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Application of a potentiometric electronic tongue as a classification tool in food analysis

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Abstract

This paper reports on the application of a potentiometric sensor array to the food analysis field, in order to distinguish simple tastes and to classify food samples. This array is formed by a set of non-specific all-solid-state potentiometric sensors and has been used in combination with principal component analysis (PCA) for the classification of food samples in batch and in flow injection mode. First attempt was to classify synthetic samples prepared with controlled variability. Once this ability is proven, satisfactory classification results are presented for commercial waters, orange-based drinks and tea samples. An interesting correlation is achieved between the natural juice content and its first calculated component, which allows for a very simple tool for screening purposes.

Keywords: Potentiometric sensor array; Food analysis; Electronic tongue; Tea; Mineral water; Orange juice

1. Introduction

Recent trends in the analytical chemistry field, take direction opposed to those that invest efforts in the development of quasi-specific instruments. The new approach departs from the use of general-purpose devices, combined with computer processing stages, which could probably grasp looks or composition of a sample and transmit us information about it [1].

These new strategies are based on the use of sensor arrays with a generic cross response to a wide spectrum of analytes; with those, we can obtain different aspects of information about the sample. Afterwards, the use of multivariate calibration tools is used to extract the sought results from the data generated by the devices [2].

This strategy is already known, for analyte detection on aqueous samples, as electronic tongue, or for gases or headspace samples, as electronic nose. The used terminology suggests that this approximation is bioinspired on animal taste or olfaction senses, where a few receptors can respond to a large variety of substances. With taste, only generic information is used, like sweet, bitter, salted or acid; additionally, this approach entails an advanced data treatment mechanism next applied by the brain, in order finally to recognize, quantify or classify between different substances.

An interesting application of this new concept is the use of chemometric tools for multivariate calibration and multiple analyte recognition simultaneously, which is a good strategy to multiparametric determination without interference removal [3–5]. A second interesting application is their use inspired in the human brain to classify the nature of a sample. For this purpose, principal component analysis (PCA) is employed as data treatment. Significant work has been performed in this field, specially, for gas sensors, or electronic noses [6–10].

The use of non-selective potentiometric sensors arrays in combination with PCA starts to be an analytical alternative for sample classification or identification, especially in food analysis [11]. Various contributions on this topic can be found in the literature. Per example, Toko used an electronic tongue to classify and assess the quality of various drinks including wine and water [12]. Wróblewski et al. were able to distinguish between different brands of beverages using a similar approximation [13,14]. Apart, Krantz-Rülcker et al. moni-

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tored a mineral water bottling plant with a voltammetric electronic tongue [15]. Few references are, however, in respect to the use of an e-tongue for quantitative purposes; such references include the determination of various species in water and wine [16], or beer and soft drinks [17,18].

In this work, we used a potentiometric sensor array formed by generic and selective devices presenting response to other interfering species. Each sensor used neutral carriers in a polymeric PVC membrane cast on a solid inner contact, and PCA is employed as the processing tool. First, we set the device to classify manually prepared samples with certain amount of variability. Once the classification ability is proven in controlled experiments, we applied the electronic tongue in food analysis to classify various types of commercial waters, some orange-based drinks and, finally, some teas.

2. Experimental

2.1. Chemicals

The ion-selective poly(vinyl chloride) (PVC) membranes were prepared from high-molecular weight PVC (Fluka), using *bis*(1-butylpentyl)adipate (BPA), dioctylsebacate (DOS), *o*-nitrophenyloctyl ether (*o*NPOE), dioctylphenylphosphate (DOPP), tris-ethylhexyl phosphate (TEHP) and dibutylsebacate (DBS) (all from Fluka) as plasticizers. The ionophores employed to formulate the potentiometric membranes were nonactin (ammonium ionophore I, Fluka), valinomycin (potassium ionophore III, Fluka), 2,3:11,12-didecalino(16-crown-5) (ionophore DD16C5, Dojindo), lasalocid (Fluka), dibenzo(18-crown-6) (Fluka), tridodecylamine (Fluka), 2,9-di-*n*-butyl-1,10-phenantroline (TCI) and bis(bis(4-1,1,3,3-tetramethylbutyl)phenyl)phosphato calcium (II) (BBTP, Fluka). Potassium tetrakis(*p*-clorophenyl)borate (Fluka) was used, when necessary for a correct potentiometric response.

