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Electronic tongue as a screening tool for rapid analysis of beer

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ABSTRACT

An electronic tongue (ET) comprising 18 potentiometric chemical sensors was applied to the quantitative analysis of beer. Fifty Belgian and Dutch beers of different types were measured using the ET. The same samples were analyzed using conventional analytical techniques with respect to the main physicochemical parameters. Only non-correlated physicochemical parameters were retained for further analysis, which were real extract, real fermentation degree, alcohol content, pH, bitterness, color, polyphenol and CO_2 content. Relationship between the ET and physicochemical datasets was studied using Canonical Correlation Analysis (CCA). Four significant canonical variates were extracted using CCA. Correlation was observed between 6 physicochemical variables (real extract and fermentation degree, bitterness, pH, alcohol and polyphenols' content) and 14 sensors of the ET. The feasibility of the ET for the quantification of bitterness in beer was evaluated in the aqueous solutions of isomerised hop extract and in the set of 11 beers with bitterness varying between 14 and 38 EBU (European Bitterness Units). Sensors displayed good sensitivity to isomerised hop extract and good prediction of the bitterness in beer was obtained. Calibration models with respect to the physicochemical parameters using ET measurements in 50 Belgian and Dutch beer samples were calculated by Partial Least Square regression. The ET was capable of predicting such parameters as real extract, alcohol and polyphenol content and bitterness, the latter with Root Mean Square Error of Prediction (RMSEP) of 2.5.

1. Introduction

Beer is one of the oldest known alcoholic beverages produced by the yeast fermentation of the cereal extract that were germinated in water beforehand [1]. Brewing is a complex process demanding the control of many parameters to ensure reproducibility of the quality of the finished product. Determination of some of the routine parameters such as extract and fermentation degree is quite simple as it is based on the measurements of density. On the other hand, measurement of some chemical parameters, i.e., polyphenol content or bitterness requires the use of the analytical instruments and therefore is more difficult and time consuming.

Bitterness in particular is a very important quality parameter in beer production. Bitter taste in beer originates mainly from the hops added to the wort during brewing [1]. Hop contains α-acids, which undergo an isomerisation reaction during boiling resulting in the formations of more soluble and more bitter iso-α-acids [2,3]. Besides imparting bitter taste to beer, iso-α-acids also possess bacteriostatic properties and play substantial role in the enhancing the foam stability of beer due to the tensioactive properties [4–7]. Instrumental measurements of bitterness consist in the determination of the total content of iso-α-acids, which is expressed in the European Bitter Units (EBU). Analysis is carried out by a spectrophotometric measurement at 275 nm of an acidic solvent extract of beer [8]. The EBU method is quite slow due to the cumbersome sample preparation step—extraction, which introduces high uncertainty and involves the use of undesirable organic solvents.

Several efforts have been done in the last three decades aiming at the development of the fast and automated technique for routine bitterness measurements [9–11]. High performance liquid chromatography (HPLC) and liquid chromatography with ultraviolet absorbance detection or with mass spectrometry (LC-UV and LC–MS) can also be used to measure the amount of iso-α-acids in beer [12,13]. However, the EBU method continues to be widely used

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as relatively high cost and long time of analysis, and experienced personnel required prohibit routine use of HPLC in the industrial conditions.

