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Application of a potentiometric sensor array as a technique in sensory analysis

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ABSTRACT

This paper reports on the application of a potentiometric sensor array used for monitoring changes in probiotic fermented milk during storage, classification of probiotic fermented milk according to flavor and to accurately predict the results from a human sensory panel. For that purpose the potentiometric electronic tongue consisting of seven sensors and an Ag/AgCl reference electrode was used. The samples of plain, strawberry, apple-pear and forest-fruit probiotic fermented milk were stored during 20 days on two different temperatures and monitored by the electronic tongue and the human sensory panel. Various pattern recognition techniques are adapted including multivariate data processing based on principal components analysis (PCA) for monitoring changes occurring in probiotic fermented milk, artificial neural networks (ANN) for the classification of probiotic fermented milk during storage, partial least square regression (PLS) and artificial neural networks (ANN) to estimate and predict the sensory panel evaluation results.

The highest correct classification percentage (97%) was obtained for plain probiotic fermented milk and the lowest (87%) for apple-pear flavored probiotic fermented milk. The highest correlation between the sensor array and the human sensory panel was obtained for the forest-fruit flavored probiotic fermented milk both by using artificial neural networks (0.998) and partial least square regression (0.992). Results from these analyses demonstrate that the electronic tongue can be used to monitor changes in probiotic fermented milk during storage, to classify probiotic fermented milk according to flavor and to predict the sensory characteristics and their relationship to the quality of the probiotic fermented milk measured by consumer.

1. Introduction

Food and beverage profiling and characterization are accomplished by two major routes: analytical chemistry and sensory evaluation. Traditionally, these two techniques are employed separately, with sensory evaluation using human panel to evaluate sensory characteristics of food and instrumental analysis delineating the chemical and physical properties of the food. Sensory evaluation is still used as the gold standard in the industry, but the developments in the instrumental field have allowed the closer relationship to be formed between these two techniques [1]. To consistently meet consumer-defined quality, it is ideal to determine key sensory drivers for acceptance using optimization modeling techniques [2–4]. These techniques integrate sensory and consumer data, which are used to establish quality specifications for key attributes. Product quality is monitored using the specification and appropriately trained sensory panel [5].

Probiotics are living non-pathogenic micro-organisms which, when ingested, exert a positive influence on host health or physiology [6]. Probiotic fermented milk has therapeutic properties and beneficial influence on health when consumed through a longer period of time [7].

Development of the electronic tongue was obviously inspired by biological sensory system, primarily human sense of taste. The human tongue consists of a large number of non-specific receptors (sensors) that reacts to dissolved compounds and transfers stimuli via the nervous system to the brain, where a neural network processes the signal pattern. Although the electronic tongue works in liquid media, like the biological tongue, the sensitivity of the artificial system can be much higher and its capabilities much wider; this makes the performance of the electronic tongue closer to that of the sense of taste.

The interest in using electronic tongue instrument to supplement human judgment has been a topic of research and discussion during the last decade [8].

The first multi-sensor system for liquid analysis based on a non-specific sensor approach was a taste sensor introduced by Toko et al. and recently referred to by the authors also as an "electronic tongue" [9]. Electronic tongues are the sensor arrays for liquid analysis using both several non-specific, low-selective, chemical sensors with high stability and cross-sensitivity and ion-selective sensors. The main purpose of the electronic tongue is qualitative

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analysis, such as recognition, classification or identification of samples, which depends on the composition of the sensor array and the mathematical procedure adopted for data treatment [10]. The sensing mechanism of the most of potentiometric sensors is based on a membrane, made on inorganic (polycrystalline and chalcogenide glass [11,12]) or organic (plasticized polymeric matrix doped with membrane active components [13,14], Langmuir–Blodgett [15] or electropolymerised films [16], etc.) materials [17]. The membrane is the main active component but in the same time it is also one of the weak points of the sensor, because most of drawbacks, such as reproducibility and long-term stability, depend on the membrane [17]. The taste sensory system transforms information related to ionic content in a liquid sample to an electrical signal measured by a sensor array composed of ion-selective electrodes. That is, taste-like characteristics of a liquid sample can be analyzed using a potential difference between a reference electrode and indicator electrodes [18].

