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GEOGRAPHICAL ORIGIN AUTHENTICITY DISCRIMINATION OF MILK BASED ON SMART ELECTRONIC TONGUE

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ABSTRACT
The aim work was to evaluate the capacity of a smart electronic tongue in the geographical origin discrimination of milk samples of different provenance. The analysis was carried out with a smart electronic tongue formed by a polypyrrole voltammetric sensor array doped with different doping agents, a multichannel potentiostat, and an Android app installed on a smartphone for signal recording, data storage, and device control. The analyzed samples were collected in different localities belonging to different agroclimatic and environmental zones in Cordoba - Colombia. The registered voltammetric signals showed particular responses to each one of the milk samples, evidencing cross-sensitivity, which constituted a "fingerprint" of the samples with information about their characteristics. The pattern recognition analysis showed that the smart electronic tongue was able to discriminate each sample. In addition, the spatial distribution of the samples showed a certain correlation with the subregions of origin. The results made it possible to establish that the smart electronic tongue could be capable of discriminating milk samples of different geographical origins and that its classification may be related to the particularities of the areas of origin.
Keywords: Geographical Origin, Milk, Polypyrrole, Sensor array, Smart Electronic Tongue
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INTRODUCTION
Milk is one of the most popular drinks worldwide due to its high nutritional content, especially for people with undernourishment problems and in the early stages of growth. Milk is a product that can be consumed in the form of a drink with its original flavor, flavored milk, and in processed presentations such as yogurts, ice creams, and milkshakes, among others. In addition, milk has benefits for human health such as the treatment of osteoporosis and some nutritional deficiencies.[1] This has meant that world milk production has increased by 59% in recent decades, with an approximate production of 843 million tons in 2018.[2] The chemical composition and organoleptic properties of milk are affected by factors such as the type of plants or food that cows eat and therefore, the composition of the soil where they graze, and climatic and geographical conditions, among others. The foregoing makes the origin or geographical area a determining element in the chemical and sensory quality of the milk. Like other food products, milk is the subject of fraudulent practices that seek to increase economic profitability. For this reason, product traceability has become a valuable tool for detecting and avoiding possible fraud. In this type of traceability process, it is very important to be able to guarantee the geographical origin of products. Various analytical methods have been applied to establish the traceability of milk. The most prominent are the isotope ratio of bio elements, the elemental profile or multi-element analysis (Cu, Ba, Zn, Mn, Fe, Al, and Cu), and visible and near-infrared spectroscopy.[3-5] These methods have shown a good ability to discriminate milk samples of different geographical origins. However, some methods require the use of sophisticated and expensive equipment, the techniques require samples pre-treatment and processing time, and the equipment is not portable, therefore not available for in-situ analysis. In recent years, electronic tongues have been developed and tested in different types of food matrices in qualitative and quantitative analyzes. This type of equipment

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has some advantages over traditional methods (GC-MS, isotope, element fingerprints, spectroscopy, etc.); it is low cost, versatile, they can be portable, easy to handle, and allows rapid analysis as no pretreatment of samples is required. However, as it is still a technology in development, it must be tested in different analytical applications because its main disadvantage is the low quantitative sensitivity of the sensor array. An electronic tongue is an analytical device integrated with a cross-sensitivity sensor array, coupled with a multi-channel measurement device and computer equipment capable of pattern recognition.[6] The operation of electronic tongue systems is based on the use of a matrix of nonspecific chemical sensors with partial selectivity (each sensor registers a particular response against a certain sample), coupled with a system of data acquisition and processing. These devices have shown their ability to analyze samples of wine, beer, tea, milk, and coffee, among others.[7-11] Electronic tongues based on electrochemical measurements have been very well received due to their great versatility. Electrochemical measurements can use a wide variety of sensors or modified electrodes of the potentiometric or voltammetric type.[12-14] The use of electrochemical electronic tongues has been reported to discriminate samples of different geographical origins such as coffee, honey, apples, and wines, among others.[15-20] However, no reports have been found on the application of this type of device for the geographical origin discrimination of milk samples, much less the application of a portable device based on Android technology, which gives it the possibility of carrying out in-situ measurements. The purpose of this work is to present the application of a smart electronic tongue elaborated with a polypyrrole electrochemical sensor array, PSoC, and Android technology for the geographical origin discrimination of milk samples.

