Research Journal of Applied Sciences 13 (8): 460-465, 2018
ISSN: 1815-932X

Evaluation of Milk According to Geographical Origin by Voltammetric Electronic Tongue and Physicochemical Properties

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Abstract: The principal aim of this research was to evaluate the capability of a voltammetric electronic tongue based on PSoC technology and sensor array form polypyrrole for to analyze milk samples from different geographical origin. Some physicochemical parameters was too used to evaluate the geographical origin of the milk samples. The study was carried out on milk samples collected in five zones of sucre (Colombia) with differentiated climatic and environmental characteristics. Both analytical approaches allowed with principal component analysis, the discrimination of the milk samples. However, the discrimination obtained with the electronic tongue was more in accordance with the characteristics of the samples according to their geographical area of origin. In addition, the discrimination was carried out by cluster analysis, the result showed concordance with those obtained with principal component analysis. It could be established that the analysis made with the electronic tongue allowed a better discrimination because this analytical technique presents a fingerprint of the samples while with physicochemical characterization it is only possible to use a few parameters of the hundreds that contain a complex sample like the milk.

Key words: Electronic tongue, polypyrrole, milk, voltammetry, geographical origin, parameters

INTRODUCTION

The particularities of a food product can be correlated with its geographical location or origin, especially, if it is a primary product such as milk, wine, tea, etc. The environmental conditions in a geographical area are a very important factor in determining their characteristics and particularities, quality, absence or presence of diseases or contaminants. Additionally, the culture of collection or production as well as storage techniques affect the characteristics of the food. Some countries regulate the geographical origins of some products through the protection of denomination of origin (Tosato, 2013).

Milk is one of the most consumed food drinks in the world due to its high nutritional value. The geographical area where the milk is produced determines its physicochemical and organoleptic particularities. In this sense, the development of techniques and analytical methods to determine the geographical origin of milk is of great interest for the industry. Recently, some chemical techniques based on traditional chemical principles have been developed to determine the geographical origin of some products. The most outstanding techniques are the determination of the ratio of isotopes (Park *et al*., 2018; Camin *et al*., 2017; Behkami *et al*., 2017) and spectrophotometry methods (Gaspardo *et al*., 2010; Magdas *et al*., 2016; Liu *et al*., 2018). These techniques have proven to be effective, however, they present some disadvantages such as high cost of equipment, need to pre-treat samples with complex procedures, require long processing times and must be carried out by qualified personnel, among others.Therefore it is necessary to develop analytical methods to determine the geographic origin of samples quickly, cheaply and without pre-treatments.

Electronic tongues have emerged as versatile analytical tools that can be applied in the qualitative and quantitative analysis of beverages (Vlasov *et al*., 2005; Rosa *et al*., 2017; Escuder-Gilabert and Peris, 2010). In addition a wide range of measurement systems based on voltammetric, potentiometric, impedanciometric, mass sensors, among others have been developed. The electronic tongues based on voltammetric sensors have shown to be the most versatile and have the best

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performance because their sensors are easy to elaborate, they provide more information of the samples through the voltammetric curves and their electronic systems are simpler and less susceptible to noise (Winquist, 2008; Arrieta *et al*., 2016; Gonzalez-Calabuig and Valle, 2018; Pigani *et al*., 2018). The electronic tongues have been applied successfully in a great variety of beverages.

The electronic tongues have been applied to evaluate the geographical origin of some beverages such as honey, wine, coffee, among others (Hassani *et al*., 2018; Ceto *et al*., 2015; Dominguez *et al*., 2014). Its use has not been reported to analyze the geographical origin in milk samples. However, it has been used for the analysis of milk for example to monitoring of freshness, different types of treatments, adulterated milk, among others (Wei *et al*., 2013; Arrieta *et al*., 2018; Wei and Wang, 2011).

The aim of this research was to evaluate the discrimination capacity of a voltammetric electronic tongue to classify milk samples of different geographical origin and establish relationships with discrimination obtained for some physicochemical parameters of the samples under study.

MATERIALS AND METHODS

Milk sampling and reagents: Five samples of cow milk were examined which were collected in different regions of the department of Sucre (Colombia). All the samples were fresh and were collected in the 5 natural sub-regions which have different environmental and climate peculiarities, the regions were: La Mojana, San Jorge, Sabanas, Montes de Maria and Morrosquillo (Fig. 1). The reagents were of analytical quality. The dissolutions were prepared by using milli Q quality ultrapure water.