Principal components analysis (PCA) is the most common and versatile statistical method for data projection, and widely used in data analysis to display sensor array measurements [19]. Partial least square (PLS) regression method is very useful in predicting a set of dependent variables from a large set of independent variables [20]. But, one of the most often used data-processing methods is an artificial neural network (ANN), the algorithms of which are based on modeling of learning and recognition process in the human brain [21].

In this paper, the comparison of two methods: sensory analysis and electronic tongue for monitoring changes and classification of commercial brands of probiotic fermented milk, has been presented. Measurements were performed using a trained sensory panel and instrument analysis using the αAstree electronic tongue produced by Alpha M.O.S. (France). This work presents also, multivariate data processing based on PCA, PLS and ANN to visually classify data patterns detected by the sensory system.

2. Materials and methods

2.1. Samples

Analysis was performed on four types of probiotic fermented milk (plain flavored, strawberry flavored, forest-fruit flavored and apple-pear flavored). All probiotic fermented milk samples were purchased on the local Croatian market freshly delivered from the producer. Samples were stored at +4 °C and +25 °C during 20 days. Hydrochloric acid (w = 37%, ISO-For Analysis grade) was purchased from Carlo Erba Reagents.

2.2. The αAstree electronic tongue

The αAstree liquid and taste analyzer was purchased from Alpha M.O.S., France. The electronic tongue uses an 16-position 730 Sample Changer and 759 Swing Head for sampling, both from Metrohm, Ltd., an interface electronic module and a sensor kit developed by Alpha M.O.S., France, a reference Ag/AgCl electrode and a mechanical stirrer both from Metrohm Ltd. The sensor kit #1 has been developed specially for food analysis to insure good sensitivity and selectivity of the sensors [22]. It comprises of seven non-specific, cross-selective potentiometric sensors (named JB, BA, BB, CA, GA, HA and ZZ) which are chemically modified field effect transistors (CHEMFETs). The active electrode area of the sensors is covered by an organic coating. By variation in composition of this organic coating different sensitivity and selectivity for each sensor to various substances was obtained [23]. Cross-sensitivity in electronic tongue systems ensures a wider response to different substances and thus a more accurate reflection of the analyte's chemical image. Cross-sensitivity can be obtained by employing different sensing materials or by combining various ionophores [24].

The potentiometric sensors use an electrochemical interface to convert the ionic activity on the surface of the transducer to a change of charge. This change of charge results in a potential change on the transducer which is then measured against a reference electrode [25]. In the case of CHEMFETs the transducer element is a field effect transistor [24] and the origin of the potential change is the complexation of the analyte with the ion carrier at the outer phase boundary on the membrane/aqueous solution interface [26]. The αAstree electronic tongue was connected to a computer built according to instructions (Alpha mos manual) with the Astree II software (Alpha M.O.S.,Version 3.0.1., 2003) installed. The Astree II software automatically gathers and stores the sensors output data.

2.3. Instrumental measurements

Four types of probiotic fermented milk were stored for 20 days at two different temperatures (+4 °C and +25 °C) and analyzed by the electronic tongue every 5th day. During the experiment 120 measurements were performed. All samples were analyzed at +25 °C. Hydrochloric acid diluted in deionized water (0.01 mol/L) was analyzed as a reference sample together with fermented milk samples to follow and later correct the drift of the sensors in time. Conditioning of the potentiometric sensor array with probiotic fermented milk was performed prior to each analysis session. The conditioning of the sensor array consisted of analyzing plain probiotic fermented milk until the response of each sensor changed less than 20 mV in 100 s.

The samples were randomly inserted in the autosampler and each sample was analyzed 300 s in 1 s intervals. Three runs were performed during every analysis session giving a total of three measurements for each sample. Typical sensor stabilities after conditioning are shown in Fig. 1. The sensors were rinsed with deionized water for 30 s between measurements.

