Research Journal of Applied Sciences 13 (8): 452-459, 2018
ISSN: 1815-932X

Classification and Analytical Evaluation of Raw Milk Contaminated with Herbicides Through the Application of a Voltammetric Electronic Tongue

[1]Arrieta Alvaro, [1]Arrieta Pedro and [2]Palencia Manuel
[1]Departamento de Biologia y Quimica, Universidad de Sucre,
Carrera 28 N°5-267 Barrio Puerta Roja, Sincelejo, Colombia
[2]Departamento de Quimica, Universidad del Valle, Calle 13 N°100-00,
Campus Melendez, Cali, Colombia

Abstract: The aim of this research was to evaluate the application of a voltammetric electronic tongue device based on PSoC technology and polypyrrole sensor array in the analysis of milk samples contaminated with herbicides. The analysis was carried out on samples contaminated with glyphosate and terbutryn and a voltammetric electronic tongue device equipped with a sensor array made of modified polypyrrole with different doping ions. The data were analyzed by principal component analysis and cluster analysis to evaluate the discrimination capacity and with partial least squares to explore the capacity of prediction of contaminant concentration. The results showed an excellent discrimination capacity when applying the methods of principal component analysis and cluster analysis. In addition, in both cases the spatial distribution of the samples was consistent with expected with the contaminant concentration. On the other hand when applying the partial least squares analysis, a good correlation and concentration values predicted by the electronic tongue were very close to the real concentrations. The electronic tongue device can be used as an analytical alternative to determine contaminants such as glyphosate and terbutryn.

Key words: Electronic tongue, polypyrrole, milk, herbicides, electrochemistry, device

INTRODUCTION

Milk is a drink with great nutritional value and high nutritional content. In this sense, milk can contribute to the nutritional requirements of people because it provides calcium, magnesium, zinc, iron, rivoflamines, sugars, fats and vitamins (Pereira, 2014; Haug *et al*., 2007). However, in milk it is possible to find some undesirable substances such as herbicides, antibiotics, fungicides, etc. (Frazier, 2007; Aytenfsu *et al*., 2016; Balduini *et al*., 2003).

Herbicides are substances widely used by the agricultural industry to control some plants know as weeds. These substances accumulate in the environment and being fat-soluble they can accumulate in the fatty tissues of animals and milk fat (Balduini *et al*., 2003; Eenennaam and Young, 2017). Therefore, it is possible to find herbicides in bovine milk and its derived products.

The demand for milk like a product of daily consumption grows continuously due to the population increase. According to FAO estimates, milk production exceeded 800 mln tons in 2017 (Anonymous, 2017). This great growing demand means that controls on its quality are increasingly strict and that new control strategies are implemented on the presence of harmful substances for human consumption.

A large number of efforts have been made to develop analytical methods to determine residues of herbicides in milk. Chromatographic methods being the most used today, specifically liquid-liquid, liquid-solid phase extraction. Other techniques are also used such as supercritical extraction, subcritical extraction and extraction with pressurized fluids (Hong *et al*., 2018; Islas *et al*., 2018; Guo *et al*., 2018; Shamsipur *et al*., 2016).

All these extraction processes involve the use of solvents such as acetonitrile, acetone, hexane, ethanol, among others. The procedures require a lot of time and some expertise is required to complete them. In addition to expensive equipment and complex handling. All the above has led to the exploration of new analytical methods to reduce the complexity of current methods, reduce their cost, shorten the analysis time and make them more accessible for use in laboratories and sites that do not require a complex infrastructure.

Corresponding Author: Arrieta Alvaro, Departamento de Biologia y Quimica, Universidad de Sucre, Carrera 28 N°5-267 Barrio Puerta Roja, Sincelejo, Colombia

A recently introduced technique is the use of multi-sensor analytical devices know as electronic tongues. These devices take their name from the gustatory system in which taste cells of the tongue which possess partial specificity, produce signals that are sent to the brain by different nerve channels to be interpreted by recognizing patterns that allow identification of drinking in contact with the tongue (Vlasov *et al*., 2005; Tahara and Toko, 2013). Thus, electronic tongues are devices composed of chemical sensors array with crossed selectivity coupled to a multichannel measurement system that uses pattern recognition analysis or multivariate statistical methods. This type of equipment has been used successfully in the analysis of beverages (Pigani *et al*., 2018; Arrieta *et al*., 2015, 2016a, b, 2010; Nery and Kubota, 2016).

