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Possible application of an electronic tongue for rapid gluten detection in gluten free flours

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Abstract

In this study a commercially available electronic tongue (αAstree, Alpha M.O.S.) was employed as a technique for gluten free and regular flour samples classification. Additionally, rapid determination of gluten content and other physicochemical parameters including protein content, acidity, reducing sugar content and total reducing sugar content was performed. The classification performance of the sensor array was assessed by multivariate exploratory techniques. The physicochemical characterization of gluten free and regular flours, including gluten content prediction, was obtained by artificial neural networks (ANN) modelling. The reference values of gluten content in flour samples were determined by the ELISA method, while reference values of protein content, acidity, reducing sugar content and total reducing sugar content were determined by standard analytical methods. The application of the electronic tongue, combined with ANN, in the differentiation of gluten free and regular flour samples resulted in 95.2% and 100% correct classifications, respectively. The developed ANN models for the prediction of gluten content in flour samples as well as protein content, acidity, reducing sugar content and total reducing sugar content, showed high potential of the electronic tongue as a simple and rapid technique for the prediction of gluten content and other physicochemical parameters of gluten free and regular flour samples.

The results of this work implicate that the electronic tongue can be employed in the evaluation of gluten content and characterization of different flours, without time-consuming sample preparation, chemicals involved and without additional time or costs, except the initial measurements required for ANN model development.

Keywords: *gluten, electronic tongue, PCA, CCA, ANN*

Introduction

Celiac disease (CD) is a chronic inflammatory multisystemic disorder that occurs as a result of an immune response to ingested gluten, a protein found in wheat, rye and barley in genetically predisposed individuals. Gluten, found in the endosperm of wheat, rye and barley, is composed of distinct portions of monomeric gliadins (which are prolamins), and polymeric glutenins. Celiac disease is characterized by villous atrophy of the small intestinal mucosa caused by gluten (Heap and van Heel, 2009; Murray, 1999). In Croatia, the cumulative incidence of celiac disease is 1.9 per 1000 live births, which, in comparison to other countries, is one of the highest in Europe (Matek et al., 1999). Currently, the only scientifically proven treatment for celiac disease is a strict lifelong adherence to a gluten-free diet. All foods and medications containing gluten from wheat, rye, barley or their derivatives are eliminated as even small quantities of gluten may be harmful. Such a diet improves quality of life in terms of decreased symptoms and normalised microvilli, which are most important for optimal gastrointestinal function (Cook et al., 2000). Complete removal of gluten from the diet will result in symptomatic, serologic, and histological remission in the majority of celiac patients (Pietzak, 2005). Therefore, people with celiac disease are forced to use gluten free cereals/products in their daily diet.

It is considered that the main gluten free cereals recommended for celiac patients have negative effects on product quality when used even at concentrations of 10-20%, and that commercial gluten free foodstuffs present undesirable properties (Blanco et al., 2011; Cabrera-Chávez et al., 2010). Gluten is important for appearance and crumb structure of cereal-based products, because it is essential to form a strong protein network required for the desired viscoelasticity. Upon that, prolamin provides viscosity and extensibility in a dough system, while glutenin is responsible for elastic and cohesive properties of dough (Demirkesen et al., 2010a). Many gluten free breads available on the market are of low quality, exhibiting a dry crumbling crumb, resulting in poor mouthfeel and flavour. Thus, the removal of gluten from bakery products negates bread quality and so the use of different substances that mimic the viscoelastic properties of gluten is often required (Gallagher et al., 2003). Many experimental designs have been developed to improve functional, nutritive and sensory properties (appearance, odour, taste, aroma and texture) of gluten free formulations (Blanco et al., 2011; Chillo et al., 2007; Demirkesen et al., 2010a; Demirkesen et al., 2010b; İbanoğlu et al., 2006; Kiskini et al., 2007; Lazaridou et al., 2007; Sedej et al., 2011; Torbica et al., 2010).

