



# Rapid honey characterization and botanical classification by an electronic tongue

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## ABSTRACT

In this paper a commercial electronic tongue ( $\alpha$ Astree, Alpha M.O.S.) was applied for botanical classification and physicochemical characterization of honey samples. The electronic tongue was comprised of seven potentiometric sensors coupled with an Ag/AgCl reference electrode. Botanical classification was performed by PCA, CCA and ANN modeling on 12 samples of acacia, chestnut and honeydew honey. The physicochemical characterization of honey was obtained by ANN modeling and the parameters included were electrical conductivity, acidity, water content, invert sugar and total sugar. The initial reference values for the physicochemical parameters observed were determined by traditional methods. Botanical classification of honey samples obtained by ANN was 100% accurate while the highest correlation between observed and predicted values was obtained for electrical conductivity (0.999), followed by acidity (0.997), water content (0.994), invert sugar content (0.988) and total sugar content (0.979).

All developed ANN models for rapid honey characterization and botanical classification performed excellently showing the potential of the electronic tongue as a tool in rapid honey analysis and characterization. The advantage of using such a technique is a simple sample preparation procedure, there are no chemicals involved and there are no additional costs except the initial measurements required for ANN model development.

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## 1. Introduction

Honey is produced by honey bees from nectar of plants or from honey dew. The composition of honey is influenced by the maturation process, nectar type and compounds added by the honey bees. It can vary with seasonal climatic changes or even be influenced by geographical origin [1]. Honey has been used since ancient times as a medicine to treat wounds and various gastrointestinal diseases and modern science verified the beneficial properties of honey on human health [2]. Nowadays, people are paying more attention to the quality of food products. The food products have to satisfy numerous quality and certification criterions before commercialization and honey is no exception.

Characterisation of unifloral honeys is a hard task initiated in Europe in response to consumer's demands. Those have demanded not only a basic quality level, but also a clear determination of geographical and botanical origin [3]. In Europe more than 100 botanical species are known to produce unifloral honey. Most of them are produced occasionally or are only of local interest, whereas others are part of the import–export market between different European countries [4]. The International Honey Commission (IHC) is a network created in 1990, under the umbrella

of Apimondia, for the enhancement of knowledge on honey quality research. The main objective of the work of the IHC is to improve honey analysis methods and to propose new quality criteria. The results achieved by the IHC were taken into account in the recent revision of the Codex Alimentarius honey standard and of the European Honey Directive [5]. Honey quality control requires the determination of any parameter that could unequivocally establish origin and calls for efforts to improve honey characterisation [3,6–9]. Conti et al. [10] differentiated among three types of Italian honey (acacia, multifloral and honeydew honey) using quality parameters (pH, sugar content, humidity) and mineral content (Na, K, Ca, Mg, Cu, Fe, and Mn) combined with multivariate data analysis. All samples of acacia and honeydew honey, and 97.7% of multifloral honey have been correctly classified [10]. Bentabol Manzanares et al. [11], similarly, obtained a high percentage of correct blossom and suspected honeydew honey classifications (96.1% after cross-validation) by applying multivariate data analysis on physicochemical parameters (moisture, water activity, electric conductivity, colour, hydroxymethylfurfural, acidity, pH, proline, diastase and invertase), sugar composition (fructose, glucose, sucrose, maltose, isomaltose, trehalose, turanose and melezitose) and palinological parameters [11]. Despite of the good honey classification results achieved by combining physicochemical parameters with multivariate statistical techniques, melissopalynology, the analysis and identification of pollen grains contained in honey was the first technique to be used for honey

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identification and still is a reference tool for this purpose [6]. The Commission of the EU encourages the development of new methods for honey authentication [1].

Electronic tongues are mainly used for qualitative analysis of foodstuffs [12–14] but recently the application of such devices in rapid quantitative determination of food constituents is increasing [15–17]. The interest in sensor arrays as alternatives to traditional methods in food analysis is due to short analysis time, non-invasive technique, little or no sample preparation and simplicity of the procedure. Other advantages of sensor arrays over traditional methods are multicomponent analysis and combined qualitative and quantitative sample characterization from a single measurement [18]. Multivariate data analysis in such applications include Principal Component Analysis (PCA) [19], Partial Least Squares (PLS) regression [20] and Artificial Neural Network (ANN) modeling [21].