The materials used to prepare the solid electrical contact were the epoxy resin components Araldite M and Hardener HR (both from Ciba–Geigy), and graphite powder (100 μm, BDH) as conductive filler.

Imidazole (Fluka), Tris base, and the salts NH_4Cl, KCl, NaCl, LiCl and $CaCl_2$ (reagent-grade, Merck), were used as background electrolytes and calibration species, respectively. All solutions were made in deionised, highly purified water (16–18 MΩ cm resistivity, Milli Q, Millipore).

2.2. Sensor array

The sensors were all-solid-state ion selective electrodes (ISEs) with a solid electrical contact made from conductive composite. They were constructed by following standard procedures developed from our laboratory [4,19]. Each device was constructed from a PVC cylinder of 100 mm length and 6 mm internal diameter. Polymeric PVC membranes were formed by solvent casting of a mixture further diluted with tetrahydrofuran (1 ml per 20 mg PVC) on a 1:1 epoxy–graphite composite solid contact. Membranes were obtaining by eight cumulative-additions followed by evaporation of 50 μL drops of each cocktail membrane. Once formed, membranes were conditioned in a 0.1 M solution of their primary ion for 24 h. Arbitrarily, for the generic electrodes, we used ammonium ion for its conditioning. Each device was constructed by triplicate and was previously evaluated to check if presented correct response characteristics. The specific formulation of the different membranes is detailed in Table 1.

2.3. Apparatus

Potentiometric measurements were performed with a laboratory-made data acquisition system consisting of 32 input channels made with differential instrumentation amplifiers (INA116, Burr-Brown, USA) that adapted the impedance for each sensor. Measurements were referred to an Ag/AgCl reference electrode (Thermo Orion 90-02-00). Each channel was noise-shielded with its signal guard. The output of each amplified channel was filtered with a second order low pass active filter centered at a 2 Hz frequency and connected to an Advantech PC-Lab 813 A/D conversion card installed in a PC. Readings were acquired by using custom software developed by the authors in Microsoft QuickBasic Version 4.5.

Potentiometric measurements were made by using solutions containing 0.010 M imidazole buffer at pH 6.50 and Tris at pH 7.5, 8.0 and 8.5 as background media depending on each application.

Table 1
Compositions of the used potentiometric sensors, percentages are by weight, unless specified

Electrode	Ionophore	PVC (%)	Plasticizer
H^+(1)	Tridodecylamine 1% (10% molar potassium tetrakis(*p*-clorophenyl)borate (KT*p*ClPB))	33	DOS 66%
Li^+	2,9-Di-*n*-butyl-1,10-phenantroline 1.4% (66% molar KT*p*ClPB)	27.8	*o*NPOE 69.8%
NH_4^+	Nonactin 1%	33	BPA 66%
K^+	Valinomycin 3%	30	DOS 67%
Na^+	DD16C5 3% (10% molar KT*p*ClPB)	29.1	TEHP 67.9%
Ca^{2+}	BBTP 4.3%	30.3	DOPP 65.4%
GENERIC 1	Dibenzo(18-crown-6) 4%	29	DOS 67%
GENERIC 2	Lasalocid 3%	27	DBS 70%
H^+(2)	Tridodecylamine 1% (10% molar KT*p*ClPB)	33	DBS 66%

2.4. Data processing

The processing of multicomponent data generated by non-specific sensors has been performed using principal component analysis (PCA). The aim is to reduce the amount of variables to new latent variables (principal components) in a reduced variable space to facilitate identification or classification; additionally, this new space simplifies the way to explain the variability contained in the available information. Here, the difficulty is to find an explanation to these principal components according to the sample composition [20]. The PCA analysis and evaluation process was performed with the software SPSS 11.