The reagents used were: Pyrrole (Py) Sodium Dodecylbenzenesulfonate (DBS) Sodium sulphate (SO_4) Ammonium persulphate (SF) Potassium Ferrocyanide (FCN) Lithium Perchlorate (PC) Anthraquinone-2, 6-Disulfonic Acid Disodium Salt (AQDS) p-Toluenesulfonic Acid (TSA). The dissolutions were prepared by using ultrapure water quality.

Electronic tongue device: The experiments were performed with an electronic tongue device made in the laboratory. The sensor array of this device comprises of seven voltammetric electrochemical sensors from polypyrrole electrodeposited on platinum electrodes of a card AC9C BVT Technologies. Each voltammetric sensor was composed of polypyrrole doped with different counter ions. The conditions used in the elaboration of the sensors have been previously studied and previously reported. In addition, its sensitivity and cross-selectivity has been evaluated in milk samples (Arrieta *et al*., 2016, 2017, 2018).

Fig. 1: Geographical origin of the examined milk samples: yellow (region of Morrosquillo) pink (region of Montes de Maria) green (region of Sabana) orange (region of San Jorge) and lila (region of La mojana)

Table 1: Electropolymerization conditions of the polypyrrole sensor array

Sensor	Acronym	Counterion concentration (mol/L^{-1})	Time (sec)
S1	PPy/DBS	0.10	45
S2	PPy/SO_4	0.05	60
S3	PPy/SF	0.05	70
S4	PPy/FCN	0.10	50
S5	PPy/PC	0.10	60
S6	PPy/AQDS	0.05	70
S7	PPy/pTS	0.10	60

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

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). Figure 1 is presented an image of the electronic tongue device.

Physicochemical characterization: The physicochemical parameters analyzed were pH, fat, lactose, protein, total solids, solids not-fat, acidity and density. The pH of the milk samples was determined at the collection point using a digital pH-m (Metrohm 780 pH m). The pH meter device was calibrated using buffers of pH 7.0 and 4.0 each time before the pH of milk sample was measured. The fat content was determined by Gerber method. Lactose content was determined by ferricyanide method. The crude protein content of milk samples were determined by Kjeldahl method.The total solid and solid not-fat were determined by gravimetric method. Acidity of the milk samples was determined by volumetric method. The density determination was accomplished using a thermolactodensimeter (GerberSmall Model). All milk samples were analyzed by triplicated and the value was averaged.

Measurementsand multivariate data analysis: The measurements with electronic tongue were made at room temperature in 5 mL of sample. The milk samples were measured in triplicate by cyclic voltammetry with scan rate of 0.1 $Vsec^{-1}$ and a potential range of -1.0 to -0.5 V. The data were organized in matrix form, using as inputs data the current values registered in the cyclic voltammetric signals of the sensor array.

On the other hand, a matrix was made with the data from all the physicochemical parameter measurements performed. The matrix was formed by 8 columns (one for each physicochemical parameter) and by 15 rows (5 samples with 3 replicas of each sample).

The statistic multivariate analyzes (Principal component analysis and cluster analysis) were carried out to the data matrices obtained with the electronic tongue and the physicochemical characterization without any type of pre-treatment with the MINITAB V 18 Software.

RESULTS AND DISCUSSION

Electronic tongueresponse: The first stage consisted in evaluate the voltammetric responses for each of the sensors towards milk samples to assure that the generated voltammetric signals are different enough and the supplied data provide rich information enough to be used in to milk samples discrimination. Figure 2 shows the responses of sensor array toward a milk sample. It can be observed that each sensor in the array shows peaks related with redox processes of the polypyrrole, since, this conducting polymer have electroactive properties.

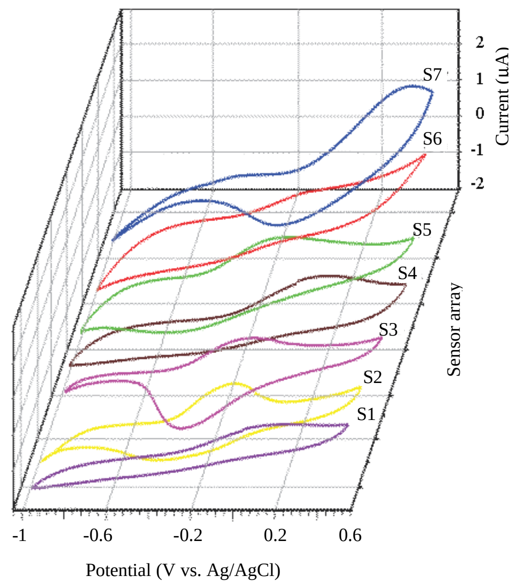

Fig. 2: Voltammetric response of polypyrrole sensor array: purple (PPy/DBS) yellow (PPy/SO4) lilac (PPy/SF) black (PPy/FCN) green (PPy/PC) red (PPy/AQDS) blue (PPy/pTS)