2.4. Sensory evaluation

The sensory panel consisted of five trained panelists. The evaluation of the sensory characteristics (appearance, consistency, color, odor and flavor) of probiotic fermented milk was performed according to ISO 6658:2005 [27]. The sensory panel rated the sensory characteristics of the samples and the overall value was taken for further analysis. Each panelist rated a sample with a score from 1 to 5 for each characteristic, which was then multiplied with an impact factor for the given characteristic. A sample was rated with a sum of maximum 20 points, where a higher number meant better sen-

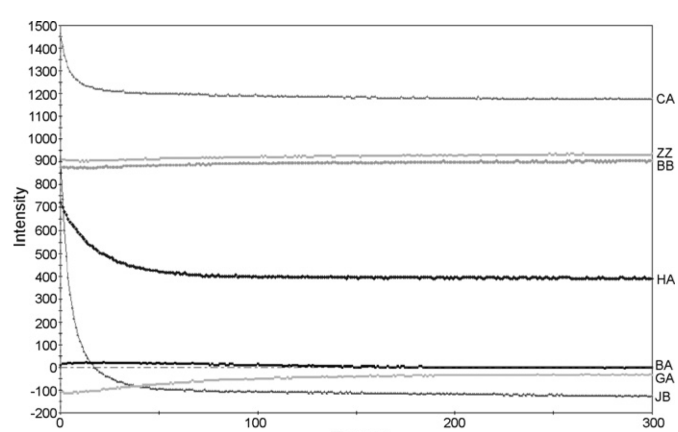

Fig. 1. Sensor responses to an apple-pear sample after conditioning.

sory characteristics. The obtained results were used to assess the performance of the potentiometric sensor array as a technique in sensory analysis.

2.5. Data analysis

The sensor outputs collected by the Astree II software (Alpha M.O.S.) were imported to Microsoft Excel (Microsoft Excel 2002, SP-2) and centered [28]. The sensor drift correction was performed using data obtained by the analysis of the reference sample (hydrochloric acid, 0.01 mol/L). The mean value of three measurements per sample was calculated and subjected to evaluation with principal component analysis (PCA). PCA was used as an unsupervised linear feature extraction and dimensionality reduction method in order to find patterns in the obtained data. PCA is widely used by authors [19,24,29] because it uses the most expressive features, which are the eigenvectors with the largest eigenvalues, to effectively approximate data by a linear subspace using the mean square criterion [30]. PCA was employed as a statistical method for the evaluation of the data in order to recognize patterns in the sensor outputs which could be associated with the occurring changes during storage of probiotic fermented milk samples at different temperatures. The PCA was performed using Statistica 7.1 (StatSoft, Inc., 2005). For the classification of the four types of probiotic fermented milk ANN were performed on the centered and corrected data using Statistica 7.1 (StatSoft, Inc., 2005). ANN have the ability to learn complex nonlinear input–output relationships, use sequential training procedures and adapt themselves to the data. However ANN are equivalent or similar to classical statistical pattern recognition methods in spite of the seemingly different underlying principles [30]. In this approach choosing the training set becomes a liability in terms of accurately describing the classification model [30] because in the food industry, especially the dairy industry, products change in composition daily. To assess the correlation between the sensory panel analysis results and the data obtained by the potentiometric sensor array PLS regression and ANN regression were employed. PLS and ANN regression were performed using Statistica 7.1 (StatSoft, Inc., 2005). The PLS regression method is employed because of its particular usefulness in predicting a set of dependent variables from a large set of independent variables [20]. ANN provide novel nonlinear algorithms for feature extraction. Those algorithms offer several advantages, as unified approaches for feature extraction and flexible procedures for finding nonlinear solutions. In the case of neural networks, both regression and classification problems can be seen as particular cases of function approximation. In case of classification problems the functions that need to be approximated are the probabilities of membership of the different classes expressed as functions of the input variables, while in the case of regression problems it is the regression function that needs to be approximated [31].

