An electronic tongue taste evaluation: Identification of goat milk adulteration with bovine milk

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A B S T R A C T

An electronic tongue with 36 cross-sensibility sensors was built allowing a successful recognition of the five basic taste standards, showing high sensibility to acid, salty and umami taste substances and lower performance to bitter and sweet tastes. The taste recognition capability was afterwards tested in the detection of goat milk adulteration with bovine milk, which is a problem for the dairy industry. This new methodology is an alternative to the classical analytical methods used to detect caprine milk adulterations with bovine milk, being a simpler, faster and economical procedure. The different signal profiles recorded by the e-tongue device together with linear discriminant analysis allowed the implementation of a model that could distinguish between raw skim milk groups (goat, cow and goat/cow) with an overall sensibility and specificity of 97% and 93%, respectively. Furthermore, cross-validation showed that the model was able to correct classify unknown milk samples with a sensibility and specificity of 87% and 70%, respectively. Additionally, the model robustness was confirmed since it correctly or incorrectly classified milk samples with, respectively, higher and lower probabilities than those that could be expected by chance.

Keywords:
Electronic tongue
Taste standards
Milk adulteration
Cow milk
Goat milk
Principal components analysis
Linear discriminant analysis

1. Introduction

Electronic tongues are sensor arrays for liquid analysis using both several non-specific, low-selective, chemical sensors with high stability and cross-sensitivity and ion-selective sensors [1]. The main purpose of electronic tongues is qualitative 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 [1]. An electronic tongue (e-tongue) device is generally tested by evaluating its recognition capability to the basic standard tastes: sweet, acid, bitter, salty and umami [2]. Classification models can be constructed from signal processing procedures using non-supervised techniques like principal components analysis (PCA) or supervised techniques such as linear discriminant analysis (LDA) and artificial neural network [1,2]. When compared with other analytical methodologies, this kind of devices also present interesting practical properties such as lower calibration costs, satisfactory accuracy for reasonable small sizes of the calibration data set and easy adaptability to different working conditions [3].

In the last decade, potentiometric sensor arrays have been widely used in food analysis namely, for milk recognition and classification [2,4–6], wine recognition and quantitative analysis and its correlation with human sensory perception [7], beer recognition [2,8], plant samples recognition and classification [9], beverage analysis [2,10–12], soy sauce taste analysis [13] and honey classification according to the pollen type [14]. In these works, several types of sensors have been tested in the potentiometric devices, namely lipid membranes [2,13], chalcogenide sensors [15], cation and anion-sensitive PVC based membranes [5,11], cation and anion-sensitive and partially selective electrodes [4,6,8,12,16], and polymeric membranes formed on solid conducting silver supports [14].

Still, a small number of works on the application of sensor array devices for milk analysis are available in the literature, especially concerning e-tongue devices, although it is possible to find several works on electronic noses [3,17–20]. Winquist and co-workers [21] used an e-tongue for the determination of bacterial counts in

Table 1
Membrane additives and plasticizers used in the polymeric membranes preparation.

Additive substance	Membrane additive identification	Plasticizer substance	Plasticizer identification
Octadecylamine	1	Bis(2-ethylhexyl)phthalate	A
Bis(2-ethylhexyl)phosphate	2	Bis(1-butylpentyl) adipate	B
Oleyl alcohol	3	Tris(2-ethylhexyl)phosphate	C
Methyltrioctylammonium chloride	4	Dibutyl sebacate	D
Tridodecylmethylammonium chloride	5	2-Nitrophenyl-octylether	E
Oleic acid	6	Dioctyl phenylphosphonate	F

fresh milk during storage. Collier and co-workers [19] also used a screen-printed electrochemical array to discriminate among four milk samples, among four yoghurt samples, and among four cultured and non-cultured dairy products, but constrained by the execution of all the measurements in a single experiment. Mabrook and co-workers [22] have proposed a new method for detecting added water to dairy products, based on ac electrical admittance measurements. Ciosek and co-workers [4,6] have developed and applied an e-tongue for milk classification according to the milk's fat content and brand, without sample pre-treatment, being able to correctly classify (predict) 97% of the milk samples.

The aims of this work were: (i) to construct an all-solid-state potentiometric e-tongue with two units of 20 polymeric membranes each (18 different and 2 replicates of selected membranes), working in parallel, in which the polymeric mixtures were applied on solid conducting silver-epoxy supports, (ii) to evaluate, by means of principal components analysis, its ability to differentiate the five basic taste standards (sweet, acid, salty, bitter and umami tastes), and finally, (iii) as a practical application for the dairy industry, to use the taste recognition ability of the e-tongue to detect raw goat milk adulterations with raw cow milk by means of linear discriminant analysis. This statistical approach intended not only to classify raw skim milks according to animal provenience (goat, cow and respective mixtures), but also to compare the model classifications with those that could be expected by chance, in order to verify if the proposed potentiometric methodology could be used reliably by cheese producers for the detection of goat milk adulterations with cow milk. This technique was used for the classification of vinegars based on their polyalcohols content [23], the varietal differentiation of red wines in the Spanish Valencian region [24], the determination of geographic origin of potatoes using their content in mineral and trace elements [25], to discriminate between table olives according to their mineral nutrient composition [26], to discriminate between bovine breeds and production sub-system based on the raw meat fatty acids profile [27], and to differentiate honey samples according to their pollen content [14].