In the literature are reported mainly devices based on electrochemical analysis techniques such as potentiometry and voltammetry. Potentiometric sensors most commonly used in electronic tongues are non-selective electrodes of lipid/polymer membranes and ion-selective electrodes (Vlasov *et al*., 2005; Tahara and Toko, 2013, Nery and Kubota, 2016; Cruz *et al*., 2018; Lipkowitz *et al*., 2018). The sensors used in the voltammetric electronic tongues are mainly metallic electrode array (Au, Pt, Ag, etc.) (Pigani *et al*., 2018; Arrieta *et al*., 2015, 2016a, b, 2010; Winquist and Wide, 1997) and modified electrode array with conducting polymers (Arrieta *et al*., 2015, 2016a, b, 2010, Antunes *et al*., 2005; Gonzalez-Calabuig and Valle, 2018; Pigani *et al*., 2018). Due to the robustness, simplicity, sensitivity and versatility of the voltammetric electrodes, the electronic tongues based on this technique have been shown to have a wider range of applications because their signals (voltammetric curves) offer more information than the potential values offered by the pontentiometric electrodes. They are also less sensitive to electronic noise and are not limited to substances with high ionic content. On the other hand, the possibility of modifying the voltammetric electrodes with conductive polymers such as polypyrrole, allows to have sensors with signals richer in information product of the interaction of the analyzed substance and the sensitive electroactive polymer.

Electronic tongue devices with voltammetric sensors modified with polypyrrole have largely shown their ability to analyze substances such as coffee, wine, beer, water, among others (Pigani *et al*., 2018; Arrieta *et al*., 2015, 2016a, b, 2010; Winquist and Wide, 1997; Antunes *et al*., 2005; Gonzalez-Calabuig and Valle, 2018; Pigani *et al*., 2018). Recently, some electronic tongue application work has been done in milk analysis to detect adulterants, sample origin and types of commercial milks (Arrieta *et al*., 2015, 2016a, b, 2010; Bueno *et al*., 2014; Bougrini *et al*., 2014). However, the application of electronic tongue devices in the analysis of samples of milk contaminated with herbicides has not been reported. In this study, the application of an electronic tongue equipped with a polypyrrole sensors array is applied in the analytical evaluation of samples of milk contaminated with glyphosate (N-(phosphonomethyl)glycine) and terbutryn (2-N-tert-butyl-4-N-ethyl-6-methylsulfanyl-1, 3, 5-triazine-2, 4-diamine), two of the most common herbicides that generate great controversy due to their persistence in the environment.

MATERIALS AND METHODS

Reagents and samples: All the reagents used were of analytical quality. The solutions were prepared by using milli-Q quality ultrapure water. The samples analyzed consisted of samples of fresh and raw milk. The 50 mL samples were prepared which were contaminated from the addition of herbicides (glyphosate and terbutryn) in concentrations of 0.1, 0.3, 1, 3 and 5%. An uncontaminated sample (0%) was also used. All samples were prepared in triplicate. The herbicides glyphosate (N-phosphonomethyl glycine) and terbutryn (2-N-tert-butyl-4-N-ethyl-6-methylsulfanyl-1, 3, 5-triazine-2, 4-diamine) were purchased in the local commerce.

Electronic tongue device: The electronic tongue device consists of three parts an electronic measurement module, a data storage and control module and a cross-selectivity sensor array.

The sensor array with cross-selectivity was developed on a card with screen-printed electrodes AC9C from BVT Technologies. This card consists of a common Ag/AgCl reference electrode and eight platinum electrodes of which one was used as a counter electrode and the remaining seven electrodes were modified by electrodeposition of polypyrrole doped with different counterions. In this way, the array consisted of seven polypyrrole sensors doped with different counterions; Sodium sulphate (SO_4), sodium Dodecyl Benzene Sulfonate (DBS), potassium Ferrocyanide (FCN), Anthraquinone-2, 6-disulfonic acid, Disodium Salt (AQDS), lithium Perchlorate (PC), p-Toluenesulfonic Acid (TSA), ammonium persulphate (SF).