3. Results and discussion

Classification experiments were first performed with standards in water, then with commercial mineral waters, orange beverages and finally with teas and related preparations.

3.1. Synthetic samples

Thirty-eight different samples were manually prepared in order to generate a model with some controlled variability. Three different pH levels were obtained with Tris 0.01 M buffer at pH 7.5, 8.0 or 8.5; ammonium, potassium and calcium ion mixtures were prepared adding 1, 2 or 3 ions simultaneously at low (0.1 mM), medium (1 mM) or high (10 mM) level concentration preventing that only one ion would be at high-level concentration at the same time in order to facilitate further sample grouping. In addition, some samples were duplicated, adding low and medium levels of sodium an lithium ions introducing some new variability; finally, a sample was prepared with high ammonium and potassium ions simultaneously. Sample measurements were made in random order and by direct potentiometry.

In order to improve the classification ability of the approximation, a PCA was applied to the manually designed data set, expecting to reduce the system into a few new variables. All nine potentiometric sensor data measurements were used as inputs and were automatically autoscaled by SPSS software. The performed PCA explained with its first three components (PC1-PC3) quite the whole variability of the system. The highest variability was explained with PC1 (45%) (principal component 1), PC2 and PC3 explained 27 and 23%, respectively. The total amount of represented variability with these three features accounted for 95%.

When the scores plot PC1 versus PC2 or PC1 versus PC3 were represented, no direct grouping of samples was observed; the clearest view could be observed with the PC2 versus PC3 plot, as indicated in Fig. 1. This figure sketches how PC2 classified the samples according to pH value, meanwhile PC3 direction discriminated between high-level calcium ion and high-level of ammonium ion.

Furthermore, it is important to reconsider PC1 effect because of its significance. For this reason, its loadings can be revised, as detailed in Table 2. Here, the biggest contributions, i.e. the biggest classification effects, have been highlighted. From these values, it is easy to deduct the role of PC1 to explain the global amount of alkaline ions.

Finally, it should be summarized that the system classifies the initial groups of samples, as it was expected, by the alkaline ions effect (group II), the pH level (groups I, II and III) and finally between high levels of calcium (group IV) or ammonium (group V). A cleaver grouping was not obtained because of the large variability used, as too many ions var-

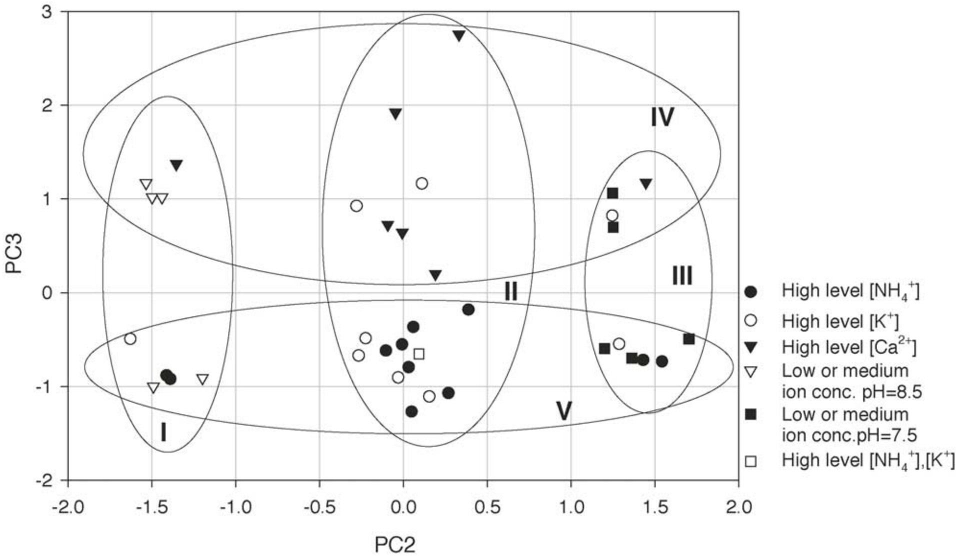

Fig. 1. PC2 vs. PC3 scores plot of the PCA from the manually prepared samples with controlled variability. Groupings are: (I) samples at pH 8.5, (II) samples at pH 8.0, (III) samples at pH 7.5, (IV) samples with high-level calcium concentration and (V) samples with high-level ammonium concentration.