To the best knowledge of the authors it is the first time that an e-tongue device is applied to detect the adulteration of raw caprine skim milk with raw bovine skim milk. In fact, the adulteration of goat milk with bovine is quite frequent, due to the seasonal fluctuations of the production of goat milk and to the higher price of this compared with bovine; the replacement is also a chance for the milk producers to get rid of their overproduction of bovine milk without loss of profit. Therefore, it is important to establish and validate easy and reliable methodologies that can be used to detect this kind of adulterations. In recent years, several analytical methods (urea-polyacrylamide gel electrophoretic techniques, isoelectric focusing, high-performance liquid chromatography, immunochromatography, immunological methods, capillary electrophoresis) [28–33] have been reported for the detection and/or quantification of milk and cheese adulterations, and in some cases even to determine the regional provenance of dairy products unambiguously (polymerase chain reaction, near infrared, mid infrared, front face fluorescence spectroscopy, stable isotope and nuclear magnetic resonance-coupled with chemometric tools) [34–38]. Although these methods are quite precise and commonly used, they are very time-consuming and expensive, requiring complex pre-treatment of the samples, specialized equipment and qualified personal. Therefore, the development of a potentiometric multisensor system (e-tongue) that could be used in the dairy industry by cheese makers, to evaluate in a real time basis the possible adulterations of their "raw materials" is of major importance.

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade and used as purchased. The membrane components were from Fluka: poly(vinylchloride) high molecular weight (PVC), octadecylamine, bis(2-ethylhexyl)phosphate, oleyl alcohol, methyltrioctylammonium chloride, tridodecylmethylammonium chloride, oleic acid, 2-nitrophenyl-octylether, dioctyl phenylphosphonate, bis(2-ethylhexyl)phthalate, dibutyl sebacate, bis(1-butylpentyl) adipate, tris(2-ethylhexyl)phosphate and tetrahydrofurane. Deionised water was used for all sample dilutions and standard solutions preparation.

Six commercial buffer standard solutions (pH at 25 °C equal to 2.00, 3.00, 4.01, 6.98, 8.96 and 9.94, from Panreac and Fixanal) were used to test the polymeric membrane behaviour with pH variation.

The reagents used in the preparation of the basic taste solutions to assess the multi-sensor system performance for taste distinction and polymeric membranes sensibility were: as sweet flavors, fructose (Panreac), glucose (Fluka) and sucrose (Panreac); as acid flavors, ascorbic acid (Panreac), citric acid (Fisher Scientific) and HCl (Riedel-de Haën); as bitter taste, caffeine (Panreac), urea (usb) and $MgSO_4$ (Panreac); as salty flavor, NaCl (Panreac), KCl (Panreac) and NH_4Cl (Riedel-de Haën). The umami taste, the fifth basic taste, was tested using the reagent monosodium glutamate, MSG (Fluka) [2,39]. The concentrations of the solutions ranged from 1×10^{-5} to 1×10^{-1} mol/L.

2.2. Membrane preparation

The polymeric membranes were prepared using poly(vinylchloride) as the polymeric matrix, with membrane additives and plasticizers as indicated in Table 1, and tetrahydrofurane, as solvent for the solid membrane mixture dissolution. Membranes were prepared using approximately 31.9–32.3% of PVC, 64.7–65.2% of the plasticizer compound and 2.8–3.2% of the sensor compound. As Table 1 shows, six plasticizer compounds and six sensor compounds were tested, giving 36 different sensor membrane mixtures. The sensor compounds were used and tested by Toko [2] for beverage analysis with an e-tongue device. However, in the present work, to improve the polymeric membrane taste sensibility, six plasticizers were used and incorporated in the polymeric membranes and its response tested against different taste standard solutions.

2.3. Samples

Raw bovine and caprine milks (from Friesian and Portuguese Serrana breeds, respectively) were obtained directly from the producers. Nine milk samples of each animal breed, obtained from different animals, were collected during a 3-week period.

2.4. Sample preparation

After sample reception, bovine/caprine solutions were prepared by mixing different levels (%, v/v) of each milk type. The milk mixtures as well as the whole bovine and caprine milks were transferred into Falkon tubes and stored in the refrigerator, during 1 h, to achieve 5 °C. All milk samples were centrifuged at 2000 × *g*, at 6 °C, during 30 min, and stored at −20 °C until use, without further treatment. Before the experimental assays, the frozen fat content at the top of each Falkon tube was removed by cutting the respective tube section. Before analysis, the milk samples were allowed to reach ambient temperature and, afterwards, analysed for training and evaluating the multi-sensor device capacity to identify the adulteration of caprine milk with bovine milk. The milk mixture levels were in the range of 1–99%. For this purpose, mixed milk solutions were prepared using five different milk samples of each animal breed. Measurements with the sensor array device were always carried out in diluted solutions samples (4 mL of each skim milk sample were diluted to 100 mL with deionised water). These diluted solutions of skim milk samples were prepared and analysed, most of then twice, giving a total of 142 milk analyses (19, 16 and 107 samples for goat, cow and goat/cow skimmed milks, respectively).