One of the alternatives for traditional techniques may be multisensor systems or electronic tongues (ET), which were developed during last two decades by several research groups [14–16]. The ET typically comprises an array of sensors with partial selectivity or cross-sensitivity to the components of interest, data acquisition device and data processing tools. Sensors based on different measuring principles can be employed in the ET systems including potentiometric, amperometric, piezoelectric and optical. The ETs have been extensively applied in food research to the tasks of recognition and classification, quantification of components and prediction of the sensory properties. Analyzed foodstuffs varied widely and included milk and dairy products, fruit juices, vegetable oils, alcoholic and non-alcoholic beverages, coffee, and tea ([14–16] and references therein). Applications of the ETs to the analysis of beer, which were reported up to date, focused mainly on the discrimination of the samples and prediction of the sensory attributes [17–20]. Recognition of beer according to the brand and main organoleptic characteristics was carried using arrays of all solid-state potentiometric sensors with plasticized polymeric membranes [17–19] and optical sensors [20]. Application of a single quartz crystal microbalance (QCM) sensor with polymeric coating for the prediction of such the sensory attributes as body, smoothness, bitterness and astringency has been reported in [21–23]. Prediction of 20 sensory attributes of beer including bitterness, sweet, sour, fruity, caramel, artificial, burnt, intensity and body was done using an array of potentiometric sensors [24]. As such, feasibility of the ET systems for the analysis of beer was demonstrated, however no applications to the quantitative analysis of beer have been reported in the literature up to date.

Therefore, the objective of the present study was the application of the ET based on potentiometric sensors to the quantitative analysis of beer. This research is a continuation of the previous work published in [24], where the ET system was used for the prediction of beer sensory attributes as scored by the sensory panel. In the present research the same set of 50 Belgian and Dutch beers was analyzed using conventional laboratory methods with respect to the main physicochemical parameters, which were subsequently used for the calibration of the ET. Special attention has been paid to the prediction of the instrumental bitterness—one of the most important beer's parameter. Therefore, a preliminary study was done, which included the evaluation of the sensors sensitivity to isomerised hop extract and testing of a method of bitterness prediction in a small set of beer samples. Next, the prediction of bitterness was done on extended set of beer samples. This research was aimed at the evaluation of the potential of the potentiometric electronic tongue as a tool for fast analysis of several physicochemical parameters including bitterness of beer.

2. Materials and methods

2.1. Samples

Measurements with the ET were carried out on commercial beers and model solutions of the isomerised hop extract. Beer samples were divided into two sets according to the purpose of the study.

The first sample set was used for calibrating the ET with respect to the physicochemical parameters that are routinely measured in beer. This set comprised 50 Belgian and Dutch beers of different types: dark and lager beers, ales, white (wheat), lambic fruit and trappist beers. The description of the first sample set together with ranges of the physicochemical parameters measured by the reference techniques are shown in Table 1.

The second set of samples was used for developing the methodology of measuring beer bitterness using a sensor system. This set comprised 11 beers produced in Denmark and UK, which were chosen to cover a large range of bitterness intensity (14–37.9 EBU). The list of the second set of samples and their corresponding bitterness values and SD are shown in Table 2.

Aqueous solutions of the 30% ethanolic isomerised hop extract were measured using the ET. Concentration range was 0.1–100 of the recommended dose for the lager beer that is 0.005–5% (v/v) or ca. 1–1000 mg/L of the iso-α-acids. Solutions of isomerised hop extract were prepared on the background of a 0.01 M HEPES buffer at pH 6.2 to avoid pH changes during measurements.

2.2. Reference procedures

In total 13 physicochemical parameters were measured: original, apparent and real extracts, apparent and real fermentation degrees, density, energetic value, color, pH, bitterness, alcohol, polyphenols and CO_2 content. All standard beer analyses were performed according to [25]. The color of beer was measured according to method 9.6, at 430 nm in a 10 mm cell after filtration of the beer. The bitterness was determined by the extraction of the bitter substances with iso-octane from acidified beer. After centrifugation, the absorbance of the iso-octane layer was measured at 275 nm, against a reference of pure iso-octane according to method 9.8. The results were expressed as European Bitter Units (EBU). The analysis of the total polyphenol content of the beer was performed spectrophotometrically at 600 nm after treatment with a solution from carboxymethyl cellulose and EDTA, followed by reaction with ferric ions in alkaline solution as described by method 9.11. The extracts, fermentation degrees, energetic value, density and the alcohol content were measured with a DMA 4500 density meter and an Alcolyzer plus (Anton Paar, Graz, Austria) [26]. The pH of beer was measured with a multichannel analyzer (Consort C831) after filtration of the beer. The dissolved CO_2 content in beer was calculated from the pressure and the temperature in the bottle as described by method Beer-13 [27]. Average values of two determinations were used for data analysis.