Therefore, the present investigation is aimed at possible application of electronic tongue to the recognition of differ-

ences between conventional and gluten free flour. After browsing of different analytical methodologies for testing of gluten free foods and determination of gliadin (as a measure of gluten in foods) such as: acid- and sodium dodecyl sulphate-polyacrylamide gel electrophoresis, capillary electrophoresis, flow cytometry, fluorescence correlation spectroscopy, fluorimmunoassays and fluorimetric methods, immunologically based sandwich or competitive enzyme-linked immunosorbent assay techniques, matrix-assisted laser desorption ionisation/time-of-flight mass tandem spectrometry, polymerase chain reaction systems, and reversed-phase and size-exclusion high-performance liquid chromatography, Peres et al. pointed out the strong need for the development of low-cost, fast, sensitive and user-friendly analytical systems to detect gliadins in foodstuffs (Peres et al., 2011). Although electronic tongue systems have been widely used recent years in food analyses, mostly water and beverages (Ciosek et al., 2006; Dias et al., 2011; Moreno et al., 2006; Peres et al., 2009; Winquist et al., 2011), wines (Gay et al., 2010; Gutiérrez et al., 2011; Novakowski et al., 2011; Parra et al., 2006; Rudnitskaya et al., 2007), milk samples (Ciosek and Wróblewski, 2008; Dias et al., 2009; Hruškar et al., 2010; Wei and Wang, 2011), beer (Arrieta at al., 2010; Polshin et al., 2010; Rudnitskaya et al., 2009), vegetables (Beullens et al., 2006, Beullens et al., 2008), fruits (Kantor et al., 2008; Rudnitskaya et al., 2006) and honey (Major et al., 2011; Wei et al., 2009), only a few published investigations reported results of detection of gliadins in food by sensor arrays. De Stefano et al. (2006) designed optical biosensor based on nanostructure porous silicon for the detection of trace amounts of gliadin in food. Peres et al. (2011) used an all-solid-state potentiometric electronic tongue with 36 polymeric membranes for the detection of gliadins in "Gluten-free" and "Gluten-containing" foodstuffs.

The aim of this investigation was to assess gluten free flour as the base for many gluten free products on the market. Gluten free flour was assessed by the electronic tongue combined with artificial neural networks (ANN) and compared to regular flours currently found on Croatian market. Additionally, the characterization of physicochemical properties of both regular and gluten free flours was performed. The obtained results were used to assess the performance of the electronic tongue combined with artificial neural networks (ANN) in regular and gluten free flour analysis.

Material and methods

Sampling

Samples of flours were obtained from health care institutions in Zagreb, Croatia and local supermarkets. Seven samples of flours labelled as "gluten free" flours (sample 1GF: gluten-free wheat flour; sample 2GF: potato starch, maize starch, maize flour, rice flour; sample 3GF: maize starch, rice flour; sample 4GF: maize starch, maize flour; sample 5GF: maize flour; sample 6GF: maize starch, soy flour; sample 7GF: maize starch, soy flour) and six samples of regular flours with very low gluten content (<100 mg gluten/kg), mainly produced from maize, wheat, rye, soy and rice (sample 1: maize flour; sample 2: rye flour; sample 3: mix of six different flours such as rye, wheat, barley, oat, millet and buckwheat; sample 4: maize starch; sample 5: rice flour; sample 6: soy flour), were analysed.

Reference physicochemical analyses and determination of gluten by ELISA technique

In all of the flour samples, regular and gluten free, reducing sugar content (%), total reducing sugar content (%), acidity, protein (crude) content (%) and gluten content (mg/kg) were determined. Sugars (reducing and nonreducing) were determined according to Luff-Schoorl method using a Luff-Solution. For the determination of acidity, 10 g of flour was diluted in 100 mL of 67% ethanol solution and, after filtration and addition of 3% ethanol-phenolphthalein solution, titrated with 0.1M NaOH solution. Acidity was expressed as mL of NaOH required to neutralize free fatty acids in 100g of flour. Nitrogen and protein (crude) content was determined according to the Kjeldahl method. Crude protein content was calculated using the factor of 6.25, or 5.7 for wheat flour.