Table 2
Loadings from the PCA analysis from the manually prepared, controlled variability samples, highlighting the biggest classification effects

	PC1	PC2	PC3
Li^+	0.03346	**0.988034**	0.05546
H^+(1)	−0.02749	**0.996193**	0.05134
Na^+	**0.673687**	0.139783	0.085112
Ca^{2+}	−0.06691	0.040746	**0.982199**
$NH_4{}^+$	**0.728791**	0.228485	**0.614573**
K^+	**0.901987**	−0.10284	0.092178
GENERIC 1	**0.917396**	−0.12597	−0.05115
GENERIC 2	**0.818043**	−0.04314	−0.15788
H^+(2)	−0.03406	**0.995738**	0.053677

ied independently. The 3D plot using the three mentioned components did not bring cleaver results either.

3.2. Commercial water samples

In order to classify between different mineral waters, a principal component data reduction analysis was performed with all data from the nine potentiometric sensors, this time corrected with its background solution measurement. This background solution was an imidazole buffer 0.01 M at pH 6.5, and its purpose was to compensate any drift. Twenty-three different commercial water samples were analysed. Samples selected comprised natural mineral water, natural sparkling mineral water, added CO_2 mineral water, lemonade, an added gas mineral water, and also one tap water sample. All potential readings were made in triplicate.

Each measurement cycle comprised cleaning of electrodes by 5 min stirring in deionised water, measurement of the background solution, 5 min stirring in deionised water and, finally, sample measurement. The initial set of data was formed with the corrected values, each sample measurement corrected with its background solution value. After automatic autoscaling done by the software used, a first PCA analysis was performed, where it resulted that the system variability could be explained with the first two principal components. After constructing the score plot of PC1 (49%) versus PC2 (41%) (Fig. 2), the sample grouping is clearly seen. As it can be distinguished, PC1 separates in function of acidity while PC2 separates depending of total amount of alkaline ions. Studying the loadings, we can add to this conclusion that PC1 also separates between monovalent and divalent ions. PC2 separates between high K^+ and Na^+ amount, normally at high level in natural sparkling mineral water, ions acquired in the natural process of carbonation. The loadings are here detailed as a function of each original variables in the new space:

$$\begin{aligned}\mathrm{PC1} = {} & 0.893E_{\mathrm{NH_4}^+} + 0.817E_{\mathrm{Na}^+} + 0.818E_{\mathrm{K}^+} \\ & + 0.871E_{\mathrm{Li}^+} + 0.869E_{\mathrm{H}^+} + 0.813E_{\mathrm{H}^+(2)} \\ & - 0.604E_{\mathrm{Ca}^{2+}} + 0.524E_{\mathrm{GENERIC\,1}} \\ & + 0.796E_{\mathrm{GENERIC\,2}}\end{aligned}$$

$$\begin{aligned}\mathrm{PC2} = {} & -0.336E_{\mathrm{NH_4}^+} + 0.501E_{\mathrm{Na}^+} + 0.558E_{\mathrm{K}^+} \\ & -0.491E_{\mathrm{Li}^+} - 0.449E_{\mathrm{H}^+} - 0.497E_{\mathrm{H}^+(2)} \\ & + 0.385E_{\mathrm{Ca}^{2+}} + 0.806E_{\mathrm{GENERIC\,1}} \\ & +0.598E_{\mathrm{GENERIC\,2}}\end{aligned}$$

As a summary, the system classified satisfactorily the initial groups of samples, separating between natural mineral water (I), sparkling mineral water (II), added CO_2 mineral water (III), and finally the more differentiated lemonade (IV).