2.3. ET measurements

The ET comprised 29 potentiometric chemical sensors with plasticized PVC and chalcogenide glass membranes and a pH glass electrode. The potentials of the sensors were measured vs. a conventional Ag/AgCl reference electrode with a precision of 0.1 V. The measurements were carried out using a custom-made high input impedance multichannel voltmeter connected to a PC.

The beer samples were degassed by filtration through a Kieselguhr filter, diluted by distilled water with a ratio of 30–70 ml and thermostated at $27 \pm 1\,^{\circ}\mathrm{C}$ prior to the measurements. Sensors were dipped in the sample and potential values were recorded after 3 min. Between the measurements the sensors were washed with distilled water until stable sensor readings were reached.

Seven replicate measurements were run on each sample of the first sample set and three replicate measurements were run on each sample of the second sample set—resulting into 383 measurements in total. Samples and their replicates were run in random order. At least three replicate calibration measurements were run on the individual solutions of isomerised hop extract.

2.4. Data analysis

Variable selection was performed on both physicochemical and ET datasets prior to the calculations. Three groups of highly correlated (correlation coefficients of 0.9–1) parameters were identified in the physicochemical dataset: original extract, alcohol content

Table 1

Description of Belgian and Dutch beer samples (1st set) and data of physicochemical parameters measured by the reference techniques.

Parameters	Beer ID								
	1–50	3, 17, 18, 38	1, 6–13, 21–23, 25, 29, 30, 39, 42–44, 48	4, 5, 14, 15, 26–28, 31, 36, 40, 41, 47, 50	2, 19, 24, 46, 49	16, 20	32, 37	33	34, 35
	All samples	Amber ale	Blonde ale	Dark ale	Lager	Lambic fruit	Wheat beer	Low alcohol	Trappist
Apparent extract (Plato°)	0.82–7.20	2.39–4.14	0.90–4.15	1.56–4.94	1.82–2.59	4.93–7.20	2.73–2.92	0.82	2.85–2.99
Original extract (Plato°)	3.75–25.31	11.30–19.88	12.53–21.64	14.53–25.31	11.09–12.50	13.52–14.13	11.11–11.51	3.75	15.89–2 0.17
Real extract (Plato°)	1.39–8.72	4.69–7.09	4.11–7.11	4.16–8.72	3.72–4.50	6.60–8.55	4.35–4.57	1.39	5.47–6.15
Apparent fermentation degree (%)	49.01–95.70	72.61–85.01	74.69–95.70	73.22–89.71	77.78–84.41	49.01–63.53	74.67–75.42	78.18	81.16–85.88
Real fermentation degree (%)	41.28–79.53	60.04–70.57	61.88–79.53	61.06–74.16	64.15–69.52	41.28–53.03	61.72–62.26	63.44	67.51–71.81
Alcohol volume (%)	1.48–11.90	4.37–9.07	5.02–11.21	5.98–11.90	4.56–5.29	3.79–4.65	4.44–4.56	1.48	7.03–9.69
Bitterness (EBU)	9.38–36.65	17.30–32.20	13.75–27.15	16.4–31.30	15.85–23.60	9.38–11.68	12.50–20.90	10.00	32.88–36.65
Polyphenols (mg/L)	88.97–860.59	182.00–266.50	118.90–316.00	195.16–351.00	128.00–164.82	637.55–860.59	92.25–158.00	88.97	303.00–320.00
Relative density	1.00–1.03	1.01–1.02	1.00–1.02	1.01–1.02	1.01	1.02–1.03	1.01	1.00	1.01
Energetical value (kJ/100 ml)	54.72–405.41	171.09–310.34	189.15–340.51	221.45–405.41	166.85–189.01	207.00–218.38	167.42–173.75	54.72	243.74–314.83
pH	3.35–4.56	4.20–4.50	3.85–4.50	4.30–4.56	4.17–4.51	3.35–3.59	4.36–4.52	4.38	4.2–4.30
Color (EBC)	5.00–113.55	12.45–40.80	8.58–27.30	40.60–113.55	6.10–8.30	29.63–38.48	5.00–6.58	77.40	12.20–76.20
CO_2 (g/L)	4.78–10.35	5.06–6.46	4.78–10.20	5.65–10.35	5.30–5.68	5.40	5.33–5.85	5.54	8.43–9.03