The experimental conditions used for the elaboration of the sensor array were previously optimized for its specific application in milk and the results have been reported (Arrieta *et al*., 2016b). The polymerizations were

Table 1: Manufacturing conditions of the polypyrrole sensor array

Sensor	Acronym	Counterion concentration (mol/L^{-1})	Time (sec)
S1	PPy/DBS	0.10	45
S2	PPy/ AQDS	0.05	70
S3	PPy/PC	0.10	60
S4	PPy/pTS	0.10	60
S5	PPy/SF	0.05	70
S6	PPy/FCN	0.10	50
S7	PPy/SO4	0.05	60

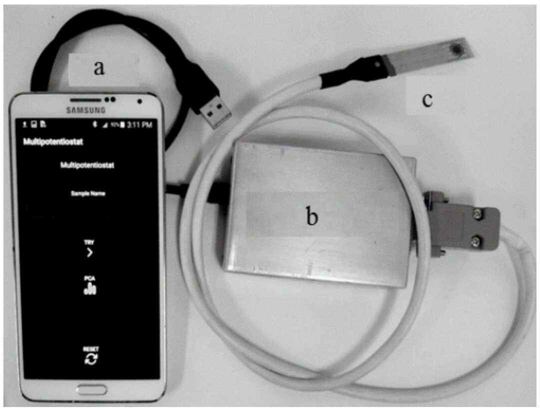

Fig. 1: Voltammetric electronic tongue device: a) Smartphone with android control application; b) Multi-potentiostat system based on PSoC and c) Polypyrrole sensor array

carried out in aqueous solutions at 0.8 V and a monomer concentration of 0.2 mol/ L^{-1} was used. Table 1 summarizes the conditions used for the development of the sensor array.

The module of the electronic tongue device used to carry out the measurements consisted of a portable multi-channel potentiostat, prepared on a FREESOC card equipped with a PSoC 5 LP microchip in which the entire measurement system was programmed through the PSoCCreator Software.

The control of the device and the data storage were made through a smartphone with an app developed as a complement to the electronic device. Details of the elaboration of the milti-potentiostat measurement system and the app have been previously reported (Arrieta *et al*., 2015, 2016a). In Fig. 1 is presented an image of the electronic tongue device.

Measurements and data analysis: All measurements were made at room temperature in 5 mL of sample. All samples were measured in triplicate with a scan rate of 100 mV sec^{-1} in a potential range of -1.0-0.5 V. Once the measurements were made, the data were organized in matrix form, using as inputs data the current values registered in the voltammetric signals of the sensor array.

The multivariate analyzes were applied to the data matrices without any type of pre-treatment. Principal Component Analysis (PCA), Cluster Analysis (CA) and Partial Least Squares Regression Analysis (PLS) were made with the Minitab V 18 Software.

RESULTS AND DISCUSSION

Electronic tongue response: The response of the sensor array to the milk samples consisted of well-defined voltammetric signals free of noise and with redox processes resulting from the interaction of the polymer on the surface of the sensors and their respective counter-ion with the sample analyzed.

The voltammetric signals consist of an oxidation and a reduction wave in which some peaks produced by the oxidation/reduction of the polypyrrole are observed through the ion exchange with the ionic compounds and interaction with the nonionic compounds present in the milk samples.

In Fig. 2, the response of the sensor array to a milk sample is presented. It is the figure that can be clearly observed that each sensor offers a different signal which indicates that each one contributes information regarding the sample analyzed.

On the other hand, it is also important that each sensor must offer differentiating responses from each of the samples. That is each sample can be differentiated by each of the sensors through different voltammetric signals which has been defined as cross-selectivity.

To show this fact, the response of the PPy/AQDS sensor is presented in Fig. 3, compared to a sample uncontaminated and samples contaminated with 0.1 and 0.3% of glyphosate, only three voltammograms are presented for the sake of simplicity and better contrast in the signals. It can be observed that there are differences in the position of the anodic and cathodic peaks and also in the form of the signal at the global level this because the presence of the contaminant may generate changes in the chemical equilibria of the samples and therefore in its global behavior at the ionic and non-ionic level which generates a change in the sensors response.

However, this pattern of responses of the sensors to the samples is not sufficient to visualize the discrimination. For this reason, a principal component analysis was carried out to analyze the discrimination capacity of the electronic tongue.