Enzyme-linked immunosorbent assay (ELISA) was used for determination of gliadin, as a measure of gluten, in investigated flour samples. In this Double Antibody Sandwich (DAS) reaction of antigen-antibody, capture monoclonal antibodies specific on prolamins of wheat, rye and barley (but non-specific on oat), and also enzyme-labeled (Horseradish peroxidase, HRPO) monoclonal antibodies specific on prolamins of wheat, rye and barley, were used. After the incubation of gliadins from the flour samples with the immobilized monoclonal antibodies, enzyme-labeled antibodies were added. Gliadin from flour samples formed a complex sandwiched between antibody attached to well and antibody labelled with enzyme. Amount of analyte was determined by adding enzymatic substrate (urea peroxide) and chromogen (tetramethylbenzidine). Washing steps were incorporated after each interaction stage to remove any nonimobilized species. After addition of stopping reagent and conversion of blue colour into yellow, read-outs were performed on reader at 450 nm. The responses were compared with that observed with 5 standard concentrations and positive and negative control.

The αAstree electronic tongue measurements

The commercial electronic tongue α*Astree* (Alpha M.O.S.) was employed in assessing regular and gluten free flours, 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 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 (Alpha M.O.S., 2003). All samples solutions were analyzed in triplicate by the electronic tongue and each analysis cycle lasted for 300 seconds giving a sum of 39 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 a 5% flour solution to obtain stable sensor responses.

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 (Daszykowski et al., 2007) and drift correction (Hruškar et al., 2010) were performed. The centered and corrected data was transferred to Statistica 9 (Statsoft, Inc., 2010) where Principal Component Analysis (PCA) (Jain et al., 2000), Canonical Correlation Analysis (CCA) and Artificial Neural Networks (ANN) regression and classification (Samarasinghe, 2007) were performed. A data matrix consisting of 39 rows (13 samples x 3 measurements) and 7 columns (7 sensor outputs: JB, BA, BB, HA, ZZ, CA and GA) was used to perform the PCA method. In order to assess the performance of the electronic tongue in distinguishing between regular and gluten free flours an ANN model was created using a data matrix of 39 rows (also 12 samples x 3 measurements) and 8 columns (7 sensor outputs and whether the samples were regular or gluten free). The sensors outputs were used to create ANN models for the prediction of 5 physicochemical properties of flour samples (gluten content, reducing sugar content, total reducing sugar content, acidity and protein content). CCA was performed on two variable sets, one comprising of 7 sensor outputs and the other comprising of 5 physicochemical parameters.

Results and discussion

Classification of gluten free and regular flour samples by the electronic tongue

The electronic tongue measurements of regular and gluten free flour, processed by PCA, are featured in Figure 1. A total of 39 measurements were processed by PCA which included 7 gluten free and 6 regular flour samples, all in triplicate. The results show the separation of regular flour samples from the gluten free samples on the first two principal components (76.3% of the variance explained) (Table 1, Figure 1). The exception was Sample 3 which grouped with the gluten free flour samples, but this sample is uncommon sample of regular flours because represents mix of six different flours such as rye, wheat, barley, oat, millet and buckwheat. Other samples of analysed regular flours, separated from the group of gluten free samples.