2.5. Multi-sensor system

Two cylindrical potentiometric sensor arrays were built on acrylic bodies (diameter of 1.5 cm and length of 6 cm), with 20 holes (3 mm of diameter) filled with conducting silver-epoxy resin (EPO-TEK E4110) connected to copper electric wires. Membranes were formed by deposition of the membrane solution on the silver conducting surface, drop by drop, in each one of the 20 holes of the acrylic body, as described in a previous work [14]. In the first sensor array, membranes were prepared with membrane additives 1–6 and with plasticizers A, B and C. In the second array, membranes with membrane additives 1–6 and with plasticizers D, E and F were used. In the unused two holes of each array, two polymeric membranes that showed a non-crystalline visual aspect of the membrane surface were duplicated: membranes A2 and A3 for the first system and membranes D4 and E4 for the second one. In the data treatment, only one of the repeated polymeric membranes, the one with best results in sensibility, was considered.

2.6. Measurements

The multi-sensor system includes the two sensor array sets, together with a reference electrode Ag/AgCl with double junction (3 mol/L KCl as external solution), and was connected to a multiplexer Agilent Data Acquisition/Switch Unit model 34970A. Sensor signals were acquired using the Agilent BenchLink Data Logger software installed in a PC computer. The electric potential signals of the 40 sensors were imported to an Excel spreadsheet and then analysed using a multivariate statistical software.

All measurements were performed in a double wall glass cell thermostatized at 25 °C, using a Tectron Bio thermostatic bath from Selecta. Each solution was analysed during a 7–10 min period.

The sensor sensibility evaluation was carried out using basic taste solutions with concentrations ranging from 1×10^{-5} to 1×10^{-1} mol/L, according to the known detection levels of several taste substances [39]. For this purpose, the e-tongue was immersed in the glass cell containing 50 mL of a 1×10^{-5} mol/L solution and, after signal stabilization, small volumes of the more concentrated solutions (1×10^{-3} and 1×10^{-1} mol/L) were added for concentration increase. Finally, the solution with concentration of 1×10^{-1} mol/L was measured.

To assess the effectiveness of the multi-sensor device to distinguish the basic standard tastes, solutions of 1×10^{-3} mol/L were prepared for each standard taste compounds and measured.

2.7. Statistical analysis

Principal components analysis was applied for reducing the number of variables (36 sensors) to a smaller number of new derived variables (principal components or factors) that adequately summarize the original information, i.e., the five basic standard tastes. Moreover, it allowed recognising patterns in the data by plotting them in a multidimensional space, using the new derived variables as dimensions (factor scores). The aim of the PCA is to produce components suitable to be used as predictors or response variables in subsequent analysis. The number of factors to keep in data treatment was evaluated by the Scree plot and also by the total percentage of variance explained by the number of components selected [40].

Linear discriminant analysis was performed to obtain classification rules for differentiation between raw milk goat and cow samples and goat/cow mixtures. It provides a classification model, characterized by a linear dependence of the classification scores with respect to the descriptors (groups defined previously), which maximize the ratio between-class variance and minimize the ratio of within-class variance. LDA assumes an a priori knowledge of the group membership of each sample in a training set. In LDA, groups are supposed to follow a multivariate normal distribution and to be linearly independent [26,40,41]. The classification power of the model derived can be evaluated using the original grouped cases or using a "leaving one-out" cross-validation procedure. In the former procedure, the same samples are employed for the deduction of the linear functions and to test their ability, which can give overoptimistic results. In the latter procedure, the sample data minus one observation are used for the estimation of the discriminant functions, and then the omitted variable is classified from them; the procedure was repeated for all observations and so each sample was classified by discriminant functions which were estimated without its contribution [26,40]. Moreover, for both procedures, the sensibility and specificity of the discriminant model were computed based on the number of individuals correctly predicted as belonging to an assigned group. Sensibility was calculated by dividing the number of samples of a specific group correctly classified by the total number of samples belonging to that specific group. Specificity was calculated by dividing the number of samples of a specific group classified as belonging to that group by the total number of samples of any group classified as belonging to that specific group. In this work, both procedures for LDA implementation were performed using the SPSS software.

Traditionally, the computation of the confusion matrix has been the final step in the discriminant analysis. However, the confusion matrix, when viewed as a contingency table, may be subject to further analysis, namely with respect to the observed correct overall classification, to group differences and to the classification and misclassification within groups (cells in the confusion matrix), to compare the predicted classification using the model to that expected from chance alone [26,42]. To evaluate the overall LDA classification, the conventional chi-square test for a contingency

table was applied. In cases where the expected (theoretical) number of samples classified in a specific group (cell) was lower than one or if in more than 20% of the cells of the confusion matrix the expected number of samples was higher than one but lower than five, the Fisher's exact test was used as an alternative to the chi-square test. For the cases where a correlation between the actual group and the predicted one was found, its intensity was evaluated by means of the Phi, Cramer's V and contingency coefficient.