EXPERIMENTAL

Material and Methods

The reagents used were from Sigma-Aldrich (analytical quality), and synthesis solutions were made with ultra-pure water. The reagents used in the elaboration of the polypyrrole sensor array were: Pyrrole was used as a monomer. As doping agents were used ammonium persulphate (SF), sodium dodecylbenzene sulfonate (DBS), lithium perchlorate (PC), sodium sulfate (SO_4), potassium ferrocyanide (FCN), anthraquinone-2,6-disulfonic acid disodium salt (AQDS), and p-toluene sulfonic acid (TSA). The milk samples consisted of raw cow's milk samples. To eliminate variability factors, the samples were collected from cows of the same breed *(Holstein*), age (6 years), lactation period (10 weeks), and date. The samples were collected in 8 different towns of the department of Córdoba (Colombia), on the Caribbean Plain at 7° 22' and 9° 26' north latitude and 74° 47' and 76° 30' longitude west of Greenwich. 5 replicas of each sample were collected, for a total of 40 samples (8 different geographic origins x 5 replicas of each). The department of Córdoba is crossed by the Sinu River and has different environmental and agroclimatic subregions with particularities that can influence the global characteristics of the milk produced. The samples were collected in 8 municipalities that belong to 4 subregions; Monteria (8°45′36″N 75°53′08″O), Cienaga de Oro (8°52′30″N 75°37′16″O), and San Pelayo (8°57′28″N 75°50′15″O) that belongs to the Sinu Medio subregion, which is characterized by presenting tropical dry forest, extensive alluvial soils generated by the dynamics of the Sinu River. Broadly, they are deep soils of moderate to high fertility. Lorica (9°14′19″N 75°48′50″O) and Purisima (9°14′11″N 75°43′25″O), which belong to the Bajo Sinu subregion, are areas with diverse ecosystems, bathed by the Sinu river and the big Lorica swamp, therefore it is characterized by swampy and wetland ecosystems. Sahagun (8°57′02″N 75°26′44″O), which belongs to the Savanna subregion, is characterized by flat areas (63%), undulating (32%), and ravines (5%). The soils are evolved and well-drained, so they are dry most of the year, in this subregion underground water is used, which influences the type of vegetation and agricultural activity. San Bernardo del Viento (9°21′18″N 75°57′16″O) and Puerto Escondido (9°01′09″N 76°15′41″O) belong to the Coastal subregion, which is characterized by slightly saline, dry, and flat soils, with moderate to low fertility. In Figure 1, the map of Córdoba is presented, indicating each of the sample collection areas. All agroclimatic and environmental differences in the geographical areas can be a determining factor in the characteristics of the milk produced in each of the subregions.

Smart Electronic Tongue Setup

The smart electronic tongue was elaborated in the laboratory. The device consisted of a voltammetric sensor array made up of 7 polypyrrole sensors each doped with a different doping agent, a multichannel potentiostat, and a smartphone with an Android app for signal recording, data storage, and device control. The sensor array was elaborated on an AC9C of BVT Technologies screen-printed electrochemical sensors card. Polymerization was performed by chronoamperometric electropolymerization using a potentiostat/galvanostat PAR EG&G 2263 and Power-Suite software of Princeton Applied Research.

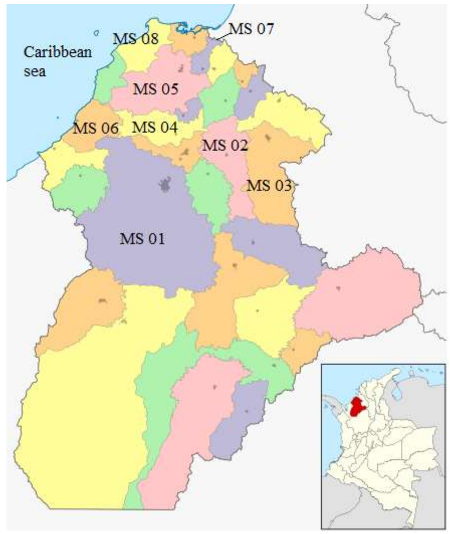

Fig.-1: Geographic Origin Map of Milk Samples Under Study: MS-01 (Monteria); MS-02 (Cienaga de Oro); MS-03 (Sahagun); MS-04 (San Pelayo); MS-05 (Lorica); MS-06 (Puerto Escondido); MS-07 (Purisima); MS-08 (San Bernardo del Viento)

In all cases, a pyrrole monomer concentration of 0.1 M was used. For the doping agents, a concentration of 0.1 M was used for DBS, PC, FCN, and TSA and 0.05 M for SF, SO4, and AQDS. The polymerization time was 50 s for DBS; 55 s for SO4; 60 s for FCN and AQDS; 65 s for SF and PC; and 70 s for TSA. The configuration of the sensor array was as follows: PPy/SF (S1), PPy/DBS (S2), PPy/PC (S3), PPy/SO4 (S4), PPy/FCN (S5), PPy/AQDS (S6) and PPy/TSA (S7). Multichannel potentiostat consisted of electronic equipment capable of producing simultaneous voltammetric signals through seven independent channels. This device was made on a FREE SOC card with a programmable microchip (PSoC LP5), programmed with PSoC creator software. In addition, the device had a built-in Bluetooth card to control and transmit wirelessly the registered voltammetric signals to a smartphone equipped with an Android app to record the signals and store the data. Details of the development of each of the components (sensor array, multichannel potentiostat, and Android app) have been previously reported.[10,19,20]