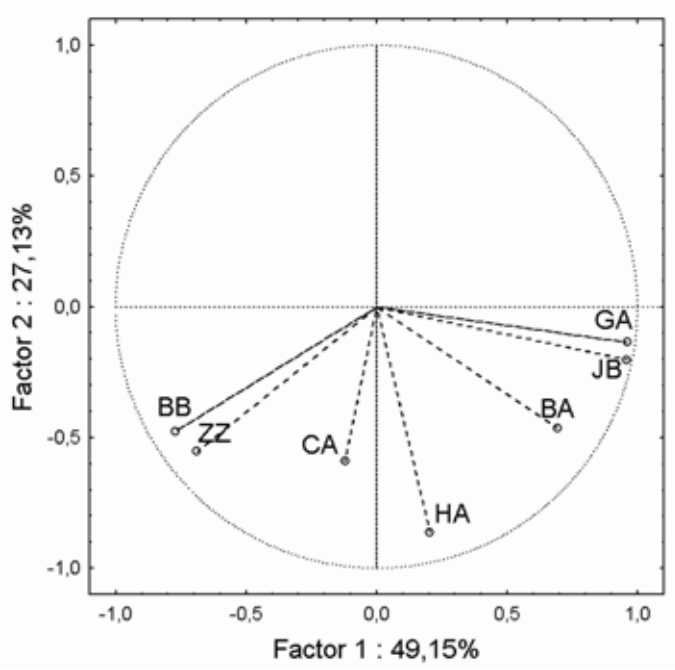

Figure 1. *PCA plot of regular and gluten free flour samples, number of replicas n = 3, number of flour samples N = 13.*

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

Factor	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
Factor 1	3.441	49.2	3.441	49.2
Factor 2	1.899	27.1	5.340	76.3
Factor 3	1.111	15.9	6.451	92.2
Factor 4	0.325	4.6	6.776	96.8
Factor 5	0.140	2.0	6.916	98.8
Factor 6	0.063	0.9	6.979	99.7
Factor 7	0.021	0.3	7.000	100.0

According to the factor-variable loadings (Table 2) sensors GA (0.267) and JB (0.266) strongly influenced sample projection on the first principal component, while sensors HA (0.391) and CA (0.184) influenced sample projection on the second principal component. Sensors BB, ZZ and BA had similar influence on both the first and second principal components (Table 2). Figure 2 further explains the influence of sensor loadings on the first two principal components on regular

and gluten free flour samples grouping. According to Figure 2 sensors BB and ZZ are the most responsible variables for regular and gluten free flour samples separation, followed by sensor CA.

Table 2. *Factor-variable loadings based on correlations obtained by Principal Component Analysis (PCA).*

Sensors	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
GA	0.267	0.010	0.000	0.005	0.305	0.206	0.207
BA	0.139	0.113	0.203	0.176	0.059	0.214	0.095
BB	0.173	0.120	0.088	0.017	0.499	0.043	0.059
HA	0.012	0.391	0.050	0.475	0.008	0.063	0.000
ZZ	0.138	0.161	0.159	0.000	0.126	0.403	0.013
CA	0.004	0.184	0.478	0.319	0.001	0.014	0.000
JB	0.266	0.022	0.021	0.007	0.002	0.057	0.625

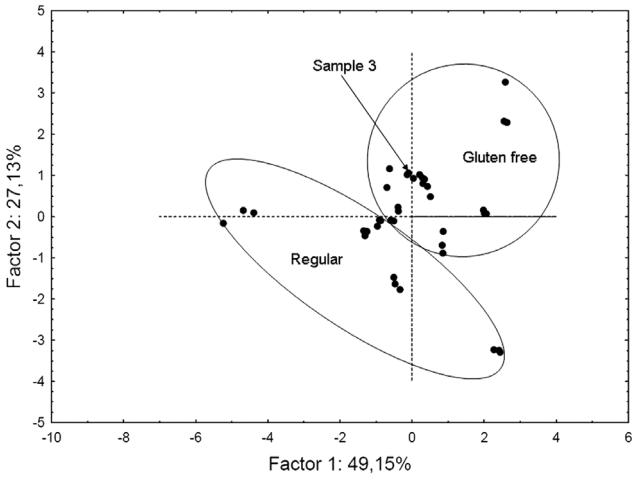

Figure 2. *Projection of sensor responses to flour samples on the Principal Component Analysis (PCA) plane.*

Characterization of gluten free and regular flour samples on the basis of the electronic tongue measurements and physicochemical analyses