Table 2
The list of the British and Danish beer samples (2nd set) and their corresponding bitterness values.

	Beer type	Bitterness (EBU)	SD
1	Brown ale	21.5	0.1
2	Pilsner	14	1
3	Pale ale	21.4	0.1
4	Pale ale	29.5	0.8
5	Pilsner	14	1
6	Brown ale	15.9	0.6
7	Pale ale	32	1
8	Porter	37.9	0.3
9	Amber ale	31.5	0.7
10	Pilsner	14	1
11	Pilsner	35	1

and energetic value; apparent and real extract and density; and apparent and real fermentation degrees. Only one parameter from each of these three groups was kept for further analysis, which were alcohol content, real extract and real fermentation degree. Selection was done taking into account relevance of the parameters to the ET measurements. The final physicochemical dataset comprised eight parameters: alcohol, real extract, real fermentation degree, color, pH, bitterness, polyphenols and CO_2 content.

Sensors that were subject to drift were removed from the ET dataset. The standard deviation of the sensor response in the replicate measurements averaged over whole dataset was used as a criterion for sensor removal. The threshold value of standard deviation was 2 mV. The final sensor array comprised 18 sensors.

The data analysis focused on the differentiation of the beer samples according to the physicochemical data, study of the interrelationship between the physicochemical and the ET datasets and calibration of the ET with respect to the physicochemical parameters.

Recognition of beer samples was done using Principal Component Analysis (PCA) [28], which was run on the physicochemical dataset.

Correlations between the ET and physicochemical dataset were studied using Canonical Correlation Analysis (CCA). CCA is a multivariate statistical technique used for studying the interrelationship between two sets of variables [29]. It consists in calculation of a number of independent canonical functions that maximize the correlation between canonical variates. Strength of the overall relationships between the pair of the canonical variates is measured by the canonical correlation, which represents the bivariate correlation between the two canonical variates.

Calibration models with respect to the physicochemical parameters were made using Partial Least Square regression (PLS) [28]. Calibration models were built for each parameter separately and validated using either a test set, which comprised one-third of the data, or a segmented cross-validation. All replicate measurements in the same sample were always included in the same cross-validation segment or either in the test or the calibration set depending on the validation method used.

Replicate measurements with the ET were averaged for CCA while all replicate measurements were used for calculating calibration models using PLS. Both datasets were standardized prior to the calculations.

Unscrambler 9.7 (CAMO ASA, Norway) was used for PCA and PLS and MATLAB 5.3 (Mathworks Inc, USA) for CCA.

3. Results and discussion

3.1. Discrimination of beer samples using physicochemical data

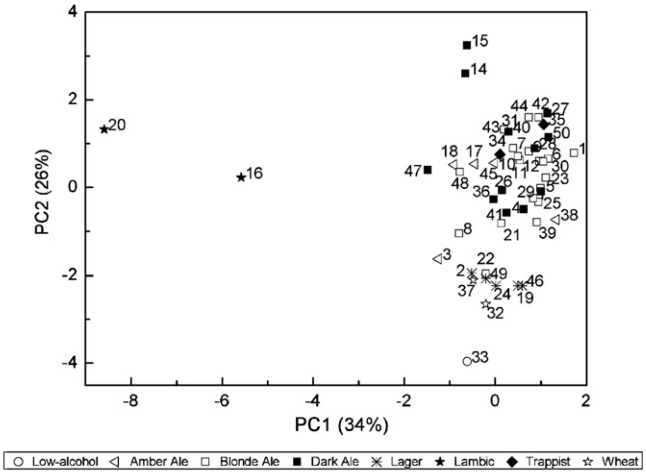

Fig. 1. PCA scores plot of 50 beer samples measured by the reference techniques along the 1st and the 2nd PCs.