Classification of contaminated milk samples: Figure 4 presents the results of the principal component analysis

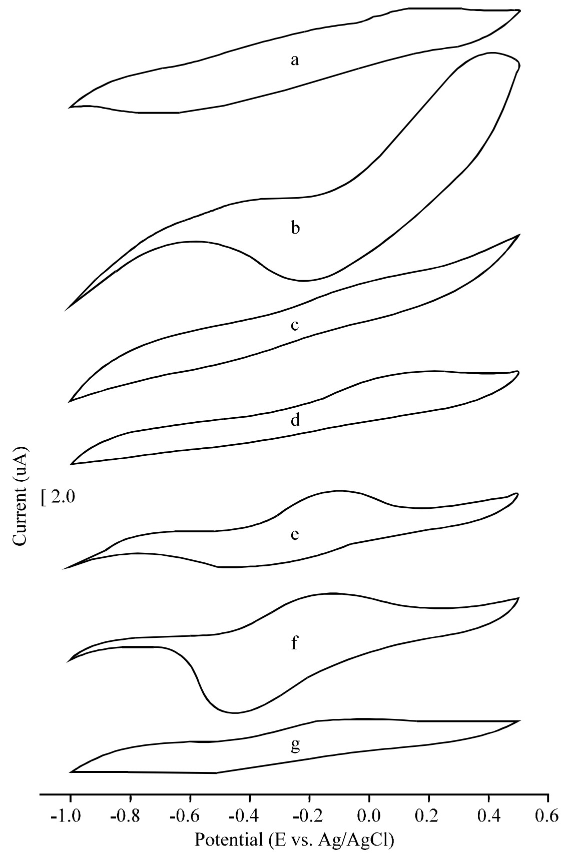

Fig. 2: Cyclic voltammetric response of: a) Ppy/PC; b) PPy/SO4; c) PPy/SF; d) PPy/pTS; e) PPy/FCN; f) PPy/AQDS and g) PPy/DBS in a milk sample uncontaminated (0%)

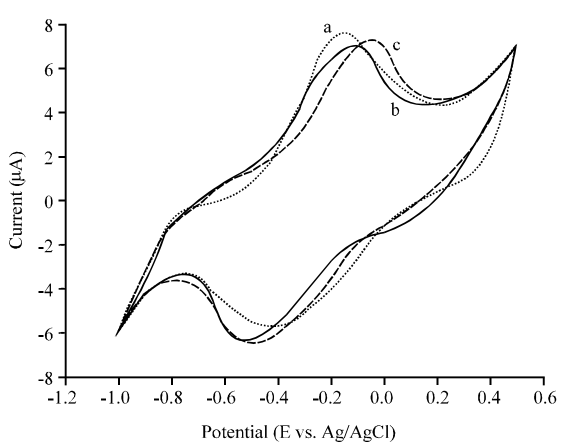

Fig. 3: Voltammetric response of PPy/AQDS in milk samples contaminates with: a) 0%; b) 0.1 % and c) 0.3 % of glyphosate