Regarding group difference's test, the Morrison [42] likelihood analysis was used to compare the proportion of correctly classified observations with the proportion expected by chance. The proportion expected by chance, c_{pro}, was calculated as [26]:

$$c_{pro} = p_{row} \times \alpha_{column} + (1 - p_{row}) \times (1 - \alpha_{column}) \quad (1)$$

where p_{row} is the true proportion of each type (or milk group) in the total sample, and α_{column} is the proportion of each type (milk group) in the whole sample categorized in that type (milk group) by the model.

The relationship between chance and observed proportions can be tested using a Z statistic of the form:

$$Z_i = \frac{p_{cc} - c_{pro}}{\sqrt{c_{pro}(1 - c_{pro})/n}} \quad (2)$$

where p_{cc} is the overall percent observations correctly classified in the sample.

Classification and misclassification within groups was used to establish the source of deviation and was determined using the maximum chance criterion, c_{max}, defined as the minimum expected correct classification for a select group of interest, being calculated assuming that all observations are categorized as coming from that group [26,42].

A Z statistic was also used to test this relationship for all the cells in the confusion matrix:

$$Z_{ij} = \frac{o_{cc} - c_{max}}{\sqrt{c_{max}(1 - c_{max})/n}} \quad (3)$$

where o_{cc} stands for observed correct (incorrect) classification of the specific cell.

3. Results and discussion

In this study, the capability of the built e-tongue to recognise the five basic standard tastes (sweet, acid, bitter, salty and umami), as well as to classify raw skim milk samples (bovine, caprine and bovine/caprine mixtures) based on the e-tongue taste skill, were investigated.

3.1. Sensor performance evaluation of the basic standard taste substances

Six buffer solutions with pH values in the range of 2–10 were used to evaluate the response of each sensor of the multi-sensor device to pH. Distinct pH sensibilities were observed for the 36 sensors used: weak sensibility, with slopes between −3.0 and 2.2 mV/decade, for sensors A1, B2, B6, C1, D1, E2, E3, E6, F3 and F4; reasonable dependence for sensors A4, D5, E1 and F2, with slopes in the range of −8.0 to −3.8 mV/decade; and, high sensibility, with slopes between −34.3 and −15.6 mV/decade, for the other sensors. In general, the majority of these two last group sensors, showed a satisfactory pH dependence with correlation coefficients higher than 0.9. Furthermore, most of the sensors used in this work were sensitive to the composition of the buffer solutions. In fact, removing buffer solutions from the calibration results in an increase of the correlation coefficients in the pH calibration.

For assessing the ability of the sensors to distinguish different flavors, solutions were used for sweet, salty, acid, bitter and umami tastes (except for the last one, each kind of taste was tested with three different compounds, see above). The aim was to verify how the sensors respond to taste solution concentrations (varying from 1×10^{-5} mol/L to 1×10^{-1} mol/L, by successive additions), and to evaluate their sensibility by calculating the slope of the sensor signal in relation to the concentrations, in a logarithmic scale. Fig. 1 shows the slope values obtained for the signal response of each sensor towards the logarithmic of the concentration of each taste solution.

Globally, the results in Fig. 1 show that the sensors used in this work present a wide range of sensibility (from −51.8 to 45.5 mV/decade) towards each basic standard taste solution concentrations, being less sensible for glucose (0.1–13.2 mV/decade), fructose (−4.7 to 11.3 mV/decade), sucrose (−4.7 and 11.1 mV/decade), caffeine (1.7 and 19.3 mV/decade) and urea (−6.8 and 3.3 mV/decade) than for $MgSO_4$, and the acid and salty basic standard tastes. In fact, for these latter taste compounds, a higher sensor sensibility was observed, with slope amplitudes from 33.1 to 53.6 mV/decade. Moreover, the sensor slope profiles for the four ionic compounds studied (NaCl, KCl, NH_4Cl and $MgSO_4$) were similar, although quite different from the other analyzed substances. Overall, for this case, slopes between −45.8 and 11.0 mV/decade were obtained, most of them being negative. It should be noticed that, although $MgSO_4$ is an ionic compound, in this work it was used as a bitter taste standard in the sensory classification, as suggested by Briggs et al. [39]. For acid substances, the values of the slopes were, in general, positive and high, varying between −51.8 and 45.5 mV/decade, the sensor response being more sensitive to HCl concentration variations. The MSG analysis showed mostly negative slopes, in the range of −25.1 to 8.4 mV/decade.

Moreover, the signal stability as well as the repeatability in time of the responses towards the standard taste compounds for each sensor were studied. Concerning the signal stability, it was observed that the different sensor signals recorded during 5 min, after a stabilization period of 5–10 min, showed a maximum variation coefficient (CV) lower than 1%, between 1 and 3% and from 3 to 5%, for 17, 19 and 4 sensors, respectively, for all the taste compounds evaluated. Regarding the repeatability, responses for three solutions of caffeine, with the same concentration, were recorded for all the sensors used, showing CV between 0.5 and 15%. These results showed that the multi-array sensor device present a satisfactory signal behavior in time.

Globally, the results obtained from the 36 sensors show that the e-tongue built in this work could distinguish the five basic standard tastes, based on the different signal sensor profiles recorded, although with less efficiency for all the sweet taste substances, caffeine and urea.