The scope of this paper was to evaluate the application of a commercially available electronic tongue ( $\alpha$ Astree, Alpha M.O.S.) as a rapid technique for botanical classification of honey and determination of honey physicochemical properties. The classification of honey botanical origin was performed by PCA, CCA and ANN, while the determination of physicochemical properties was carried out by ANN.

## 2. Material and methods

### 2.1. Honey samples and reagents

The analysis was performed on 12 honey samples of which 5 samples were acacia honey, 4 samples honeydew honey and 3 samples were chestnut honey. The samples were obtained directly from the producers. The samples were stored and analyzed at +20 °C. Hydrochloric acid ( $w = 37\%$ , ISO—for Analysis grade) was purchased from Carlo Erba Reagents.

### 2.2. Reference determination of honey samples physicochemical properties

In all honey samples, the physicochemical parameters were determined according to the methods proposed by the International Honey Commission [22]. Electrical conductivity of honey is measured by dissolving 20 g of honey dry matter in 100 ml deionized water using a conductivity meter. The results were expressed in miliSiemens per centimeter (mS/cm) IHC, 2002). Free acidity of honey samples, defined as the content of all free acids expressed in milliequivalents per kilogram (meq/kg) of honey, was determined by titration of honey samples dissolved in water with 0.1 M sodium hydroxide solution [22]. Water content was determined refractometrically as the value determined from the refractive index of the honey samples by reference to a standard table [22]. Invert sugar content and total sugar content of the honey samples were determined by the method based on the modification of the Lane and Eynon procedure, involving the reduction of Soxhlet's modification of Fehling's solution by titration against a solution of reducing sugars in honey using methylene blue as an internal indicator [22].

### 2.3. The $\alpha$ Astree electronic tongue measurements

In order to accomplish the characterization and classification of honey the commercial electronic tongue  $\alpha$ Astree (Alpha M.O.S.) was employed consisting of 7 potentiometric sensors designated as JB, BA, BB, HA, ZZ, CA and GA by the manufacturer (Alpha M.O.S.), an Ag/AgCl reference electrode (Metrohm, Ltd.), a mechanical stirrer (Metrohm, Ltd.), a 16-position Sample Changer and a 759 Swing Head for sampling (Metrohm, Ltd.), an interface electronic module for signal amplification and analog to digital conversion (Alpha

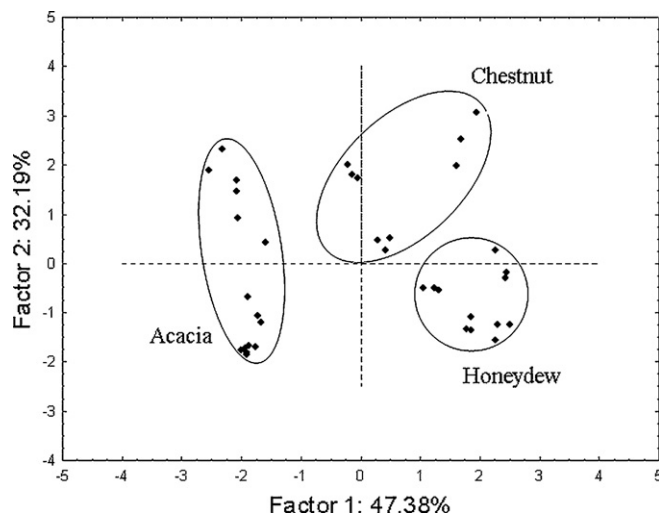


Fig. 1. PCA plot of acacia, chestnut and honeydew honey measurements, number of replicas  $n = 3$ , number of honey samples  $N = 12$ .

M.O.S.). The electronic tongue was connected to a personal computer with the Astree II software (Alpha M.O.S., Version 3.0.1., 2003) installed. The software automatically gathers and stores the outputs of the sensors. The sensors used in this study are chemically sensitive field-effect transistors (chemFET). The sensors were specially designed by the manufacturer for food and beverage analysis [23]. All samples were analyzed in triplicate by the electronic tongue and each analysis cycle lasted for 300 s giving a sum of 36 measurements. After every sample measurement a reference sample was analyzed consisting of hydrochloric acid diluted in deionized water (0.01 mol/l) to monitor and correct the drift of sensors in time. The sensors were rinsed with deionized water after every analysis cycle. Prior to each sample measurement the sensor array was conditioned in honey to obtain stable sensor responses.