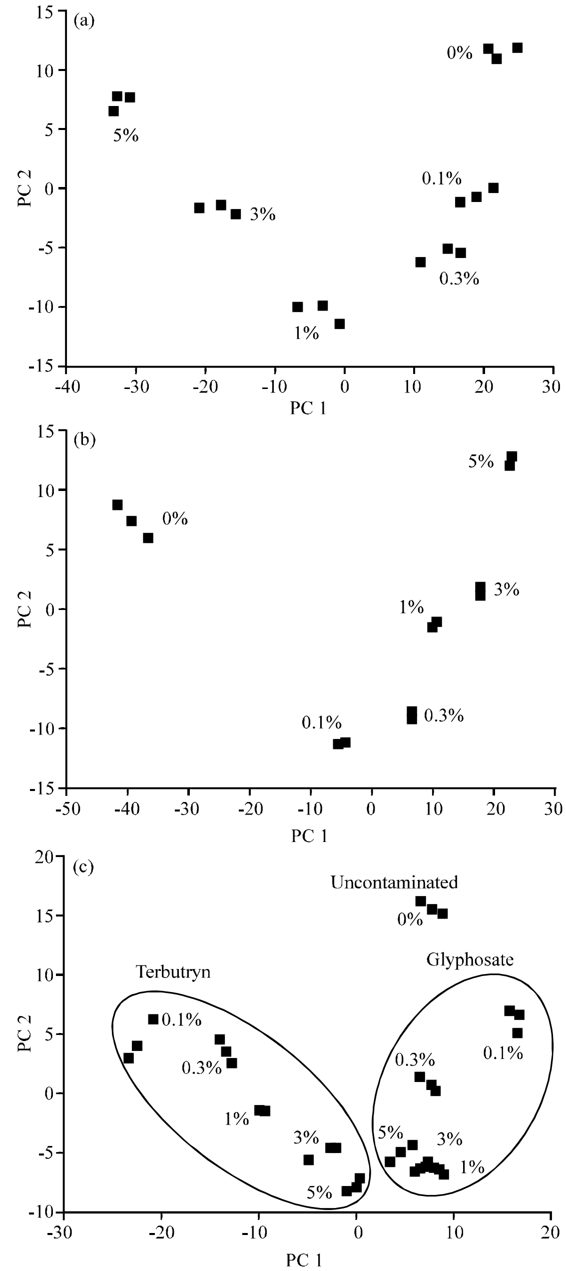

Fig. 4: PCA score plot obtained by mean of milk samples contaminated with: a) Glyphosate; b) Terbutryn and c) Glyphosate and terbutryn

applied to the data matrix obtained from measurements made on the samples contaminated with the herbicides; Fig. 4a shows the result of the analysis carried out on the samples contaminated with glyphosate, Fig. 4b shows the result of the analysis carried out on the samples contaminated with terbutryn and Fig. 4c shows the result of the analysis carried out on the total set of samples that includes both pollutants.

In Fig. 4a, it can be clearly seen that the electronic tongue is able to discriminate milk samples contaminated with glyphosate. The two principal components describe a 92.45% of information, the first component summarizes a 63.81% and the second one a 28.64%. In the spatial distribution, it is observed that the sample uncontaminated (sample 0%) is far from the samples containing glyphosate in the positive quadrant of the first and second component.

In the positive quadrant of the first component and negative of the second are the samples that contain 0.1 and 0.3% glyphosate. The samples with 1 and 3% were observed in the negative quadrant of both components while the sample with 5% glyphosate was located separately in the negative quadrant of the first component and positive in the second component.

In the principal component analysis of the samples containing terbutryn, a well-marked discrimination was obtained with the sample uncontaminated (sample 0%) separated in the negative quadrant of the first component and positive in the second while the samples with 0.1, 0.3, 1 and 3% were located in the lower part of the figure in the negative part of the second component, the sample with 5% of terbutryn was located in the positive quadrants of both components. The information collected in the two components was 90.86% with 59.78% in the first and 31.08% in the second.

Although, this spatial distribution of the samples contaminated with terbutryn is different to that found with the samples contaminated with glyphosate, some similarities can be established regarding the organization of the samples because in both cases, the uncontaminated sample is markedly separated from the rest, just as the sample containing 5% of contaminant is located a little away from the samples with less amount of contaminant.

To explore the behavior of the electronic tongue in the discrimination of the two groups of samples combined, a principal component analysis was carried out on the joint data matrix of the samples contaminated with glyphosate and terbutryn (Fig. 4c). The figure shows a distribution in which the samples containing glyphosate and terbutryn make up separate macro-groups in which the samples with different degrees of contamination are discriminated into separate subgroups. The macro-groups are discriminated in the first component in the positive part the samples contaminated with glyphosate were located and in the negative part those contaminated with terbutryn.

On the other hand, the sample without contaminant is markedly separated from the rest and closer to the macro-group of samples containing glyphosate. These results allow establishing that the electronic tongue discriminates very clearly samples with glyphosate and terbutryn and can also discriminate different degrees or concentrations of these contaminants.