The non-supervised PCA method was applied to the sensors signals profile measured in 1×10^{-3} mol/L solutions of the taste standards to display its variability. Principal components analysis showed that 97.6% of the total variance of the data could be explained using only three principal components. Fig. 2 shows the three-dimensional representation of the three principal component factor scores obtained for the flavour solutions.

As can be inferred by the results shown in Fig. 2, the five basic standard tastes could be separated in five different groups, confirming the satisfactory performance achieved with the e-tongue device. The first principal component factor allowed the separation of the acidic taste substances in the positive region and the MSG substance (umami taste) on the negative region; the second factor separates the sweet taste (glucose, fructose and sucrose are present in the positive region) from the other taste substances. The urea

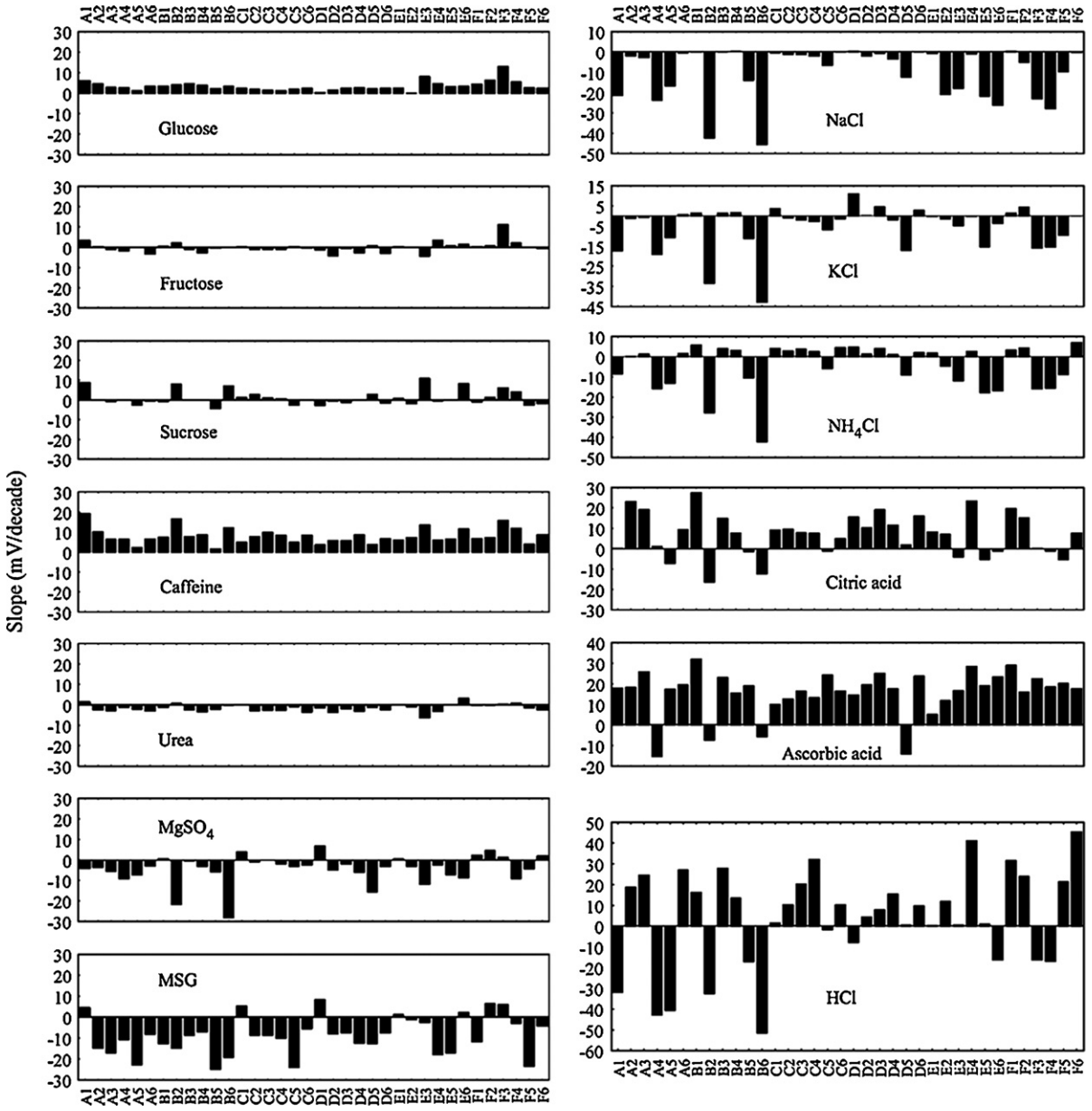

Fig. 1. Sensibility of all sensors to the basic standard taste solutions.

and caffeine are close to the interception of these two factors axes (no relevant contribution from the factors), while NaCl, KCl, NH_4Cl and $MgSO_4$ are grouped in the region with low values of these two factors. The $MgSO_4$, used as a bitter substance, was grouped with the salty taste compounds, due to its ionic nature. Factor 3 allows a weak separation between compounds with high dissociation degree (positive region) and molecular/low dissociation degree compounds (negative region) with exception of glucose, which is in the positive region. Globally, these results are in accordance with the sensor sensibility evaluation presented and discussed above.