3. Results and discussion

3.1. Monitoring changes in probiotic fermented milk during storage by the potentiometric sensor array

The aim of the experiment was to assess the performance of a potentiometric sensor array in the recognition of occurring changes in probiotic fermented milk samples during storage.

The PCA scores of the measurements are shown in Fig. 2. The total variances shown by the first two principal components (Factor 1, Factor 2) for plain, strawberry flavored, forest-fruit flavored and apple-pear flavored fermented milk are 97.58%, 97.52%, 97.86% and 97.95%, respectively (Fig. 2). The main source of variance between the samples, as shown by the first principal component (Factor 1), is the duration of storage. All sensors had similar contributions to the first principal component except sensor CA which had the highest contribution to the second principal component (Factor 2) (Table 1). The second principal component shows that the second most important source of variance between the samples is the storing temperature. The changes occurring in probiotic fermented milks during storage are more pronounced at +25 °C and are visible as early as at the 5th day of storage. Samples stored at +4 °C show little change during the first 10 days of storage, but change significantly between the 10th and 15th day. The samples stored at +25 °C also show significant change between the 10th and 15th day of storage (Fig. 2). As shown by other authors [32,33] the changes in composition of fermented milks are strongly influenced by storage time and storing temperature. The electronic tongue used in this experimental session was capable of tracking changes in composi-

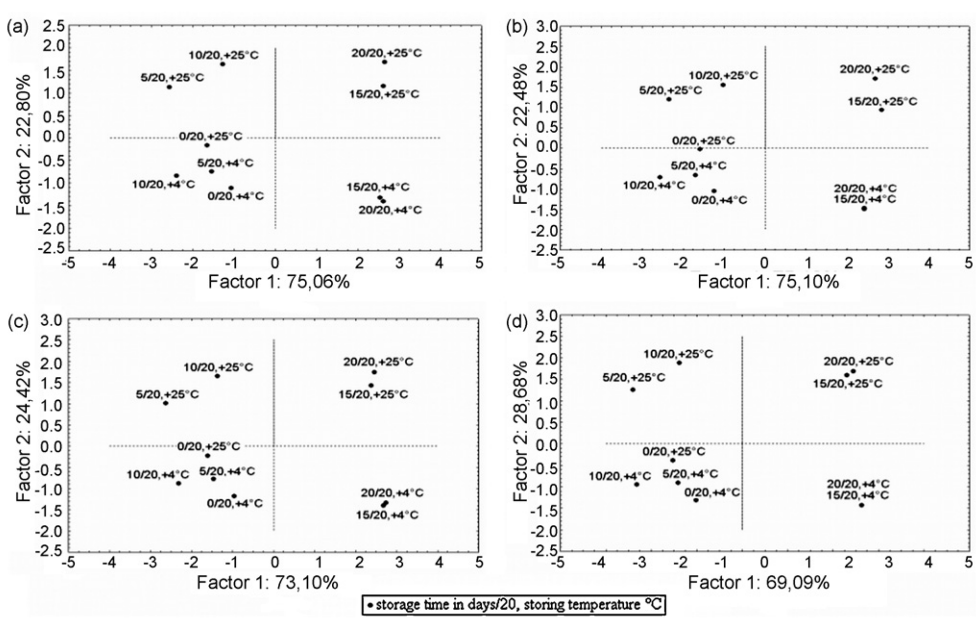

Fig. 2. PCA plot of (a) forest-fruit flavored, (b) plain, (c) strawberry flavored and (d) apple-pear flavored probiotic fermented milk measurements during 20 days of storage at two different temperatures, number of replicas $n = 3$, 10 samples per type of probiotic fermented milk.

Table 1
Contribution of the sensors to principal components, based on correlations, according to flavor of probiotic fermented milk.