### 2.4. Data analysis

The sensor outputs stored by the Astree II software were exported to Microsoft Excel (Microsoft Excel 2002, SP-2) where operations of centering [24] and drift correction [16] were performed. The centered and corrected data was transferred to Statistica 7.1 (Statsoft, Inc., 2005) where Principal Component Analysis [25], Canonical Correlation Analysis (CCA) [26] and Artificial Neural Networks regression and classification [27] were performed. A data matrix consisting of 36 rows (12 samples  $\times$  3 measurements) and 7 columns (7 sensor outputs) was used to perform the PCA method. In order to classify honey samples according to their botanical origin an ANN model was created using a data matrix of 36 rows (also 12 samples  $\times$  3 measurements) and 8 columns (7 sensor outputs and the botanical origin of honey samples). The sensors outputs were used to create ANN models for the prediction of 5 physicochemical properties of honey samples (electrical conductivity, acidity, water content, invert sugar content and total sugar content). CCA was performed on two variable sets, one comprising of 7 sensor outputs and the other comprising of 5 physicochemical parameters.

## 3. Results and discussion

### 3.1. Botanical classification of honey samples

Fig. 1 shows a two plane PCA plot of acacia, chestnut and honeydew honey measurements performed by the electronic tongue. The first two planes (PC 1 and PC 2) represent 79.57% of the total

**Table 1**  
Eigenvalues and explained variance by principal components (factors).

	Eigenvalue	Total variance (%)	Cumulative eigenvalue	Cumulative (%)
Factor 1	3.317	47.38	3.317	47.4
Factor 2	2.254	32.19	5.570	79.6
Factor 3	1.071	15.30	6.641	94.9
Factor 4	0.245	3.50	6.886	98.4
Factor 5	0.077	1.10	6.963	99.5
Factor 6	0.026	0.37	6.988	99.8
Factor 7	0.012	0.17	7.000	100.0

variance between sample measurements (Table 1). As shown in Fig. 1, by applying the PCA method the samples grouped into three distinct clusters according to their botanical origin.

The acacia honey group is separated from the other two groups on the first principal component (PC 1—47.38% of the variance), while on the second principal component (PC 2—32.19% of the variance) the chestnut and honeydew honey samples are divided into separate groups (Fig. 1). The highest loading on the first principal component had the GA sensor (−0.884), followed by the BA (−0.794), JB (−0.762) and ZZ (0.759) sensors (Table 2). On the second principal component sensors CA, JB, HA, BB and BA had similar loadings ranging from −0.664 (CA) to −0.568 (BA), while sensors ZZ and GA had a lesser impact (−0.496 and −0.381, respectively) (Table 2).

In an attempt to further explain the behavior of the potentiometric sensor array in the assessment of honey botanical origin a comparison was made between the sensors outputs and the physicochemical properties of honey samples. To accomplish this task Canonical Correlation Analysis and Principal Components Analysis were performed. Table 3 shows the correlations obtained by CCA between individual variables from the two data sets.

According to Table 3 the GA sensor is highly correlated with acidity and electrical conductivity (−0.847 and −0.803). The sensors JB and BA are highly correlated with acidity (−0.868 and −0.864, respectively) and the sensor ZZ with electrical conductivity (−0.814), invert sugar content (−0.794) and total sugar content (−0.789) (Table 3). The high correlations between these variables indicate that the acacia honey group was separated from the chestnut and honeydew honey group primarily because of the different acidity and electrical conductivity and in a lesser degree because of the invert sugar and total sugar content. The sensor HA highly correlates with the invert sugar and total sugar content (−0.857 and −0.816, respectively) (Table 3). The CA sensor correlates moderately with invert sugar content (−0.474), total sugar content (−0.415) and water content (−0.355) (Table 3). The BB sensor correlated moderately with electrical conductivity (0.503), invert sugar content (−0.463), total sugar content (−0.427) and water content (−0.305) (Table 3). According to the obtained results the parameters responsible for the separation of chestnut and honeydew honey groups are invert sugar content, total sugar content and to a lesser degree water content.

In order to classify honey samples according to their botanical origin neural networks were employed to create a model with a very high percentage of correct classifications. The ANN model was trained using 26 sample measurements and tested using 10 sample measurements. The sample measurements used for both the training and testing subsets were randomly selected prior to model development. The architecture of the developed model was 5 input neurons, 2 hidden neurons and 3 output neurons. Table 4 shows the results obtained by training and testing the ANN classification model. As shown, all training and testing samples measurements were correctly classified according to their botanical origin providing 100% correct classifications (Table 4). While developing the ANN model for the classification of honey botanical origin a downsizing of the model was required in order to avoid over fitting. The train-

ing started with 7 inputs and 3 hidden nodes. During the training of the model the train and test errors were monitored to assure that the model retains a good generalization capability while sensitivity analysis of the input variables was performed after the initial training in order to remove the excess variables and keep the model as simple as possible. Similarly Wei et al. [14] obtained 98.43% correct classifications of honey botanical origin for the training subset and 93.75% for the testing subset. The network topology used by the authors was 7 input neurons, 12 hidden neurons and 8 output neurons [14].