3.2. Application of the multi-sensor device to classify milk samples

The e-tongue device built in this work was also used for milk samples classification purposes, based on its ability to differentiate the taste standard substances, as described above. Fig. 3 presents a typical average signal sensor profile recorded for three whole skim caprine milk samples, three 50% bovine/caprine milk mixtures and three whole skim bovine milk samples, showing also the magnitude of the standard deviation observed. This figure shows that small differences between the signal intensities are observed for the three milk samples, which indicates the need to use all the 36 sensors to classify whole or adulterated milk. Moreover, for this study, the responses of the four repeated sensors used in the two parallel sensor arrays were also included, since it has been reported that the inclusion of repeated sensors in multivariate analysis can improve model performance [43].

LDA with probabilities proportional to each group size was used to discriminate between the three milk groups: goat, cow and goat/cow raw skim milk samples. Although this method requires the normality of the data, it can deal with deviations from normality, having good robustness [44]. The number of variables (sensors signals) that could be used to obtain the maximum correct classification of the raw milk samples according to milk types was selected, retaining those which did not fail the tolerance test, allowing max-

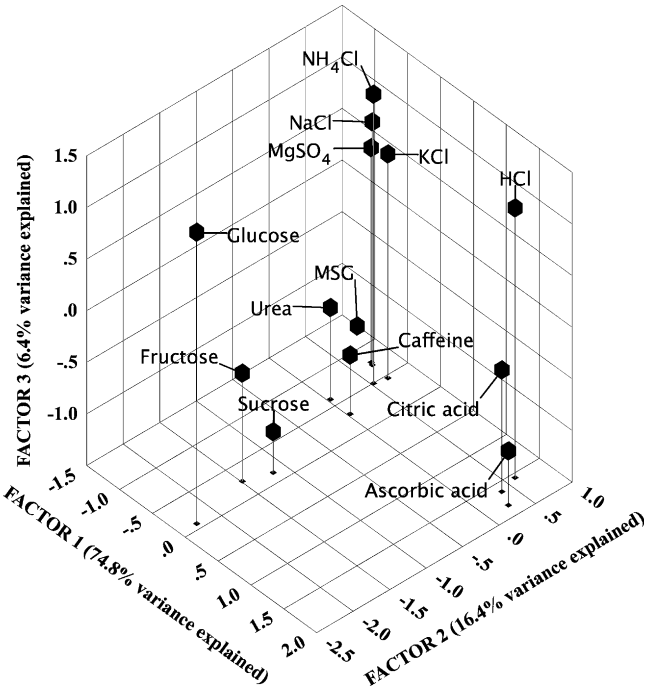

Fig. 2. Representation of the three principal component factor scores obtained for the taste solutions.

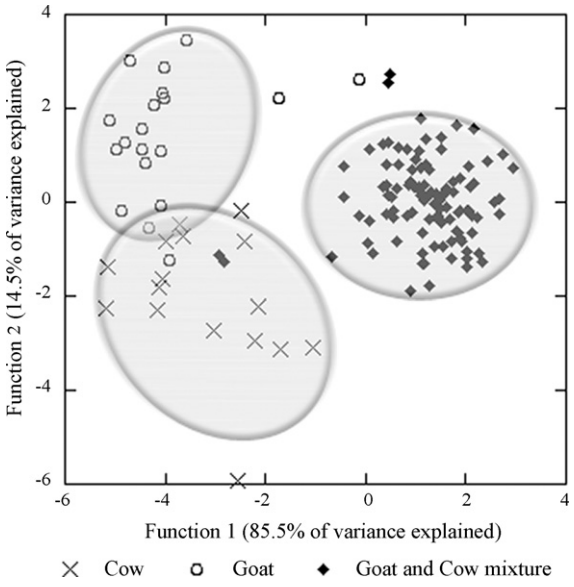

Fig. 4. Discriminant analysis obtained for the 142 skim raw milk samples.

imizing the discriminant information for classification purposes. Keeping a small number of key variables is essential for increasing the reliability of the mathematical classification, eliminating features with minor information and allowing also a visual examination of the data set by a two-dimensional plot of the key features [26]. For the linear discriminant analysis carried out, considering that the prior probabilities were proportional to each group size, all sensors, except sensors E5, E6, F2 and F4, which failed the tolerance test, were included. This test was used to identify multicolinearity in discriminant analysis. Two discriminant functions, explaining 100% of cumulative variance (85.5% and 14.5% for Functions 1 and 2, respectively), were retained, being both significant accordingly to the Wilk's Lambda test ($p \leq 0.001$). Taking into account the coefficients of the canonical discriminant functions, standardized by the variance within groups, it can be stated that the most outstanding contribution to discrimination in the first function, in decreasing order of importance, was obtained from sensors F1, A1, B1, A5, B3, B4, C4, C5, D4, C1, A2, F5, A4, B5 and A3. Regarding the second discriminant function, sensors D3, A6, E2, E4, D5, D4, D2, F3, E3, B2, B6, D6, A3 and C2 showed the largest absolute correlation. Applying these functions to the sensor signals obtained for the different raw skim milk samples analyzed, the corresponding scores for each function were calculated and plotted versus the canonical functions, allowing the visualization of their ability to discriminate among the three skim milk groups considered (Fig. 4). While raw skim bovine milk samples were characterized by negative values of both Functions 1 and 2, caprine milk samples were characterized by negative values of Function 1 and mainly positive values of Function 2. Mixtures of cow and goat raw skim milks presented positive values of Function 1 and values ranging from −2 to +2 for Function 2. From these results and based on the relative contribution of each sensor to discriminant Function 1, it can be stated that the sensors 1, 4 and 5 (with plasticizers A, B, C and D or F) are those that most contribute for the discrimination between caprine/bovine skimmed milk mixtures and whole skim bovine or caprine milks. Moreover, sensor 2 (with plasticizers B, C, D and E), sensor 3 (with plasticizers A, D, E and F) and sensor 6 (with plasticizers A, B and D) are those that most contribute to discriminant Function 2, facilitating the differentiation between whole skim bovine and caprine milks.