The curves showing two processes, one in the direct cathodic sweep (oxidation) and one in the reverse anodic scan (reduction). The detail of this redox processes had been reporter in previous research (Arrieta *et al*., 2015, 2016, 2017, 2018; Almario and Fuentes, 2016). Voltammograms of milk samples exhibited oxidation and reduction peaks dependent on geographical origin, this is named as cross-selectivity. Agree to IUPAC, cross-sensitivity is a capability of a non-selective sensor array to respond to a number of different analytes in the complex sample (Vlasov *et al*., 2005).

The data of variation in current supplied by voltammetric signals from polypyrrole sensor array obtained of measurement of milk samples were collected in a matrix. The matrix was used to carry out the Principal Component Analysis (PCA) which is an unsupervised method was established using 15 samples (5 milk samples×3 replicas) to determine

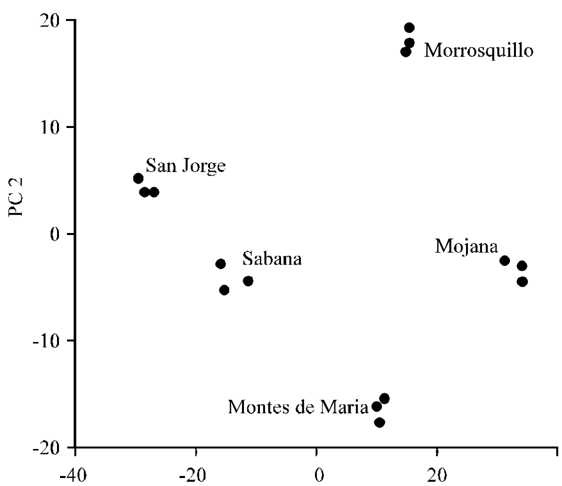

Fig. 3: PCA score plot of obtained by mean of milk samples from different geographical origin with electronic tongue

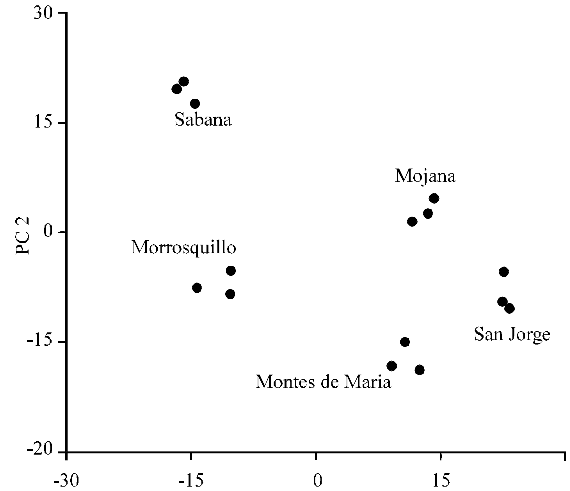

Fig. 4: PCA score plot of obtained by mean of milk samples from different geographical origin with physicochemical parameters

the capability of the electronic tongue to distinguish between milk samples from different geographical areas.

Figure 3 shows a PCA score plot of the measurements performed by the voltammetric electronic tongue in which the first two principal components explain nearly 89.3% of the total variance, 51.8% the first Component (CP 1) and 37.5% the second Component (CP 2). As can to be observed it was possible to discriminate clearly among the different milk samples. The scores plot reveals a separation among all milk samples which can be attributed to the differences in the chemical composition of milk according to their geographical origin.

The spatial distribution of the milk samples in the graph obtained with the principal component analysis (Fig. 3) shows a good correlation with what is expected by the climatic and environmental characteristics of the zones of origin of the samples because the samples from San Jorge and Sabana that are areas with certain geographical similarities are closer while the other samples that belong to the rest of the zones that are geographical different are more separated.