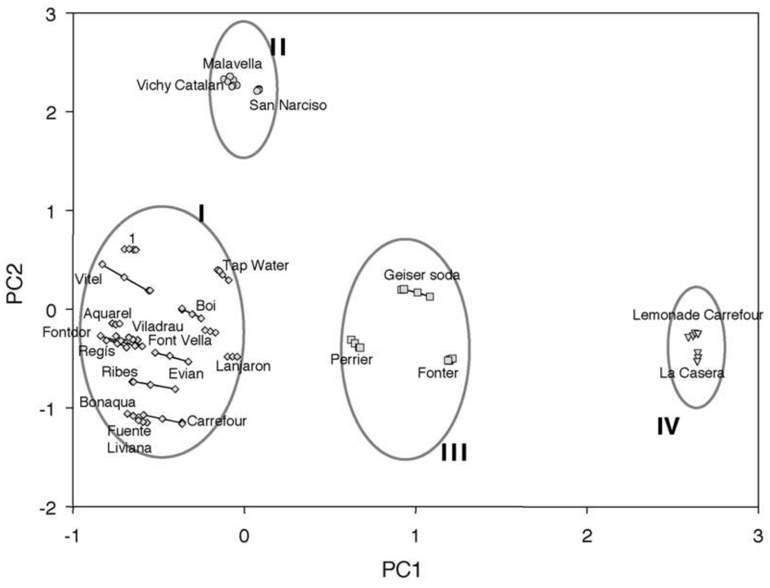

Fig. 2. PC1 vs. PC2 scores plot of the PCA performed to data from mineral waters. Groupings are: (I) mineral waters, (II) sparkling mineral waters, (III) added CO_2 mineral waters and (IV) lemonades.

3.3. Orange-based drinks

Thirty-six orange-based drinks composed by natural orange juices, sugar plus water added orange juices, orange refreshments and some orange flavour drinks were analysed using the proposed approach. As before, all potential, readings were made by triplicate and made relative to an imidazole buffer 0.01 M at pH 6.5 background solution in order to compensate any drift. A cyclic measurement scheme was again selected, comprising cleaning of electrodes by 5 min stirring in deionised water, measurement of background solution, 5 min stirring in deionised water and, finally, sample measurement.

Potentiometric data measurements of the nine devices were introduced as input information to the principal component data reduction analysis, but this time, a good classification or grouping was not obtained directly. With the aim of improving these results, an additional preprocessing was done. Thus, the autocorrelation matrix of input data was computed, where we eliminated the data of correlated sensors. The sensor array was then reduced to six elements, after removing the two hydrogen ion plus the lithium ion sensors. The PCA analysis was performed again, where obtained results indicated that the whole system variability could be explained with a single component that accounted for 75% variability. Representing the score plot of PC1 versus sample number (Fig. 3), unexpectedly, a sample distribution was observed, easily assigned to an ordering depending of natural orange juice content in the sample, as stated by its label.

Analysing the loadings of original variables in this new component we obtained:

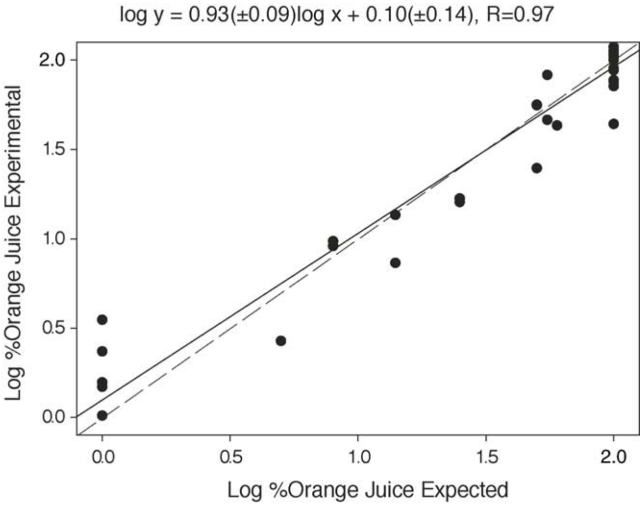

Fig. 4. Adjusted regression curve from log %orange juice expected vs. log %orange juice content. Regression curve is presented with the dashed line.