Sensors	Forest-fruit		Plain		Strawberry		Apple-pear	
	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
JB	0.187345	0.006090	0.186281	0.006424	0.190822	0.008781	0.200824	0.011280
BA	0.161791	0.054884	0.165103	0.045696	0.165448	0.043808	0.172741	0.050835
BB	0.162488	0.085893	0.164043	0.082461	0.163294	0.091768	0.155101	0.121023
HA	0.116065	0.223276	0.111726	0.238943	0.114417	0.214564	0.123486	0.180558
ZZ	0.155099	0.110346	0.150988	0.122536	0.155376	0.115729	0.138905	0.158893
CA	0.052283	0.443192	0.068673	0.391235	0.041568	0.452586	0.038208	0.395938
GA	0.164929	0.076318	0.153187	0.112704	0.169076	0.072765	0.170734	0.081473

Table 2
Classification of probiotic fermented milk according to flavor by artificial neural networks.

	Apple-pear	Strawberry	Plain	Forest-fruit
Total	30	30	30	30
Correct	26	27	29	27
Wrong	4	3	1	3
Unknown	0	0	0	0
Correct (%)	87	90	97	90
Wrong (%)	13	10	3	10
Unknown (%)	0	0	0	0

tion of different types of probiotic fermented milk during storage. Moreover, the electronic tongue showed that changes in composition occur more rapidly on higher temperatures.

3.2. Classification of probiotic fermented milk by means of artificial neural networks

The results obtained by the potentiometric sensor array were processed with artificial neural networks to classify probiotic fermented milks according to flavor. The ANN model created had a high percentage of correct classification regardless of storage duration and temperature.

The advantage of using measurements of the samples through their entire shelf life in the model is to gain a pool of data that is capable of describing the overall flavor instead the temporary flavor of the products. In the process of training neural networks 90 randomly selected sample measurements from four different types of probiotic fermented milk were used and 30 sample measurements were used for validation. The neural network created had 7 neurons in the input layer, 74 neurons in the hidden layer and 4 neurons in the output layer. The methods used for training of the model were the sampling method, *k*-nearest neighbors method and pseudo-inversion method. The model had the highest percentage of correct classification for plain probiotic fermented milk (97%), then forest-fruit and strawberry flavored (90% both) and the lowest for the apple-pear flavored probiotic fermented milk (87%) (Table 2). Upon examining the training and validation set separately, the training set used for building the ANN model had the lowest percentage of correct classification of 92% for the strawberry flavored probiotic fermented milk and the highest of 100% for forest-fruit flavored probiotic fermented milk. The validation set, used for testing the obtained model had the lowest percentage of correct classification with the apple-pear and strawberry flavors (63%, both). The highest percentage of correct classification in the validation set had plain probiotic fermented milk with 100% correct classification. Strawberry flavored probiotic fermented milk had 75% of correct classifications in the validation set. The lower percentages of correct classifications in the validation set for apple-pear and forest-fruit flavored probiotic fermented milk could be due to overtraining of the model (95% and 100% of correct classifications in the training set, respectively) [34].

Ciosek et al. [35] obtained high percentage of correct classification of orange juice, beer and milk samples (milk samples had the highest percentage of correct classification – 100% in the training set and 96.7% in the test set) by a sensor array using the BPNN (back propagation neural networks) method. Ciosek and Wroblewski [24] obtained a high correct classification percentage on milk samples (100% of correct classification) with an array consisting of both selective and partially selective sensors using only the PLS–DA method in a stationary system. The percentage of correct classification lowered significantly when only partially selective sensors were applied. This implies that selective sensors, combined with the existing partially selective sensors could improve the performance of the commercial potentiometric sensor array. Dias et al. [29] also obtained high percentage of correct classification (93%) of cow and goat milk samples with a sensor array consisting of 40 sensors using the DA method. The correct classification percentage in cross-validation was 70%.

3.3. Correlation between sensory panel and potentiometric sensor array

Sensory evaluation was carried out by a trained sensory panel and an attempt was made to find correlations between the results of the sensory panel and the measurements performed by the electronic tongue. The data acquired by the electronic tongue was compared to the sensory panel ratings of the samples during storage at two different temperatures. ANN regression and PLS regression were employed to model the sensory panel ratings and calculate correlations.