### 3.2. Rapid characterization of honey samples physicochemical properties

In order to assess the physical and chemical properties of honey ANN models were created using the data acquired by the electronic tongue combined with the actual measurements of the physical and chemical properties. The results obtained from the developed models are shown in Table 5. The plots of the physical and chemical properties observed and predicted values are shown in Fig. 2, including their respective correlations.

The best performance of the developed ANN models had the model for the determination of electrical conductivity. The train and test errors of the model were 0.021 and 0.020 respectively. The average errors were 0.001 and −0.006, the standard deviation of errors 0.025 and 0.024 and the absolute errors 0.020 and 0.021 for the training and testing subset, respectively (Table 5). The obtained correlation between the observed and predicted electrical conductivity was 0.999 (Fig. 2), with both 0.999 for the training and testing subsets (Table 5). The network topology used was 4-2-1, with the input neurons being the sensors BA, BB, ZZ and GA. Sensors JB, HA and CA were excluded during the training of the model after performing sensitivity analysis of the input variables. The model was trained by 100 epochs of gradient descent followed by 64 epochs of conjugate gradient algorithm. The low errors of prediction and the high correlation of the ANN model suggest that the electronic tongue can be successfully applied in the determination of electrical conductivity of honey. The excellent performance of the model can be attributed to the nature of the procedure since both techniques measure electrical properties of the honey samples.

The train and test error of the ANN model for the determination of acidity in honey were 0.024 and 0.029. During the training of the model the best architecture of 4-3-1 was selected. The input neurons chosen after the sensitivity analysis were JB, BB, ZZ and GA. The training consisted of 100 epochs of gradient descent algorithm followed by 156 epochs of conjugate gradient algorithm. The average errors were 0.017 and −0.058, the standard deviation of errors 0.454 and 0.546 while the absolute errors were 0.391 and 0.479 for the testing and training subset, respectively (Table 5). The developed model obtained a correlation of 0.997 between the observed and predicted values of honey acidity (Fig. 2), with correlations of both 0.997 for the training and testing subsets (Table 5). According to the obtained errors and high correlation the developed ANN model exhibited excellent performance in the prediction of honey acidity ranging from 6 meq/kg to 26 meq/kg. Such results can be

**Table 2**  
Factor-variable loadings based on correlations obtained by PCA.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
JB	−0.762	−0.620	0.156	0.003	−0.047	0.047	0.078
BA	−0.794	−0.568	0.147	−0.013	−0.124	−0.087	−0.042
BB	0.518	−0.597	0.577	−0.190	0.009	0.069	−0.037
HA	0.623	−0.598	−0.410	0.270	−0.107	0.049	−0.018
ZZ	0.759	−0.496	0.357	0.182	0.098	−0.082	0.029
CA	0.305	−0.664	−0.617	−0.284	0.058	−0.031	0.011
GA	−0.884	−0.381	−0.123	0.147	0.187	0.031	−0.035

**Table 3**  
CCA between sensors outputs and honey physicochemical parameters.

	Water content	Invert sugar content	Total sugar content	Electrical conductivity	Acidity
JB	−0.513	0.230	0.297	−0.690	−0.868
BA	−0.491	0.281	0.349	−0.727	−0.864
BB	−0.305	−0.463	−0.427	0.503	0.040
HA	0.232	−0.857	−0.816	0.694	0.490
ZZ	−0.113	−0.794	−0.789	0.814	0.339
CA	0.355	−0.474	−0.415	0.294	0.232
GA	−0.300	0.325	0.364	−0.803	−0.847

**Table 4**  
Results from the ANN model for the determination of honey botanical origin.

		Total	Correct	Wrong	Correct (%)	Wrong (%)
Acacia honey samples	Training	8	8	0	100	0
	Testing	7	7	0	100	0
Chestnut honey samples	Training	5	5	0	100	0
	Testing	4	4	0	100	0
Honeydew honey samples	Training	8	8	0	100	0
	Testing	4	4	0	100	0

explained by high sensitivity of the sensor array to acids as reported previously [16].