The obtained data of the physicochemical characterized milk samples were also analyzedfor PCA, the resulting score plot is shown in Fig. 4. The first two principal components explain nearly 96.7% of the total variance, 64.2% the firs Component (CP 1) and 32.5% the second Component (CP 2). As observed, milk samples were classified and a clear difference can be observed between them. However, the spatial distribution of the samples in the score plot does not bear much relation to what was expected for to the particularities of the samples and their geographical origin. The best response of the electronic tongue is perhaps due to the fact that the physicochemical analysis in this study only includes a few parameters and milk is a sample that comprises more than a thousand compounds.

To verify the veracity of the results obtained with the principal component analysis performedwith the two analytical approaches, a cluster analysis was carried out. Figure 5 presents the dendrogram obtained when applying the cluster analysis to the data matrix obtained from the measurements made with the electronic tongue. As can be observed a clear differentiation of each one of the milk samples was obtained and like the results of the PCA, the relationship between the samples conserves a distribution that keeps some correspondence with the climatic and environmental peculiarities of its geographical origin, maintaining less separation between samples from the San Jorge and Sabana areas.

The dendrogram obtained from the realization of the cluster analysis in the data matrix of the physicochemical parameters is presented in Fig. 6. It can be seen that it was possible to discriminate each one of the samples but its distribution not related to the particularities of the geographical origin of group of milk samples.

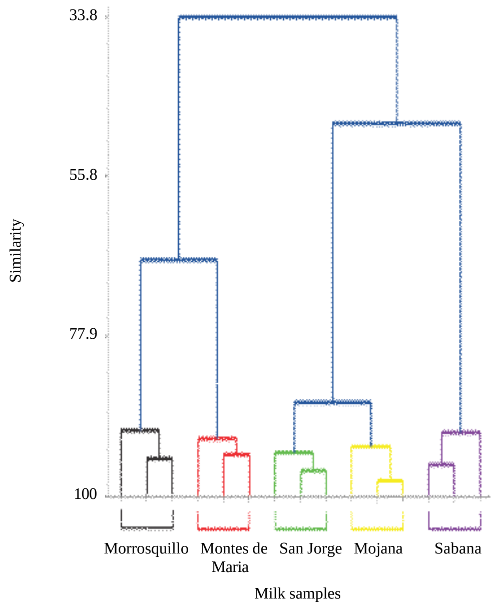

Fig. 5: Dendrogram from cluster analysis in Euclidian distance for electronic tongue analysis of milk samples: black (region of Morrosquillo) yellow (region of Mojana) red (region of Montes de Maria) purple (region of Sabana) green (region of San Jorge)

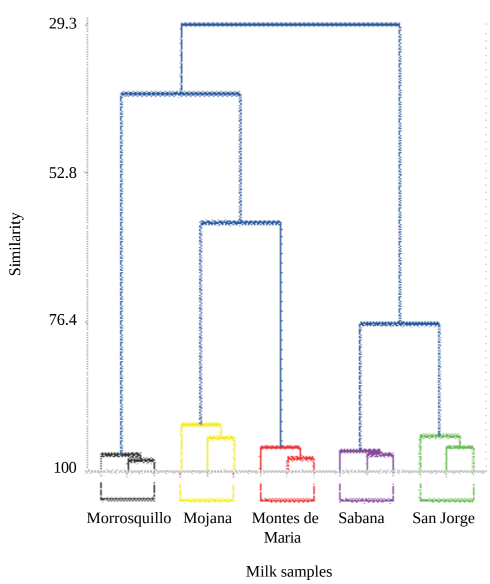

Fig. 6: Dendrogram from cluster analysis in Euclidian distance for physicochemical parameters of milk samples: black (region of Morrosquillo) red (region of Montes de Maria) green (region of San Jorge) yellow (region of Mojana), purple (region of Sabana)

CONCLUSION

A voltammetric electronic tongue based on PSoC technology with a sensor array elaborated from doped polypyrrole with different counterions was able to discriminate milk samples from different geographical origins.

The distribution of the samples obtained by Principal Component Analysis (PCA) and Cluster Analysis (CA) was related to the characteristics of the geographic areas of origin.

The physicochemical parameters used in this study allowed the classification of the samples by means of PCA and CA. Nevertheless, the distribution does not keep much relation with the particularities of the samples.

Although, it was possible to discriminate the samples with both analytical approaches, the classification made with the electronic tongue was more in agreement with the characteristics of the samples because with this approach the information collected of the sample is obtained from all the chemical complexity due the electronic tongue do not obtain information about the nature of the chemical compounds under consideration but only present a digital fingerprint of the beverage. While that the physicochemical parameters were few for a sample as complex as milk.

ACKNOWLEDGEMENT

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

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