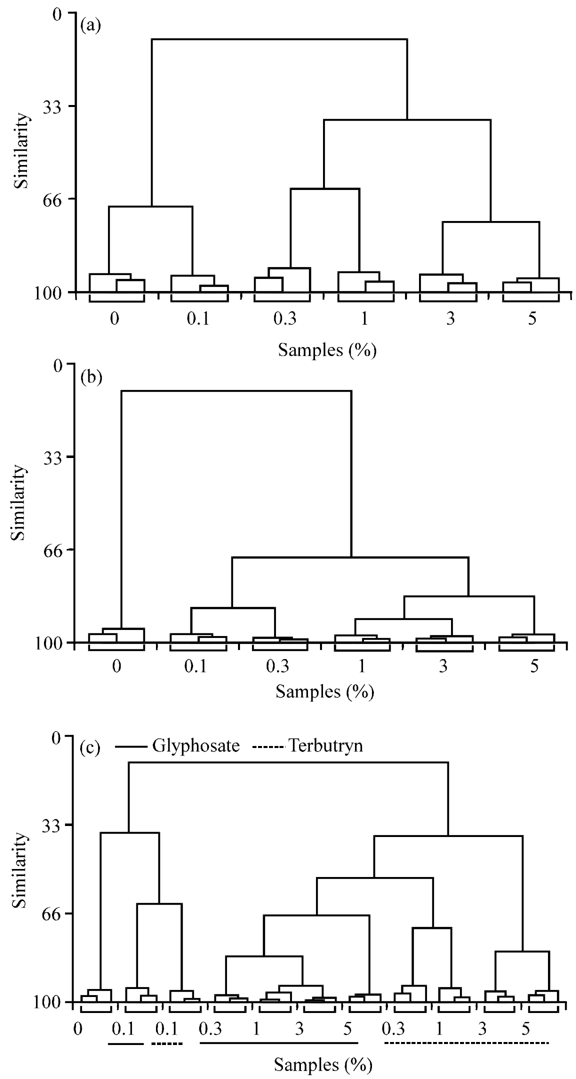

Fig. 5: Dendrogram from cluster analysis in euclidian distance for electronic tongue analysis of milk samples contaminated with: a) Glyphosate; b) Terbutryn and c) Glyphosate and terbutryn

To confirm the results of discrimination obtained with the principal component analysis, a cluster analysis method was applied (Fig. 5). The cluster analysis was applied to the matrices of the measurements made to the group of samples contaminated with glyphosate (Fig. 5a) to the group of samples contaminated with terbutryn (Fig. 5b) and to the joint matrix of the two groups of samples (Fig. 5c).

The figures shows a clear discrimination of the three replicas of each milk sample. In the dendrogram obtained with the samples contaminated with glyphosate represented in Fig. 5a, it is observed that the uncontaminated sample has a small similarity with the sample with the lowest concentration of contaminant (0.1%), the rest of the samples are completely separated forming separate groups in each concentration. The dendrogram obtained from the analysis of the samples contaminated with terbutryn was observed a very clear separation of each of the groups of samples that comprise the different concentrations of trebutryn, standing out the uncontaminated sample which have a widely marked separation.

In the analysis that integrates the two groups of contaminated samples it was possible to discriminate each group of samples in this case the sample without contamination is separated with a low degree of similarity with the two groups of samples with lower concentration of contaminant (0.1% of glyphosate and 0.1% of terbutryn). In addition there are two macro-groups formed by the samples containing glyphosate and terbutryn. In summary, it can be observed very clearly that the discrimination of the samples obtained by cluster analysis is consistent with that obtained by principal component analysis and in both cases they are related to the particularities of each group of samples.

Analysis of the herbicides concentration: In order to explore the possibility of predicting the concentrations of glyphosate and terbutryn from the measurements made with the electronic tongue, a Partial Least Squares (PLS) analysis with cross validation was carried out. The model was built with the data supplied by the electronic tongue and the concentrations of contaminant in the samples. Figure 6 shows the result of the partial least squares model carried out to predict the herbicide concentration in the milk samples.

The PLS figures shows the predicted herbicide concentration values versus the real values of the concentrations in the samples. As can be seen, a good correlation was obtained between the values of herbicide concentration predicted by the electronic tongue and the real values. The correlation coefficients were 0.981 and 0.989 for the calibration models carried out for glyphosate (Fig. 6a) and terbutryn (Fig. 6b), respectively. In addition, the prediction errors calculated by the model were quite low; 0.402% for glyphosate and 0.107% for terbutryn. These results indicate that this type of analytical approach can be applied as an alternative method to determine the presence and concentration of glyphosate and terbutryn in milk samples.