The developed ANN model for the prediction of water content in honey had the train and test errors of 0.030 and 0.040, respectively. The model employed a simple architecture consisting of 4 input neurons, 3 hidden neurons and 1 output neuron. The training consisted of 100 epochs of gradient descent algorithm, followed by 443 epoch of conjugate gradient algorithm. The input nodes selected after sensitivity analysis were sensors JB, BA, ZZ and GA. The average errors were 0.001 and −0.092, standard deviation of errors 0.204 and 0.256 and absolute errors 0.170 and 0.237 for the training and testing subsets (Table 5). The obtained correlation between the observed and predicted values of water content was 0.994 (Fig. 2). The correlation of the training subset was 0.994 and the testing subset was 0.993 (Table 5). The ANN model showed excellent prediction capability which can be explained by the relatively high water content of honey (from 14% to 21%).

The architecture of the ANN model for invert sugar content determination was 4-3-1. The input neurons selected during the training process were sensors JB, BA, ZZ and GA. The training consisted of 100 epochs of gradient descent algorithm followed by 141 epochs of conjugate gradient algorithm. The train and test errors of the ANN model were both 0.047. The obtained correlation between the observed and predicted values of invert sugar content was 0.988 (Fig. 2). The average errors of the training and testing subsets were −0.001 and −0.511, standard deviation of errors 0.727 and 0.515 and absolute errors 0.596 and 0.575, respectively (Table 5). The correlations for the training and testing subsets were 0.987 and 0.994 (Table 5). The excellent results obtained by the ANN model show that the sensor array is capable of responding and quantification of the carbohydrate content of a solution with high precision in the investigated range (from 55% to 71%).

The ANN model for the determination of total sugar content in honey had train and test errors of 0.066 and 0.060, respectively. The

**Table 5**  
Descriptive statistics of the ANN models for the determination of physicochemical properties of honey samples.

		Data mean	Data S.D.	Error mean	Error S.D.	Abs E. mean	S.D. ratio	Correlation
Electrical conductivity (mS)	Training subset	0.541	0.453	0.001	0.025	0.020	0.055	0.999
	Testing subset	0.725	0.464	−0.006	0.024	0.021	0.051	0.999
Acidity (meq/kg)	Training subset	14.937	6.419	0.017	0.454	0.391	0.071	0.997
	Testing subset	14.889	6.833	−0.058	0.546	0.479	0.080	0.997
Water content (%)	Training subset	17.046	1.871	0.001	0.204	0.170	0.109	0.994
	Testing subset	16.429	2.230	−0.092	0.256	0.237	0.115	0.993
Invert sugar content (%)	Training subset	64.187	4.521	−0.001	0.727	0.596	0.161	0.987
	Testing subset	64.888	4.528	−0.511	0.515	0.575	0.114	0.994
Total sugar content (%)	Training subset	66.347	4.401	−0.002	0.998	0.807	0.227	0.974
	Testing subset	62.802	4.786	−0.016	0.905	0.852	0.189	0.982