To demonstrate the ability of the electronic tongue system to assess the gluten content of flour samples, a comparison was made between the sensors outputs and the results of gluten evaluation by ELISA technique and also the results of other physicochemical analyses of flour samples (reference analyses) or in detail: reducing sugar content, total reducing sugar content, acidity and protein content. Canonical Correlation Analysis and Principal Components Analysis were performed to accomplish this task. Table 3 presents the correlation analysis between sensor outputs and flour physicochemical parameters. The correlation was performed to analyze the relationship between the electronic tongue's sensors and physicochemical parameters and provide insight in possible causes of regular and gluten free samples separation. According to Table 3, sensors GA and JB both highly correlate with total reducing sugar content, acidity and protein content indicating that the mentioned physicochemical parameters could be responsible for sample projection on the first principal component. Sensor HA correlates with reducing sugar content and total reducing sugar content while sensor CA does not correlate with any of the analyzed parameters. The sample projection on the second principal component could be explained by reducing sugar content to some extent, but with the sensor CA not correlating with any of the analyzed parameters indicates the existence of other substances influencing the electronic tongue measurements (Table 3). According to Table 3 sensor BB correlates with protein content and reducing sugar content, while sensor ZZ correlates with protein content and gluten content. As shown in Figure 2 the sensors most responsible for gluten free and regular flour samples separation were sensors BB and ZZ, followed by sensor CA. Therefore, the separation of the gluten free and regular flour samples can be explained by the difference in gluten content, protein content and reducing sugar content. Still, further investigation is required concerning the influence of sensor CA indicating the existence of other compounds responsible for gluten free and regular flour samples separation.

Table 3. *Canonical Correlation Analysis (CCA) between sensors outputs and flour physicochemical parameters.*

Sensors	Reducing sugar content (%)	Total reducing sugar content (%)	Acidity (mL NaOH)	Protein content (%)	Gluten content (mg/kg)
GA	0.150	-0.443*	-0.555*	-0.715*	0.005
BA	-0.040	-0.655*	-0.795*	-0.674*	-0.383
BB	-0.475*	0.211	0.302	0.629*	-0.406
HA	-0.555*	-0.486*	-0.329	-0.149	-0.417*
ZZ	-0.406	0.213	0.244	0.629*	-0.522*
CA	-0.336	-0.331	0.047	-0.069	0.170
JB	0.160	-0.583*	-0.718	-0.783*	-0.103

****p<0.01***

In order to classify flour samples according to gluten content, artificial neural networks were employed. Table 4 shows the results obtained by the ANN classification model. As shown, the model was 100% accurate in the classification of regular flours, while the classification accuracy of gluten free flour samples was 95.2% (Table 4). The developed model had the architecture of a 3-layer perceptron consisting of 5 input neurons, 2 hidden neurons and 2 output neurons. The transfer functions used were logistic, between the input and hidden neurons, and softmax between the hidden and output neurons.

Table 4. *Results from the artificial neural networks (ANN) model for flour classification according to gluten content.*

Flour type	Total	Correct	Incorrect	Correct (%)	Incorrect (%)
Gluten free flour	21	20	1	95.2	4.8
Regular flour	18	18	0	100	0
All	39	38	1	97.4	2.6

The results obtained by PCA suggest that the electronic tongue system is capable to distinguish flour samples on the basis of their gluten content and CCA additionally explained the relationship between the sensors outputs and physicochemical properties, including gluten content, of flour samples. The developed ANN model provided a 100% correct classification rate in the case of regular flours and 95.2% correct classification rate in the case of gluten free flours showing the technique potential in the determination of gluten free flour samples.

Peres et al. (2011) showed that the electronic tongue could be used in practice as a fast and economic preliminary tool to evaluate possible gluten contaminations of "Gluten-free" foodstuffs. The authors demonstrated that system could classify "Gluten-free" and "Gluten-containing" foodstuff samples, with different gliadin content, with overall sensitivities and specificities greater than 77%, and that could distinguish, with acceptable sensitivities and specificities (greater than 83%), "Gluten-free" from "Gluten-containing" food samples with only one of the 10 "Gluten-containing" foods being misclassified.