The confusion matrix (Table 2) associated with the linear discriminant analysis between milk groups showed satisfactory overall sensibility and specificity: 97% and 93%, 87% and 70%, respectively, for original and cross-validation classifications. Moreover, applying the Fisher's exact test to the results obtained for both original groups and cross-validation classifications, as the practical rule of the chi-square test was not obeyed, chi-square values of 158.50 and 106.37 were, respectively, obtained ($p < 0.0002$ for four degrees of freedom), showing a strong association between original and predicted groups. In fact, the symmetric measures (Phi, Cramer's V or contingency coefficients) calculated for both original groups and cross-validation classifications, are higher than 0.78 and 0.66, respectively, showing a powerful association between the variables with high statistical significance ($p < 0.0002$). Therefore, it can be inferred that the model performance yield a better classification into milk groups than those expected just by chance,

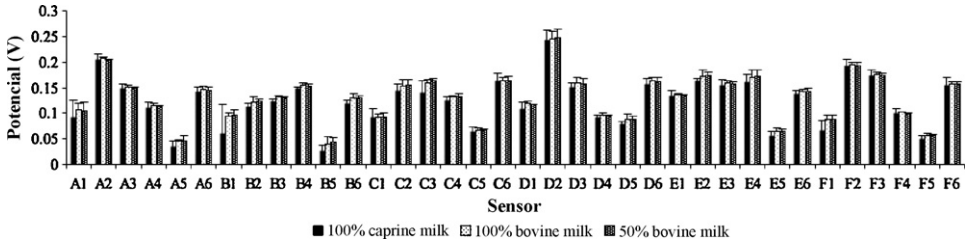

Fig. 3. Signal sensor profiles (with the standard deviation bar) for whole skim caprine milk sample, 50% bovine/caprine milk mixture and whole skim bovine milk sample.

Table 2
Confusion matrix of discriminant analysis (milk groups) according to the signals obtained from the e-tongue potentiometric device[a].

Actual group	Predicted group membership			Total	Sensibility (%)
	Goat	Cow	Goat/Cow mixtures		
Goat	16 (10)	2 (6)	1 (3)	19	84 (53)
Cow	0 (6)	16 (9)	0 (1)	16	100 (56)
Goat and Cow mixtures	0 (0)	2 (3)	105 (104)	107	98 (97)
Total	16 (16)	20 (18)	106 (108)	142	97 (87)
Specificity (%)	100 (63)	80 (50)	99 (96)	93 (70)	

[a] Results from cross-validation are given in parenthesis.

both for the samples analyzed and for the unknown skim milk samples.

Furthermore, the analysis of the results presented in Table 2 shows that globally the best characterized group was caprine/bovine skim raw milk with 99% and 98% of specificity and sensibility for the original data, and 96% and 97% for cross-validation procedure. These results clearly show that the linear discriminant model obtained was able not only to recognize differences between the sensor signals obtained with the e-tongue device for this group of samples and the other two, but also to classify unknown samples of caprine/bovine raw skim milk. However, with respect to the cow and goat groups, the model showed large deviations between specificity and sensibility values for the original and cross-validation classifications, mainly due to some misclassification observed for those types of samples in the cross-validation procedure. Therefore, although the ability of the deduced discriminate functions to detect different sensor signals among skim raw goat and cow milks was very satisfactory (84% and 100% of sensibility and 100% and 80% of specificity for goat and cow milks, respectively), its ability to classify further unknown samples was less efficient (only 53% and 56% of sensibility and 63% and 50% of specificity for goat and cow milks, respectively).