$$\mathrm{PC1} = -0.746E_{\mathrm{NH_4}^+} - 0.648E_{\mathrm{Na}^+} + 0.996E_{\mathrm{K}^+} + 0.680E_{\mathrm{Ca}^{2+}} + 0.946E_{\mathrm{GENERIC\,1}} + 0.976E_{\mathrm{GENERIC\,2}}$$

This could explain the classification ability depending of other parameters not contemplated in the potentiometric ISE array that would be modelled by generic devices.

To confirm the prediction of natural orange juice content, a new representation of the former data is shown in Fig. 4, where the predicted log %orange juice, calculated from the actual PC1 value, is plotted versus log %orange juice, as

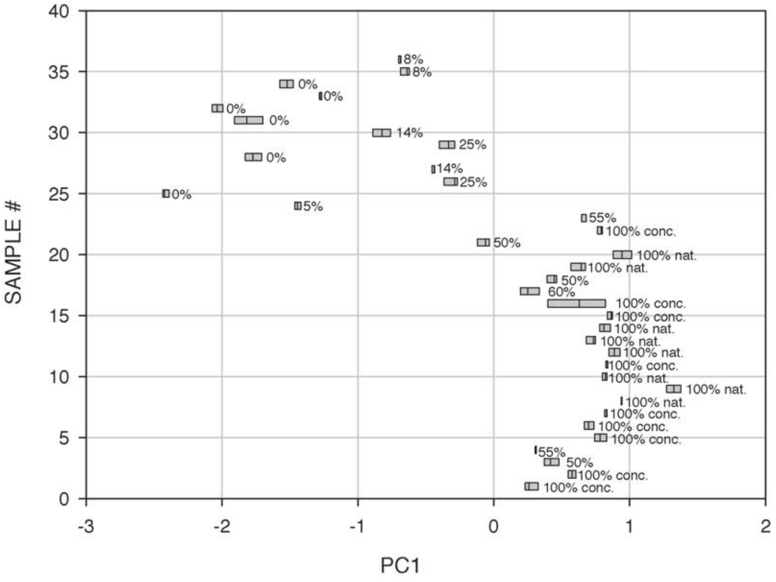

Fig. 3. PC1 vs. sample number scores box-plot of the PCA performed to data from orange-based drinks. Medium line in each box represents median value. Upper and lower quartiles are in box extremes.

stated on the label. Here we can see that the system predicts satisfactorily the nature of the sample, as we are able to predict the natural juice content in the studied samples, appearing as an interesting tool, p. ex. for screening purposes in the food industry. The utility of the fit is even more patent if we consider that no actual analysis was performed, nor is easily available. Successful classification of different orange juice brands has been described in a recent work [13], which employed a combination of selective sensors plus cross-sensitive ones fabricated with membranes containing mixtures of ionophores.

3.4. Tea samples

Thirty commercial tea samples were prepared by immersing 10 min each tea bag in initially boiling imidazole buffer 0.01 M at pH 6.5. In this case, potential readings were performed in a low-dispersion single channel flow injection system, as alternative for the automation of the cyclic referencing scheme used in Sections 3.2 and 3.3. The system was formed by an injection system constructed from solenoid valves (Biochem), a Gilson Minipuls peristaltic pump as the propulsion system (2.5 ml min^{-1}) and a homemade flow cell adapting the eight potentiometric sensors in series (one of each kind, except the hydrogen ion sensor $H^+(2)$). The sandwich flow cell, made from a perspex body and brass holders, permitted the use of the previously used batch sensors, fixed with appropriate o-rings and nuts. The inner volume of the flow cell was 800 μl. The carrier solution used was an imidazole buffer 0.01 M at pH 6.5, and the readings were the peak heights recorded for each sample relative to the background solution.