During the training of ANN models, 20 measurements were randomly selected to form and 10 measurements to validate a given

Table 3
Correlation coefficients and standard errors for the ANN regression model.

	Forest-fruit		Plain		Apple-pear		Strawberry	
	Training set	Validation set	Training set	Validation set	Training set	Validation set	Training set	Validation set
Data mean	16.885	16.570	17.360	17.570	17.440	16.540	17.700	17.250
Data S.D.	2.912	2.713	1.745	0.980	2.309	1.901	2.257	1.998
Error mean	0.000	−0.130	0.000	−0.014	0.000	0.101	−0.002	−0.332
Error S.D.	0.046	0.292	0.061	0.293	0.325	0.360	0.060	0.827
Abs E. Mean	0.039	0.275	0.046	0.226	0.238	0.279	0.047	0.613
S.D. Ratio	0.016	0.108	0.035	0.299	0.141	0.189	0.027	0.414
Correlation	0.999	0.996	0.999	0.964	0.990	0.982	0.999	0.929

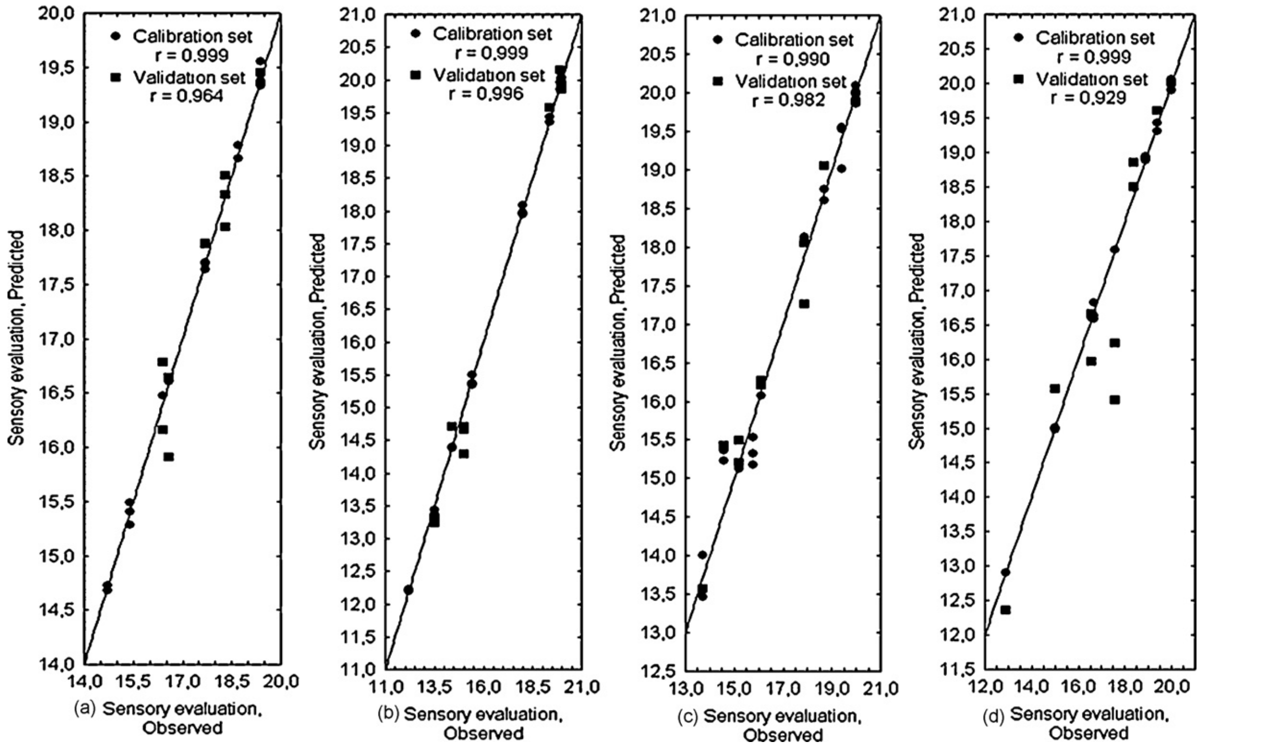

Fig. 3. ANN regression between the potentiometric sensor array and the human sensory panel for (a) plain, (b) forest-fruit flavored, (c) apple-pear flavored and (d) strawberry flavored probiotic fermented milk, number of replicas $n = 3$, 10 samples per type of fermented milk.