Smart Electronic Tongue Analysis

Smart electronic tongue measurements were carried out on 10 mL of milk sample. The device was programmed to record voltammetric signals at 100 mV s^{-1} scan rate, -1.0 V to 0.5 V potential range, and starting the sweep at 0.0 V. To convert the voltammetric signals (current signals) into data, an Android app was developed capable of taking the signals generated by the sensors, transforming them into normalized data, organizing them in a matrix and generating a text file (.txt). Details of this development were previously published.[19] To evaluate the smart electronic tongue discrimination capacity, the generated data matrix was employed to carry out a pattern recognition statistical analysis through artificial neural networks for cluster analysis and principal component analysis. These analyses were carried out in Matlab 9.6 software. Figure-2 shows an image of the smart electronic tongue where its three components are identified; the electronic measurement system (multichannel potentiostat), the smartphone with the data control, recording, and storage app, and the sensor array made up of the seven polypyrrole sensors with different dopants (S1, S2, S3, S4, S5, S6, and S7), Ag/AgCl reference electrode (RE) and platinum counter electrode (CE).

RESULTS AND DISCUSSION

The measurements made with a smart electronic tongue were carried out once the samples had been collected. The sensor array was washed with water before each measurement and a first conditioning measurement was carried out, which was discarded. The measurement time was approximately 4 minutes per sample, so it took about 160 minutes to record the voltammograms of the 40 samples. Figure-3 shows the voltammetric signals registered with a smart electronic tongue in milk samples of different geographical origins. It can be observed that each sensor presents a particular voltammetric response to each one of the milk samples so that the sensor array presented a high degree of cross-sensitivity and the set of registered signals can be considered a "fingerprint" of the samples analyzed.

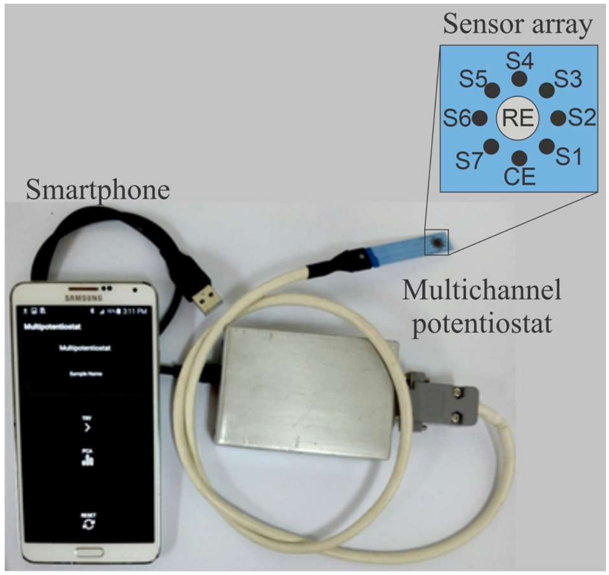

Fig.-2: Smart Electronic Tongue Conformed by Sensor Array, Multichannel Potentiostat, and Smartphone with Android App for Device Control

The voltammetric signals from the sensor array showed oxidation and reduction peaks due to the electroactivity of the polypyrrole doped with each doping agent and its interaction with the components in the complex matrix of the milk samples, which generates that the shape of the peaks varies in shape, position, intensity, and width, allowing global information to be extracted from the characteristics of the samples. Figure-4 shows the graph generated by the principal component analysis. The two principal components collected 96.1% of information (variance); 68.3% in the first principal component (CP 1) and 27.8% in the second principal component (CP 2). It can be seen that different milk samples were discriminated. The spatial distribution of the samples denotes a certain correlation with the geographical origin. The samples that were collected in the Sinu Medio subregion (Monteria – MS-01; Cienaga de Oro – MS-02 and San Pelayo – MS-04), were located in the negative quadrant of CP 1 and positive of CP 2. The samples collected in Lorica – MS-05 and Purisima -MS-07 which belong to the Bajo Sinu subregion, were located close in the negative quadrants of CP 1 and CP 2.