Owing to the rather low sensibilities and specificities obtained for cow and goat milk groups in cross-validation, and because the two dimensional plotting of the score versus the corresponding canonical functions did not lead to a completely separation of these two groups (Fig. 4 and Table 2), a more detailed evaluation of the confusion matrix was made. For this, the test based on the likelihood ratio defined by Morrison [42] was applied to evaluate the expected classification of specific milk groups (rows) using the proportional chance criteria to obtain an estimation of the expected correct classification by chance. The estimated proportional chance criteria may be compared with the overall correct classification by the Z_i score obtained for each group (milk type) according to Eq. (3) defined in Section 2. The Z_i values calculated for each milk group studied are shown in Table 3, for both the original group and cross-validation classifications. Although considering the satisfactory classification results described before, this study was only necessary for cross-validation data and for the cow and goat groups, it was made for both classification procedures and for the three milk groups to obtain a more complete view of the e-tongue performance. The results show that for the original group procedure, the classification obtained using the deduced model is significantly higher than expected by chance ($Z_i \geq 5.24$; $p < 0.0002$), since the model only misclassifies a few samples of each milk as belonging to other groups. The application of this overall test of significance to the cross-validation data shows that the deduced model can classify goat, cow and goat/cow unknown samples with higher success than that obtained by chance ($Z_i \geq 2.25$; $p \leq 0.012$), although with lower efficiency when compared with the results obtained with the original data.

A more detailed statistical analysis can be conducted in order to determine if the deduced model probability to correct or incorrect classify each raw skim milk sample is greater or lower than that expected by chance. Since divergences may be presented in any of the confusion matrix cells, each one should be tested to determine whether its proportion differs from chance [26]. The Z_{ij} values of this comparison and its associated probabilities are also shown in Table 3 for the two classification methodologies used, original data groups and cross-validation.

For the original data group classification procedure, the results obtained (Z_{ij}) reinforced the conclusion already stated showing that the correct classification of goat, cow and goat/cow samples was higher than that expected by chance ($Z_{ij} \geq 6.30$; $p < 0.0002$) and also that the misclassification obtained by the deduced model (samples of one group incorrectly classified as belonging to another group) is lower than that obtained by chance ($Z_{ij} \leq -2.84$; $p \leq 0.0023$), except for goat samples that are misclassified as cow samples by the model, with a probability similar to that obtained by chance ($Z_{ij} = -1.00$; $p = 0.1587$).

On the other hand, for the cross-validation results, the deduced model can classify unknown samples of each group in the correct group with higher probability than that obtained by chance ($Z_{ij} \geq 6.04$; $p < 0.0002$). However, it misclassified goat samples as cow and *vice-versa* with higher probability than obtained by chance (Z_{ij} equal to 6.37 and 9.89, respectively; $p < 0.0002$), meaning that the deduced model confuses some goat unknown samples with cow and *vice-versa*. Moreover, goat unknown samples were misclassified as goat/cow samples with a similar probability as by chance ($Z_{ij} = 0.84$; $p = 0.2005$). On the other hand, cow unknown samples

Table 3
Analysis of the confusion matrix[a]: group difference's test (Z_i) and classification and misclassification within groups (Z_{ij}).

Classification procedure	Current milk group	Z_i	Predicted milk group (Z_{ij})		
			Goat	Cow	Goat and Cow mixtures
Original data	Goat	5.24 (<0.0002)	24.79 (<0.0002)	−1.00 (0.1587)	−2.84 (0.0023)
	Cow	5.35 (<0.0002)	−4.25 (<0.0002)	33.44 (<0.0002)	−4.25 (<0.0002)
	Goat and Cow mixtures	8.36 (<0.0002)	−20.84 (<0.0002)	−20.32 (<0.0002)	6.30 (<0.0002)
Cross-validation	Goat	2.39 (0.0084)	13.74 (<0.0002)	6.37 (<0.0002)	0.84 (0.2005)
	Cow	2.25 (0.0122)	9.89 (<0.0002)	16.95 (<0.0002)	−1.89 (0.0294)
	Goat and Cow mixtures	5.78 (<0.0002)	−20.84 (<0.0002)	−20.06 (<0.0002)	6.04 (<0.0002)

[a] Probabilities of the values of Z_i and Z_{ij} are given in parenthesis.

were misclassified as goat/cow samples with lower probability than the one obtained by chance ($Z_{ij} = -1.89$; $p = 0.0294$). As expected, considering the results already discussed, unknown goat/cow samples were always misclassified in a significant lower proportion than that expected by chance ($p < 0.0002$).

The confusion obtained regarding the misclassification of some goat and cow raw skim milk samples could be explained by the small number of samples obtained for these two groups (19 and 16 samples analysed, respectively) when compared to the large number of samples considered for the goat/cow group (142 samples analysed). Therefore, a larger number of samples should be used in order to enhance the ability of the deduced linear discriminant model, based on the signals obtained from the e-tongue device assembled, especially to differentiate between different unknown milk samples.

4. Conclusions

This work shows that the multi-sensor device developed allows differentiation between the five basic standard tastes (sweet, salty, bitter, acid and umami tastes), being more effective towards the recognition of the acid, salty and umami tastes. When the device was applied to the study of caprine milk adulterations with bovine milk, a problem in the dairy industry, it was able to give different signal profiles associated to the specific sensory characteristics of each skimmed milk sample, allowing discrimination between goat, cow and goat/cow raw skimmed milks with satisfactory sensibilities and specificities (over than 87% and 70%, respectively). Therefore, it has been shown that this methodology can be used as a fast and economic procedure to evaluate, in a real time basis, the possible adulterations of goat raw milk with cow raw milk. However, in order to use the e-tongue as a routine methodology for caprine milk adulteration detection in the dairy industry, it is needed to improve the multi-sensor system by testing and including more sensible sensors to milk composition variations.

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