Rapid evaluation of gluten content, protein content, acidity and reducing sugar content of gluten free and regular flour samples

In order to assess the physical and chemical properties of flour samples, ANN models were created using the data acquired by the electronic tongue combined with the analytical results of gluten content, protein content, reducing sugar content, total reducing sugar content and acidity. The results obtained from the developed models are shown in Table 5. The plots of the observed and predicted values of protein content, acidity, gluten content, reducing sugar content and total reducing sugar content are shown in Figure 3, including their respective correlations.

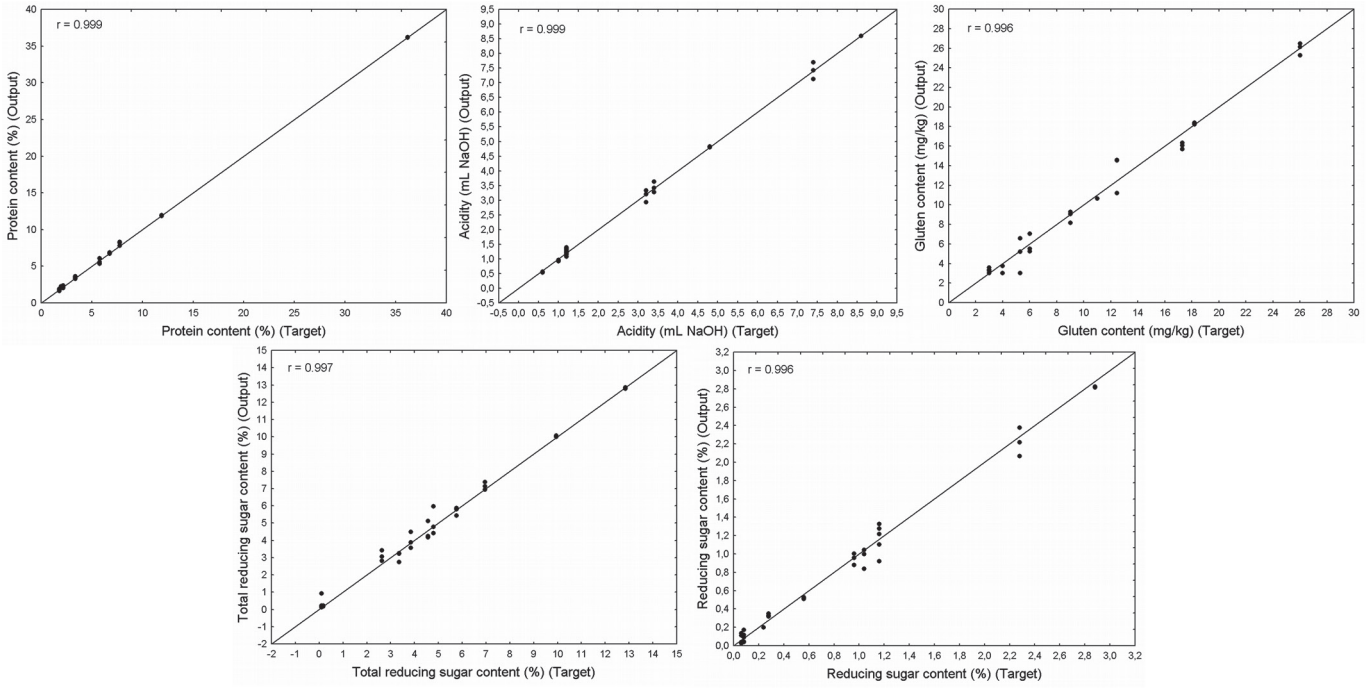

Figure 3. *Artificial neural networks (ANN) regression between the potentiometric sensor array and the physicochemical parameters in flour samples, number of replicas n = 3, number of flour samples N = 13.*

Table 5. *Descriptive statistics of the artificial neural networks (ANN) models for the determination of flour physicochemical parameters.*