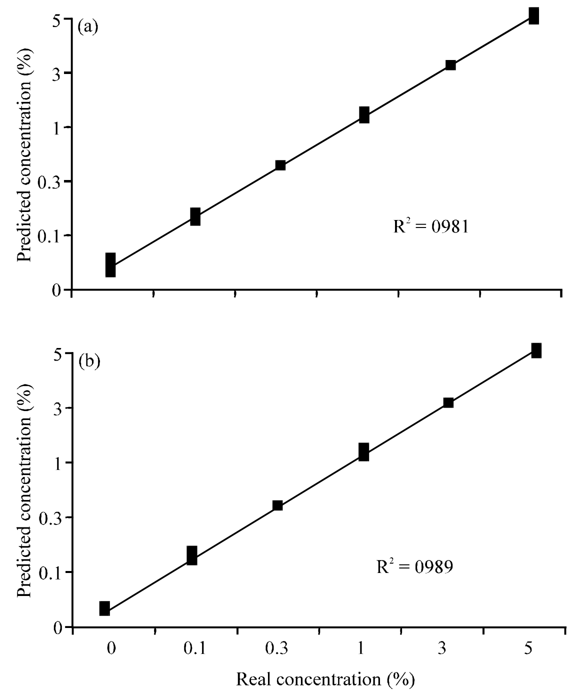

Fig. 6: Predicted vs. real concentration plot obtained of PLS Model applied to electronic tongue analysis in milk samples contaminated with: a) Glyphosate and b) Terbutryn

CONCLUSION

The use of an electronic tongue based on PSoC technology and voltammetric sensors elaborated from doped polypyrrole with different counterions it has been possible to discriminate milk samples contaminated with terbutryn and glyphosate.

The spatial discrimination obtained from the application of principal component analysis is related to the concentrations of herbicide present in the samples. In addition, the application of cluster analysis allowed to corroborate the excellent results obtained with the principal component analysis because the distribution of the samples followed a similar pattern in both statistical approaches.

The possibility of applying the analysis carried out with the electronic tongue to carry out quantitative determinations was explored based on the application of the partial least squares regression method. The results showed that the values of concentration of herbicides predicted by the measurements made with the electronic tongue are very close to the real concentrations which allows to conclude that this type of devices can be used as an analytical alternative in quantitative determinations of glyphosate and terbutryn.

ACKNOWLEDGEMENTS

The researchers acknowledge to the University of Sucre, the University of Valle and the Administrative Department of Science and Technology (Colciencias) for the financial support of this research.

REFERENCES

Anonymoys, 2017. Food outlook, biannual report on global food markets. Food and Agriculture Organization, Rome, Italy. http://www.fao.org/3/a-i7343e.pdf.

Antunes, P.A., C.M. Santana, R.F. Aroca, J.O.N. Oliveira and C.J.L. Constantino *et al*., 2005. The use of Langmuir-blodgett films of a perylene derivative and polypyrrole in the detection of trace levels of Cu2+ ions. Synth. Met., 148: 21-24.

Arrieta, A., O. Fuentes and M. Palencia, 2015. Android and PSoC technology applied to electronic tongue development. Res. J. Appl. Sci. Eng. Technol., 10: 782-788.

Arrieta, A., O. Fuentes and R. Pestana, 2016a. Portable electronic tongue based on PSoC and android technology for analysis of raw milk. J. Sci. Technol. Appl., 1: 97-105.

Arrieta, A.A., J.A. Diaz and O.C. Fuentes, 2016b. [Development and optimization of a network of electrochemical sensors for an electronic language oriented to milk analysis (In Spanish)]. Rev. Soc. Qui. Per., 82: 152-161.

Arrieta, A.A., M.L. Rodriguez-Mendez, D.J.A. Saja, C.A. Blanco and D. Nimubona, 2010. Prediction of bitterness and alcoholic strength in beer using an electronic tongue. Food Chem., 123: 642-646.

Aytenfsu, S., G. Mamo and B. Kebede, 2016. Review on chemical residues in milk and their public health concern in Ethiopia. J. Nutr. Food Sci., 6: 1-11.

Balduini, L., M. Matoga, E. Cavalli, E. Seilles and D. Riethmuller *et al*., 2003. Triazinic herbicide determination by gas chromatography-mass spectrometry in breast milk. J. Chromatogr. B., 794: 389-395.