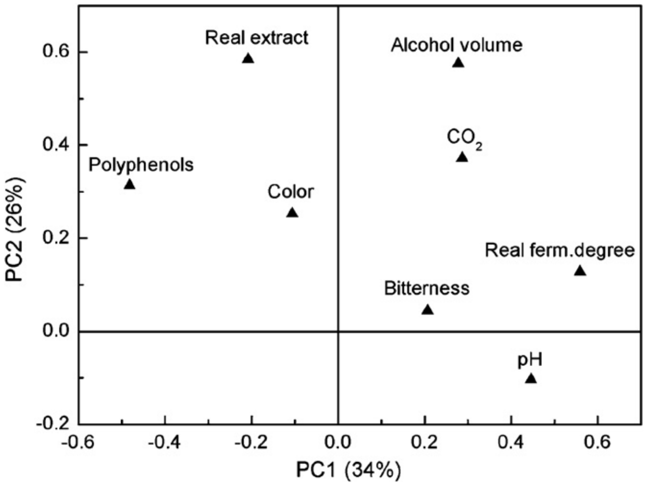

Fig. 2. PCA loadings plot of 8 physicochemical parameters measured by the reference techniques along the 1st and the 2nd PCs.

First, a PCA, an unsupervised statistical technique, was run on the data matrix of physicochemical parameters of the first set of samples (Belgium and Dutch beers). The PCA scores and the correlation loadings plots for the first two principal components are shown in Figs. 1 and 2. Two beer samples (16 and 20) are clearly separated from the rest of the set. These beers are lambic fruit beers that are produced by spontaneous fermentation with addition of fruit. The first and the second principal components account for 34 and 26% of the total variance, respectively. But more principal components have been extracted (Figs. 3 and 4) to complement previous ones, since only 60% of the total variance has been explained. The third and the fourth principal components account for 15 and 11% of the total variance, respectively, representing together with PC1 and PC2 86% in total.

The main contribution to the first component is due to the concentration of polyphenols, pH and the real degree of fermentation. The value of the former parameter is increasing along PC1. Separation along the second principal component is mainly caused by the difference in real extract and alcohol volume. The alcohol volume is increasing along PC2 from the lowest alcohol beer sample 33 (~1.5% alc.v.) to the strongest beer samples available in this set—samples 14 and 15 (~11.9% alc.v.).

PC3 is determined by the color. In general, dark beer samples are located at the right hand side of the PCA score plot, while light beer samples (including lambic fruit) are at the left hand side of the plot.

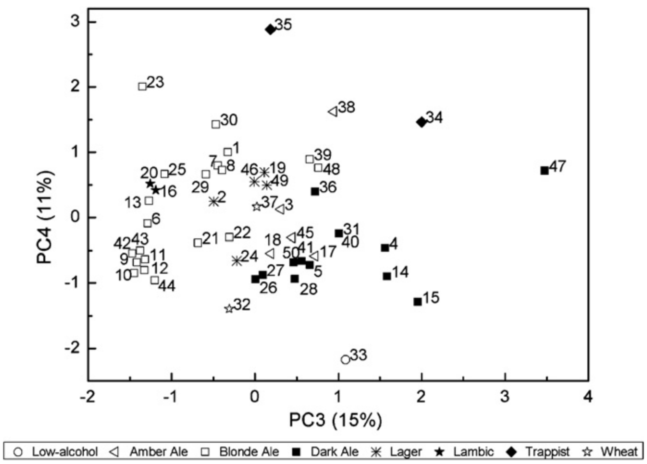

Fig. 3. PCA scores plot of 50 beer samples measured by the reference techniques along the 3rd and the 4th PCs.