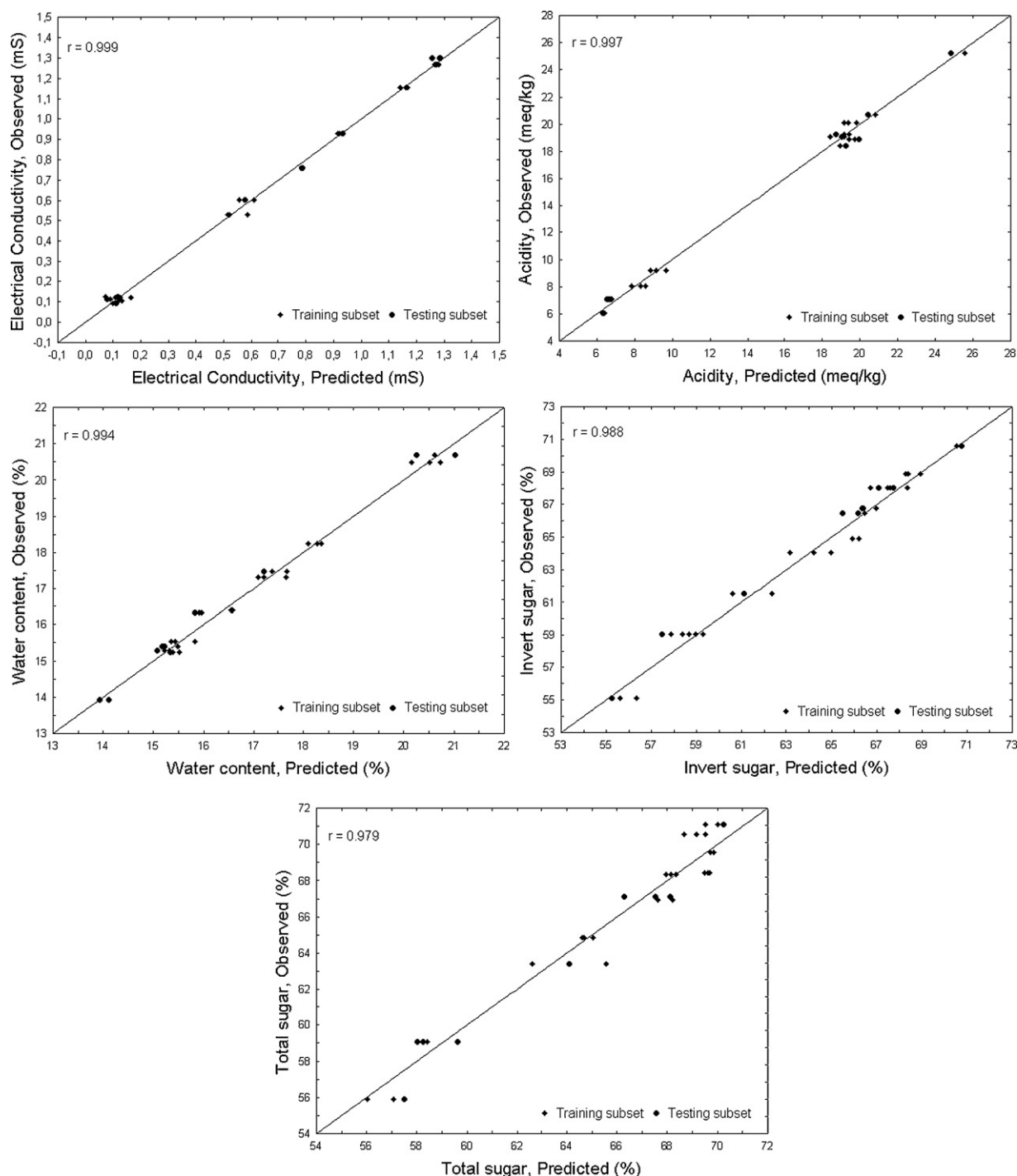


Fig. 2. ANN regression between the potentiometric sensor array and honey samples physicochemical parameters, number of replicas  $n = 3$ , number of honey samples  $N = 12$ .

model employed a simple architecture of 3-2-1. After sensitivity analysis the variables chosen as input neurons were BA, ZZ and GA. The model was trained for 100 epochs of gradient descent followed by 114 epochs of conjugate gradient algorithm. The training subset had an average error of  $-0.002$ , the standard deviation of error was 0.998 and the absolute error 0.807, while the testing subset had the average error of  $-0.016$ , standard deviation of error 0.905 and the absolute error of 0.852 (Table 5). The correlation between the observed and predicted values of total sugar content was 0.979 (Fig. 2), with the training subset's correlation being 0.974 and the testing subset's 0.982 (Table 5). The ANN model for the prediction of total sugar content in honey also exhibited excellent performance

as the ANN model for the determination of invert sugar content. The results were expected since the total sugar content (from 56% to 72%) differs only slightly from the invert sugar content of honey (from 55% to 71%).

#### 4. Conclusion

In this paper a commercially available electronic tongue was employed in the determination of honey botanical origin as well as rapid characterization of physicochemical properties of honey. The performance of the sensor array was assessed by multivariate data analysis techniques and neural networks. The application



of an electronic tongue combined with artificial neural networks as a technique for honey botanical origin classification proved to be a good alternative to standard classification methods because it offers faster results with no loss of accuracy (100% correct classifications obtained from the developed ANN model). All developed ANN models for rapid honey characterization performed excellently showing the potential of the electronic tongue as a tool in rapid honey analysis. The best performance was observed for the ANN model for the determination of electrical conductivity ( $r=0.999$ ) while the worst performing model was for the determination of total sugar content ( $r=0.979$ ). The advantage of using such a technique is a simple sample preparation procedure, there are no chemicals involved and there are no additional costs except the initial measurements required for ANN model development. The downside of the technique is the initial time consuming physical and chemical characterization of honey in order to gain enough data for meaningful ANN model development.

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