Bougrini, M., K. Tahri, Z. Haddi, E.N. Bari and E. Llobet *et al*., 2014. Aging time and brand determination of pasteurized milk using a multisensor E-nose combined with a voltammetric E-tongue. Mater. Sci. Eng. C., 45: 348-358.

Bueno, L., D.W.R. Araujo, M.O. Salles, M.Y. Kussuda and T.R. Paixao, 2014. Voltammetric electronic tongue for discrimination of milk adulterated with urea, formaldehyde and melamine. Chemosensors, 2: 251-266.

Cruz, M.G., N.S. Ferreira, M.T.S. Gomes, M.J. Botelho and S.T. Costa *et al*., 2018. Determination of paralytic shellfish toxins using potentiometric electronic tongue. Sens. Actuators B. Chem., 263: 550-556.

Eenennaam,V.A.L. and A.E. Young, 2017. Detection of dietary DNA, proteinand glyphosate in meat, milk and eggs. J. Anim. Sci., 95: 3247-3269.

Frazier, L.M., 2007. Reproductive disorders associated with pesticide exposure. J. Agromedicine, 12: 27-37.

Gonzalez-Calabuig, A. and D.M. Valle, 2018. Voltammetric electronic tongue to identify Brett character in wines: On-site quantification of its Ethylphenol metabolites. Talanta, 179: 70-74.

Guo, H., H. Wang, J. Zheng, W. Liu and J. Zhong *et al*., 2018. Sensitive and rapid determination of glyphosate, glufosinate, bialaphos and metabolites by UPLC-MS/MS using a modified quick polar pesticides extraction method. Forensic Sc. Intl., 283: 111-117.

Hassani, E.N.E.A., K. Tahri, E. Llobet, B. Bouchikhi and A. Errachid *et al*., 2018. Emerging approach for analytical characterization and geographical classification of Moroccan and French honeys by means of a voltammetric electronic tongue. Food Chem., 243: 36-42.

Haug, A., A.T. Hostmark and O.M. Harstad, 2007. Bovine milk in human Nutrition-a review. Lipids Health Dis., 6: 1-6.

Hong, Y.Q., X. Guo, G.H. Chen, J.W. Zhou and X.M. Zou *et al*., 2018. Determination of five macrolide antibiotic residues in milk by micellar electrokinetic capillary chromatography with field amplified sample stacking. J. Food Safety, 38: 1-7.

Islas, G., J.A. Rodriguez, I. Perez-Silva, J.M. Miranda and I.S. Ibarra, 2018. Solid-Phase extraction and Large-volume sample Stacking-capillary electrophoresis for determination of tetracycline residues in milk. J. Anal. Methods Chem., 2018: 1-7.

Lipkowitz, J.B., C.F. Ross, C. Diako and D.M. Smith, 2018. Discriminating aging and Protein-to-fat ratio in cheddar cheese using sensory analysis and a potentiometric electronic tongue. J. Dairy Sci., 101: 1990-2004.

Nery, E.W. and L.T. Kubota, 2016. Integrated, Paper-based potentiometric electronic tongue for the analysis of beer and wine. Anal. Chim. Acta, 918: 60-68.

Pereira, P.C., 2014. Milk nutritional composition and its role in human health. Nutrition, 30: 619-627.

Pigani, L., G.V. Simone, G. Foca, A. Ulrici and F. Masino *et al.*, 2018. Prediction of parameters related to grape ripening by multivariate calibration of voltammetric signals acquired by an electronic tongue. Talanta, 178: 178-187.

Shamsipur, M., N. Yazdanfar and M. Ghambarian, 2016. Combination of Solid-phase extraction with dispersive Liquid-liquid microextraction followed by GC-MS for determination of pesticide residues from water, milk, honey and fruit juice. Food Chem., 204: 289-297.

Tahara, Y. and K. Toko, 2013. Electronic Tonguesa review. IEEE. Sens. J., 13: 3001-3011.

Vlasov, Y., A. Legin, A. Rudnitskaya, D.C. Natale and A. D'amico, 2005. Nonspecific sensor arrays (Electronic Tongue) for chemical analysis of liquids (IUPAC technical report). Pure Appl. Chem., 77: 1965-1983.

Winquist, F., P. Wide and I. Lundstrom, 1997. An electronic tongue based on voltammetry. Anal. Chim. Acta, 357: 21-31.