Parameter		R2	Slope	RSE %
Protein content (%)	Training subset	0.999	0.999	1.12
	Testing subset	0.999	0.924	6.20
	Validation subset	0.995	0.984	5.40
	Average	0.997	0.969	4.24
Acidity (mL NaOH)	Training subset	0.998	0.998	2.82
	Testing subset	0.995	0.975	3.71
	Validation subset	0.975	0.979	7.66
	Average	0.989	0.984	4.73
Gluten content (%)	Training subset	0.985	0.994	7.43
	Testing subset	0.972	0.962	7.99
	Validation subset	0.999	0.975	3.03
	Average	0.985	0.977	6.15
Reducing sugar content (%)	Training subset	0.993	0.986	6.48
	Testing subset	0.982	0.910	12.15
	Validation subset	0.997	0.980	3.67
	Average	0.991	0.959	7.43
Total reducing sugar content (%)	Training subset	0.995	1.000	4.46
	Testing subset	0.985	0.991	7.07
	Validation subset	0.997	0.829	17.35
	Average	0.992	0.940	9.63

The obtained correlation between the observed and predicted protein content (%) in flour was 0.999 (Figure 3). The RSE of the ANN model was 4.24% with the training, testing and validation subset's RSE being 1.12, 6.20 and 5.40, respectively (Table 5). The low RSE percentages (Table 5) and the high correlation of the ANN model suggest that the electronic tongue can be successfully applied in the determination of protein content of gluten free and regular flours, within the investigated range (from 1.78 to 36.19%).

The developed ANN model for the prediction of acidity (mL of NaOH required to neutralize free fatty acids from 100g of flour) obtained a correlation of 0.999 (Figure 3). The RSE of the model was 4.73 with the training, testing and validation subset's RSE being 2.82, 3.71, and 7.66, respectively (Table 5). In the investigated range (from 0.60 to 8.60 mL of NaOH required to neutralize free fatty acids from 100g of flour), according to the obtained RSE values (Table 5) and high correlation, the developed ANN model exhibited excellent performance in the prediction of gluten free and regular flours acidity.

ANN model for the prediction of gluten content (mg/kg) in gluten free flours and regular flours showed a correlation of 0.996 (Figure 3). The RSE of the model was 6.15, with the training, testing and validation subset's RSE being 7.43, 7.99 and 3.03, respectively (Table 5). The low RSE values (Table 5) and correlation values obtained by the developed ANN model for the prediction of gluten content, indicate that the sensor array is capable of responding and quantification of the gluten content of a solution of gluten free and regular flours with high precision in the investigated range (from 3.00 to 26.00 mg/kg).

The obtained correlation between the observed and predicted reducing sugar content (%) was 0.996 (Figure 3), The RSE of the model was 7.43, with the training, testing and validation subset's RSE being 6.48, 12.15 and 3.67, respectively (Table 5). In the investigated range (from 0.06 to 2.88%), the developed ANN model for the prediction of reducing sugar content in gluten free and regular flours exhibited excellent performance with low RSE percentages (Table 5) and high correlation.

Also, the developed ANN model for the prediction of total reducing sugar content (%) showed a high correlation of 0.992 (Figure 3). The RSE of the model was 9.63, with the training, testing and validation subset's RSE being 4.46, 7.07 and 17.35, respectively (Table 5). Obtained RSE values (Table 5) and correlation value indicate also excellent performance, within the investigated range (from 0.10 to 12.85%), in the prediction of total reducing sugar content of gluten free and regular flours.

Conclusions

In this paper a commercially available electronic tongue was employed in the classification of gluten free and regular flours as well as rapid determination of physicochemical parameters including gluten content determination, protein content determination, acidity, reducing sugar content and total

reducing sugar content determination. The performance of the sensor array was assessed by multivariate data analysis techniques. The application of an electronic tongue combined with artificial neural networks proved to be a successful tool for classification of gluten free and regular flours (95.2 and 100% of correct classifications obtained by the developed ANN model, respectively). The developed ANN model for the prediction of gluten content showed high potential of the electronic tongue as a technique for the prediction of gluten content in gluten free and regular flours. All developed ANN models indicate that the electronic tongue can be successfully applied for rapid determination of protein content, acidity, reducing sugar content and total reducing sugar content in flour.

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