Potentiometric readings obtained with the flow system were used to perform a PCA analysis. Again, no initial grouping was observed at first attempt. For this reason, autocorrelated channels were identified and eliminated, in this case the two generic sensors plus the calcium sensor. Alternative procedures for the reduction of number of channels employing statistical criteria have been proposed recently [14]. In this way, from the data of the remaining five inputs, a much clearer transformation is obtained, and results obtained in the scores plot PC1 (48%) versus PC2 (45%) present a clear grouping of beverages, as it can be appreciated in Fig. 5. First proposed class included tea and lime blossom tea, *Tilia europaea* (group I) and the second class included camomile, *Matricaria chamomilla*, and pennyroyal tea, *Mentha pulegium* (group II). Additionally, the sample containing only dogrose infusion, *Rosa dumetorum*, was an outlier, dissimilar to the rest, probably due to the different nature and properties. So, a gross classification of this beverages was accomplished, although with the array of used sensors it was not possible to distinguish the different tea varieties.

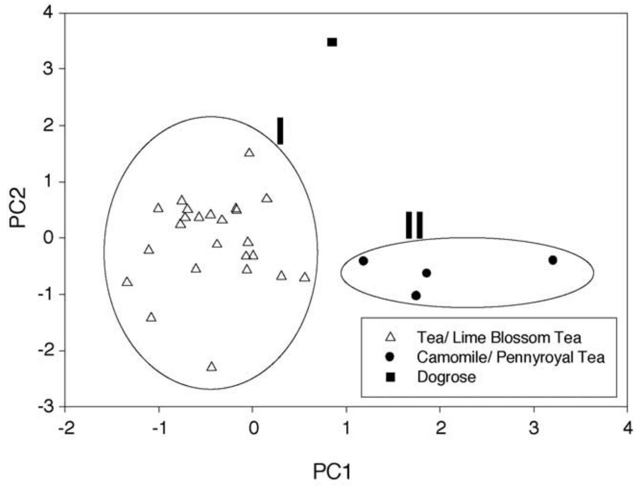

Fig. 5. PC1 vs. PC2 scores plot of the PCA performed to tea samples. Groupings are: tea and lime blossom tea (I), camomile and pennyroyal tea (II) and dogrose infusion alone.

Studying the loadings of original variables in the new components space, the values obtained were:

$$\begin{aligned}\text{PC1} = {} & 0.919E_{\text{NH}_4{}^+} + 0.836E_{\text{Na}^+} + 0.627E_{\text{K}^+} \\ & + 0.611E_{\text{Li}^+} + 0.297E_{\text{H}^+}\end{aligned}$$

$$\begin{aligned}\text{PC2} = {} & 0.313E_{\text{NH}_4{}^+} + 0.504E_{\text{Na}^+} + 0.723E_{\text{K}^+} \\ & +0.740E_{\text{Li}^+} + 0.918E_{\text{H}^+}\end{aligned}$$

From these, we can recognize that PC1 represents total ion amount, obtaining in group I a higher level of ions in this stronger beverages class, whereas in group II, a lower level for this softer infusions is observed. Dogrose, *Rosa dumetorum*, is a more acidic beverage, and is discriminated basically by PC2, where the acidity of the sample becomes obvious.

4. Conclusions

The presented approach, either in batch or in flow mode, has been able to classify satisfactorily different types of samples yielding good models. A first demonstration was made with manually prepared samples. Once this was performed, we built classification models for mineral waters, orange-based drinks, teas and related beverages. Easily, potential applications of this approximation in the food analysis field may be designed, applying this tool as a taste sensor, which can be redesigned depending of the desired application. The new concept, which employs selective plus non-specific devices with cross-sensitivity to a wide spectrum of analytes together with multicomponent analysis offers new possibilities in analytical chemistry. In this way, global methods of analysis can be devised that are non-specific to a sample, non-specific to a certain analyte, but rather they entail measurement toolboxes that can take a photograph of the global sample nature or composition and therefore give a global information, after a proper training with the cases under study. Improvement of presented results can be sought enlarging the sensor array to other analytes, i.e. anions, plus providing more elaborated tools for feature extraction.

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