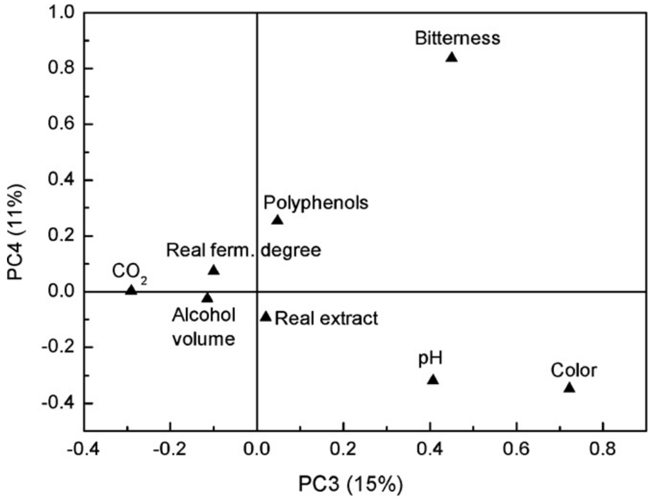

Fig. 4. PCA loadings plot of 8 physicochemical parameters measured by the reference techniques along the 3rd and the 4th PCs.

Bitterness is the most important attribute along PC4. There is a trend along the axis of PC4 from samples 9, 10, 12, 32, 33, 42 and 44, which have the lowest bitterness values, to the samples 34, 35, 38 and 47 at the right top corner of the PCA score plot, which have the highest bitterness values.

3.2. Comparison of physicochemical and the ET datasets

The study of the relationship between physicochemical and ET data has been performed using CCA. Four significant canonical variates have been extracted with correlation 0.98, 0.95, 0.92 and 0.81, respectively. The similarity map determined by the first two canonical variates for the ET and physicochemical data is shown in Fig. 5. Variables with absolute values of canonical coefficients, which were higher than 0.5, were considered important. Six physicochemical parameters (real extract and fermentation degree, bitterness, pH, alcohol and polyphenols' content) and eight sensors can be related to each other. The polyphenol content was highly positively correlated to the response of sensor C13 and negatively to that of the sensors C12 and A12. Negative correlations were found between bitterness and the response of sensor A8, and pH and response of the pH and the C4 sensor. Negative correlations between such parameters as polyphenol content and bitterness and sensors is related to the fact that most of the phenolic and bitter tasting beer components have an anionic nature and the potentiometric response toward them is therefore anionic, i.e., potential values are decreasing with the increase of the concentration. The sensor response to the hydrogen activity is cationic, i.e., the sensor potential increases with the increase of proton concentration. Since pH is a negative logarithm of H^+ activity, the observed correlation between pH value and sensor potentials was negative.

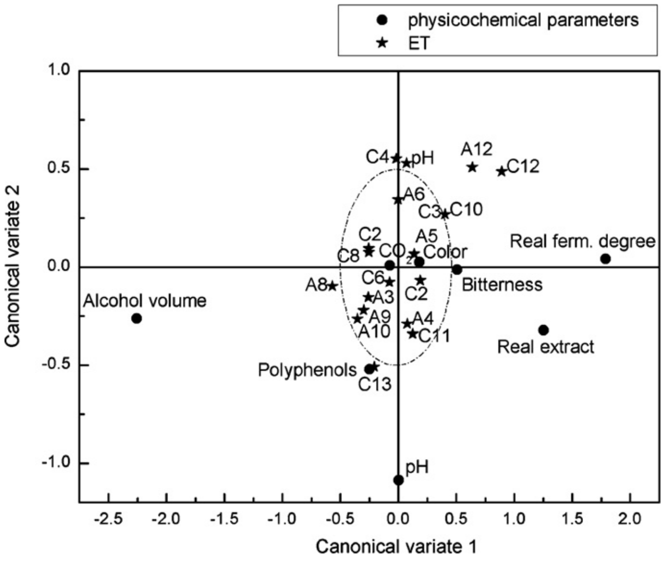

Fig. 5. Canonical correspondence analysis similarity maps for the ET and physicochemical data determined by the 1st and the 2nd CVs.