model for each type of probiotic fermented milk. The correlation coefficients and the standard errors of the ANN regression model are presented in Table 3. In Figs. 3 and 4 the regression curves of the ANN and the PLS model are shown with respective correlations. All networks were trained by a back propagation algorithm followed by a conjugate gradient algorithm. The PLS regression model employed 30 measurements to form a regression curve for each type of probiotic fermented milk. The highest correlation estab-

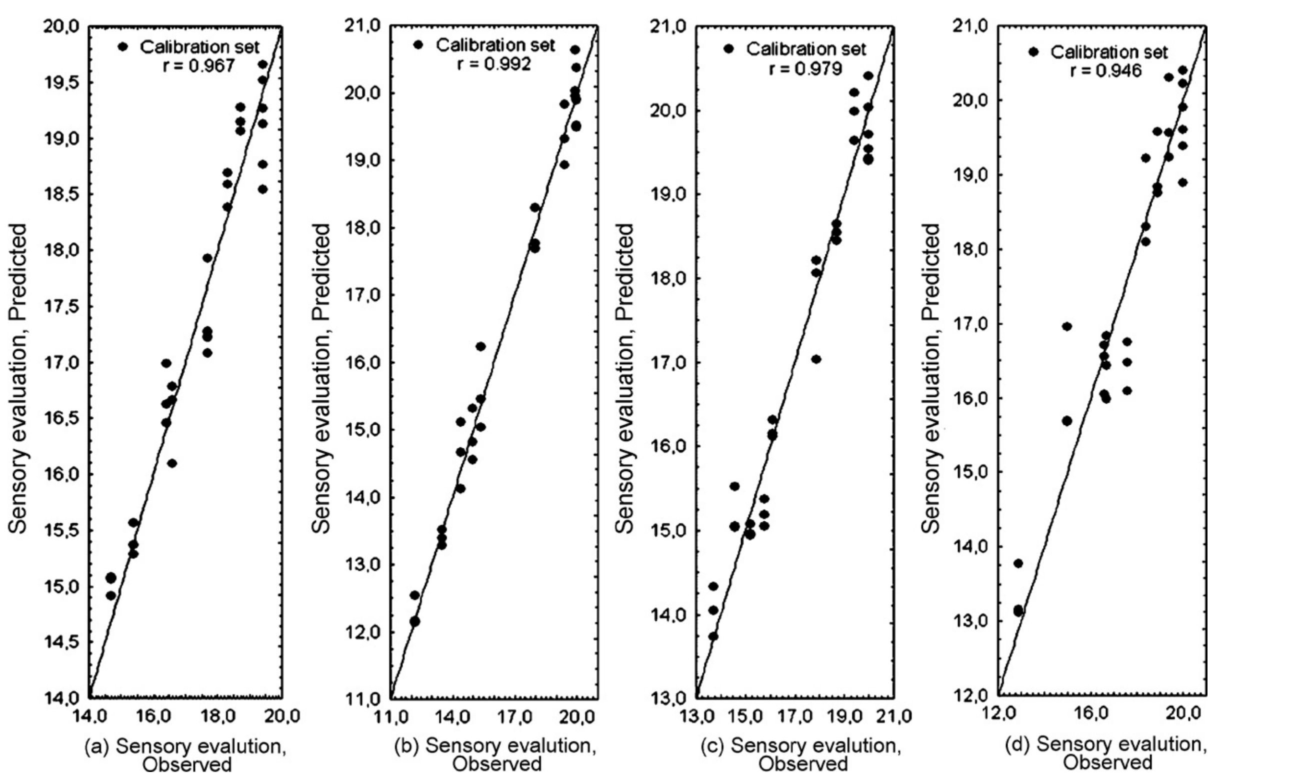

Fig. 4. PLS regression between the potentiometric sensor array and the human sensory panel for (a) plain, (b) forest-fruit flavored, (c) apple-pear flavored and (d) strawberry flavored probiotic fermented milk, number of replicas $n = 3$, 10 samples per type of fermented milk.

