputs. The presence of high colinearity means that some model inputs are not really helping to improve model performance, the higher is the colinearity the lower is the model performance (Oroian, 2015).

A sensitivity analysis was performed to investigate the effect of each input parameter on the output in terms of magnitude and direction (Table 5). In this way, it was possible to determine to what extent the models can be affected by changes in the values of the input parameters (Matignon, 2005; Shojaeefard, Akbari, Tahani, & Farhani, 2013). Frequency was the most sensitive in the case of the elastic and loss modulus followed by moisture, which implies that both parameters are critical to the models (PNN in the case of elastic modulus and MLP for the loss modulus). On the contrary, the low sensitivity of sugars and temperature suggests their low importance in these models. In the case of the MLP model of complex viscosity, moisture and temperature have the highest sensitivity; which is normal since frequency does not have a great influence on this rheological parameter taking into account the Newtonian behaviour of Mozambican honey.

The impact of the chemical parameters on the honey rheological parameters (G′, G″ and η*) can be estimated quite well based on the sensitivity analysis. It can be observed that all the parameters are influenced primarily by the frequency, followed by the moisture content. This fact is in agreement with other studies, which revealed the high influence of moisture content on the rheological parameters (Oroian, 2015; Patil & Muskan, 2009; Özcan, Arslan, & Ceylan, 2006). In the case of glucose and fructose, they had less influence on the rheological parameters than the moisture content (Table 5).

4. Conclusion

The physicochemical parameters and Newtonian behaviour of Mozambican honey are similar to those of other types of honey commercialized in parts of the world such as Africa, Europe and Middle East. In general, following the criteria of colour and conductivity, the majority of honey from Mozambique can be classed as honeydew honey. Rheological parameters, applying an artificial neural network (ANN), can be predicted as a function of temperature, frequency and chemical composition. The multilayer perceptron (MLP) is the best model for loss modulus (G") and complex viscosity (η*), while the probabilistic neural network (PNN) is apt for elastic modulus (G′).

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