The similarity map determined by the 3rd and 4th canonical variates (Fig. 6) shows that the same 6 physicochemical parameters and 14 sensors had canonical coefficient with absolute value above 0.5. Color and CO_2 content had canonical coefficients close to 0 according to all four significant canonical variates and therefore there is no correlation between them and the ET data.

3.3. Assessment of ET as a tool for measuring beer bitterness

Capability of the ET to measure bitterness of beer or concentration of the bitter iso-α-acids was first evaluated in the aqueous

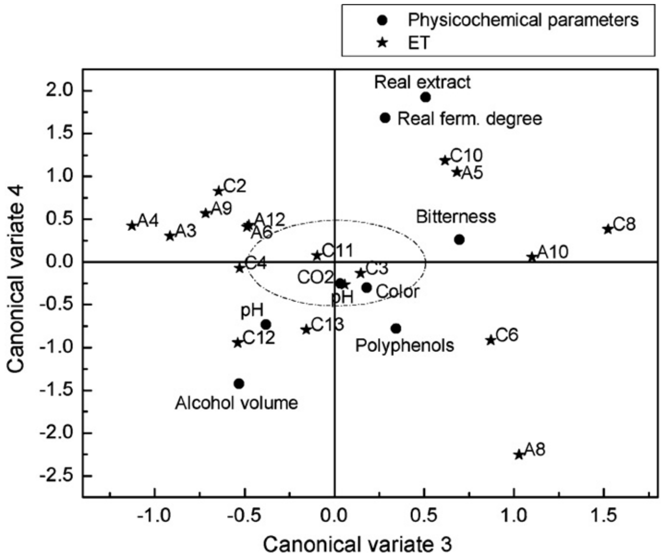

Fig. 6. Canonical correspondence analysis similarity maps for the ET and physicochemical data determined by the 3rd and the 4th CVs.

Table 3
Response parameters of chemical sensors in the individual solutions of isomerised hop extract.

Sensors	Parameters	
	Slope, mV/pX	Standard potential, mV
A2	−84 (1)	153 (1)
A3	−81 (2)	128 (3)
A5	−80 (2)	186 (5)
A8	−90 (1)	147 (4)
A11	−87 (3)	158 (2)
A12	−110 (6)	68 (2)

solutions of isomerised hop extract and in a small set of beers. Furthermore, an attempt to calibrate the ET with respect to the beer bitterness was entertained on a larger sample set.

Sensitivity of the sensor array to the iso-α-acids was studied in the aqueous solutions of isomerised hop extract. Calibration measurements were run in the concentration range 1–1000 mg/L of iso-α-acids. Parameters of the sensor response calculated using the Nernst equation (slope and standard potential) together with their standard deviation are shown in Table 3. Since measurements were made on the background of HEPES buffer, the pH was kept constant and the observed responses were related solely to the change of the iso-α-acids concentration. Observed slopes of the electrode responses were super-Nernstian (higher than 59 mV per decade for one-charged ion). Super-Nernstian potentiometric responses to lipophilic substances such as the iso-α-acids have been observed earlier. This is explained by the non-equilibrium extraction of lipophilic substances from the aqueous to the organic membrane phase. However, if this response is reproducible, as was the case here, it can be used for the analytical purposes. Detection limit of the sensors toward iso-α-acids was estimated to be 1 mg/L.

Calibration model for the prediction of bitterness in British and Danish beer samples was calculated by PLS regression using bitterness values based on spectrophotometric method as a reference. A cross-validation method was used including the replicate measurements in the same samples either in the calibration or the cross-validation set. Results of the prediction of bitterness using the ET for the calibration and the cross-validation datasets are shown in Table 4. A good correlation was observed between ET response and reference methods, but the RMSEP was comparatively high which might be due to the drift of some of the sensors' response of the ET in the presence of lipophilic organic compounds.