lished with PLS regression was found for the sensory evaluation of forest-fruit flavored probiotic fermented milk and was 0.992 (Fig. 4). With ANN regression the established correlation was 0.998 for the same sensory evaluation. During the training of the ANN model for the prediction of sensory evaluation of forest-fruit flavored probiotic fermented milk, a correlation of 0.999 was achieved for the training set and 0.996 for the test set (Table 3, Fig. 3). The network had 4 neurons in the input layer, 6 neurons in the hidden layer and 1 neuron in the output layer. The correlation obtained by ANN regression was slightly higher than the correlation obtained by PLS regression which can be explained by a better regression algorithm and input downsizing. The correlation obtained by PLS regression for the sensory evaluation of apple-pear flavored probiotic fermented milk was 0.979 (Fig. 4), while a correlation of 0.988 was achieved using ANN regression. The network created had 7 neurons in the input layer, 9 neurons in the hidden layer and 1 neuron in the input layer. The training set correlation for the apple-pear flavored probiotic fermented milk was 0.990 and the correlation for the test set was 0.982 (Table 3, Fig. 3). Again the ANN regression model proved more efficient at approximating the function of regression of the sensory evaluation and again just by a small margin. The correlation for sensory evaluation of plain probiotic fermented milk obtained by PLS regression was similar to the evaluation of apple-pear probiotic fermented milk and was 0.967 (Fig. 4). The correlation obtained by ANN regression was 0.994 which is moderately higher than the correlation obtained by the PLS regression model. During the training of the ANN regression model for the sensory evaluation of plain probiotic fermented milk the training set achieved a correlation of 0.999 and the test set achieved a correlation of 0.964 (Table 3, Fig. 3). The network consisted of 5 neurons in the input layer, 6 neurons in the hidden layer and 1 neuron in the output layer. The smallest correlation was found between the observed and predicted sensory evaluation of strawberry flavored probiotic fermented milk obtained by PLS regression and it was 0.946 (Fig. 4). In the case of ANN regression the correlation was 0.975 for the same sensory evaluation which was significantly higher than the correlation obtained by the PLS regression model. In the training process the correlation obtained for the training set was 0.999 and for the test set 0.929 (Table 3, Fig. 3). The network created had 6 neurons in the input layer, 9 neurons in the hidden layer and 1 neuron in the output layer.

ANN regression proved to be a better tool for predicting sensory evaluation scores for all analyzed types of probiotic fermented milk. The data obtained from the potentiometric sensor array achieves high correlation with the observed sensory panel scores for all four types of probiotic fermented milk, both with the ANN regression and PLS regression methods.

4. Conclusions

In this paper a commercially available potentiometric sensor array is evaluated as a simple technique in sensory analysis of probiotic fermented milk. The sensor array reported successfully on the changes that occurred in the composition during storage of probiotic fermented milk. Moreover, the sensor array was capable of tracking different rates of degradation of probiotic fermented milk stored at two different temperatures. The usefulness of this technique proved itself in the classification of the analyzed types of probiotic fermented milk during the entire shelf life. The statistical interpretation of the data acquired by the sensor array varied depending on the statistical method applied. From the statistical methods applied in this paper ANN provided the best evaluation scores which simplifies the future statistical interpretation of the acquired sensor array data of probiotic fermented milk samples. The measurements of the potentiometric sensor array achieved a high correlation with the results of the human sensory panel demonstrating the potential as a useful technique in sensory analysis.

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