3.4. *Prediction of chemical parameters in Belgium and Dutch beer samples*

Calibration models were calculated by PLS1 regression using physicochemical data as a reference. Models were validated based on a test set which comprised one-third of the data (119 samples) and covered the whole range of the reference parameters but did not included samples with maximum and minimum values. This validation method is preferable to estimate the 'true prediction accuracy' of the sensors. Since the electronic tongue consists of potentiometric sensors, the logarithm of the concentration of the iso-α-acids and polyphenols measured by the reference techniques was used in the PLS analysis.

Table 4
Results of bitterness prediction in beer (2nd sample set) using the ET.

Bitterness (EBU)	Slope	Offset	Correlation	RMSEC/RMSECV[a]	MRE[b]
Calibration	0.89	2.60	0.94	2.80	10
Cross-validation	0.78	5.30	0.92	3.50	12

[a] RMSEC and RMSECV stand for Root Mean Square Error of Calibration and Cross-Validation, respectively (expressed in the original units of the variable).
[b] MRE is a Mean Relative Error, i.e., averaged absolute deviation of the predicted values from the measured ones (expressed in percents).

Table 5
Prediction of physicochemical parameters in beer (1st sample set) using the electronic tongue. Validation was done based on a test set of 119 samples.

Parameters	Slope	Offset	Correlation	RMSEC/RMSEP[a]	MRE[b]
Real extract (Plato°)					
Calibration	0.80	1.10	0.90	0.60	10
Validation	0.68	1.80	0.76	0.78	11
Alcohol volume (%)					
Calibration	0.73	1.60	0.85	0.88	15
Validation	0.59	2.50	0.70	1.10	17
Bitterness (EBU)					
Calibration	0.86	2.80	0.93	2.10	9
Validation	0.83	3.30	0.89	2.50	10
Polyphenols (mg/L)					
Calibration	0.74	59.00	0.83	46.00	15
Validation	0.69	68.00	0.81	52.00	18

[a] RMSEC and RMSEP stand for Root Mean Square Error of Calibration and Prediction, respectively (expressed in the original units of the variable).
[b] MRE is a Mean Relative Error, i.e., averaged absolute deviation of the predicted values from the measured ones (expressed in percents).

The ET was capable of predicting four physicochemical parameters: real extracts, alcohol and polyphenol content and bitterness. Parameters of the predicted vs. measured curves together with the error measures (RMSE root mean square and relative mean errors) for the calibration and the test sets are shown in Table 5.

The capability of the ET to measure the content of ethanol [16] and polyphenols [30] was reported earlier. However, in this research the Root Mean Square Error of Prediction in case of the polyphenols is quite high and this is probably related to the fact that the set of the sensors was not sensitive enough to the polyphenols due to low concentration of them in beer. The good correlation of the ET with the real extract may be explained by the sensitivity of the sensors with respect to beer dextrins, amino acids and Maillard reaction products. The lowest relative error (10%) was found for bitterness. Measurements of bitterness using the ET are fast and involve very simple sample preparation (beer degasification) that can be easily automated. Therefore, the ET offers significant advantage over the conventional spectrometric method for measuring bitterness.

4. Conclusions

The potential of the electronic tongue to predict values of several instrumental parameters of beer quality was evaluated in the present study. The electronic tongue with 18 potentiometric chemical sensors developed at St. Petersburg University has been proven to be fast and easily applicable which requires little sample preparation and only a relatively small amount of sample for the analysis. The electronic tongue was shown to be able of predicting physicochemical parameters such as real extract, alcohol and polyphenol content and bitterness, the latter with the accuracy of 10%. The ET was demonstrated to be a promising tool for rapid assessment of some beer parameters including bitterness.

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