On the other hand, the sample collected in Sahagun, which belongs to the Savanna subregion, was located a little distance from the others, in the positive quadrant of CP 1 and negative of CP 2. The samples from the Coastal subregion collected in Puerto Escondido – MS-06 and San Bernardo del Viento – MS-08, were both located in the positive quadrant of the two principal components CP 1 and CP 2). The above indicates that in addition to having been able to make good discrimination of the samples, their distribution showed a certain correlation with the subregions of origin and therefore with the agroclimatic and environmental conditions of the zones. The discrimination of the samples and their spatial distribution in the quadrants of the principal component analysis graph showed some relationship with the agroclimatic and environmental characteristics of the geographical areas of origin. Thus, the samples MS-01, MS-02, and MS-04, belonging to the middle Sinu subregion formed a macro-group, and the samples MS-03 belonging to the savanna subregion were located a little distance from the rest. The samples from the coastal sub-region MS-06 and MS-08 were located close to each other forming a macro-group, as were the samples from the Bajo Sinu sub-region MS-05 and MS-07. As previously described, these subregions present different agroclimatic and environmental characteristics, in such a way that it is to be expected that the vegetation, water availability,

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and livestock management techniques will be different, which can generate differences in the chemical and organoleptic characteristics of milk.

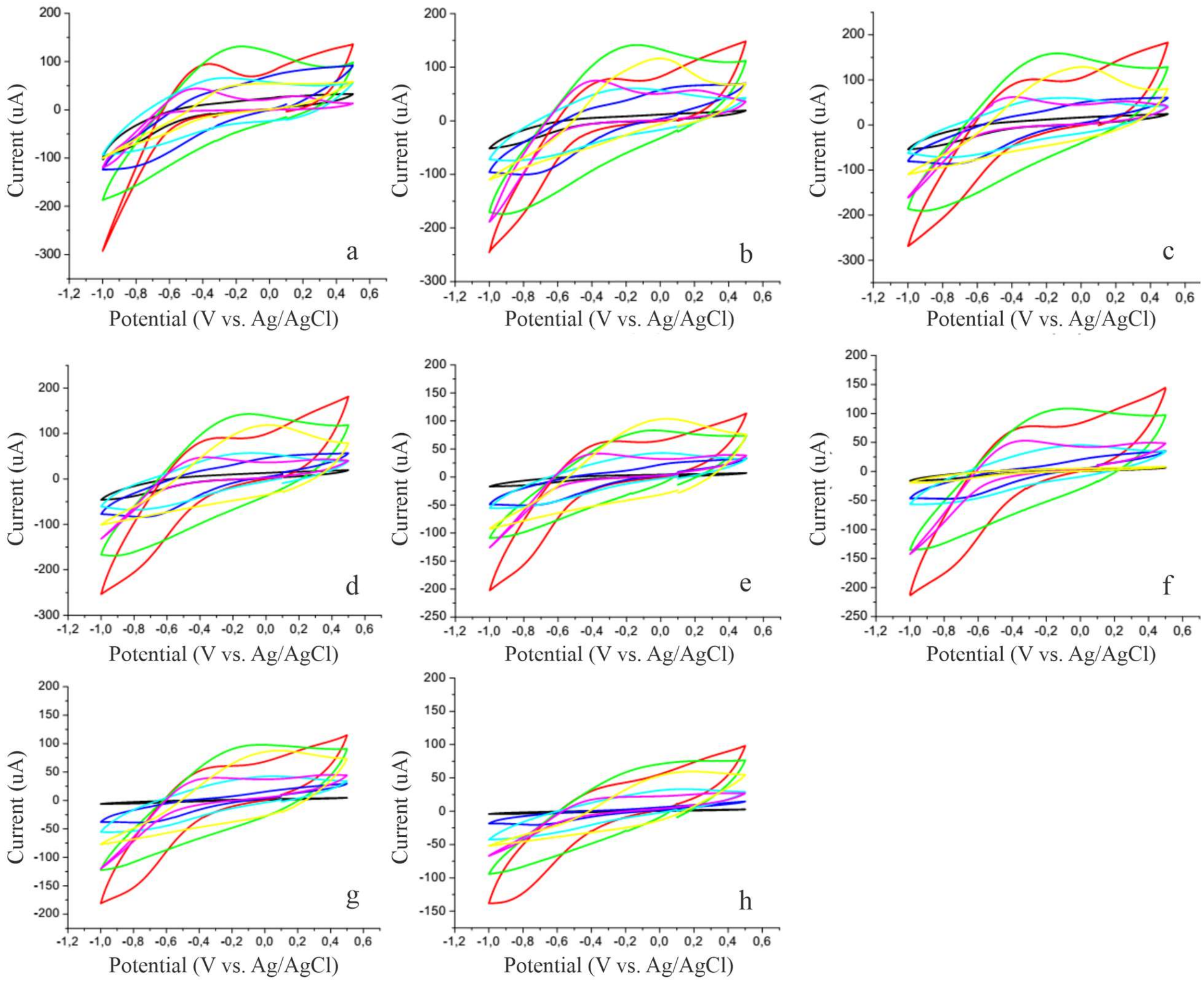

Fig.-3: Voltammetric Response of Smart Electronic Tongue Immersed in Milk Samples of Different Geographic Origin a) MS-01, b) MS-02, c) MS-03, d) MS-04, e) MS-05, f) MS-06, g) MS-07 and h) MS-08

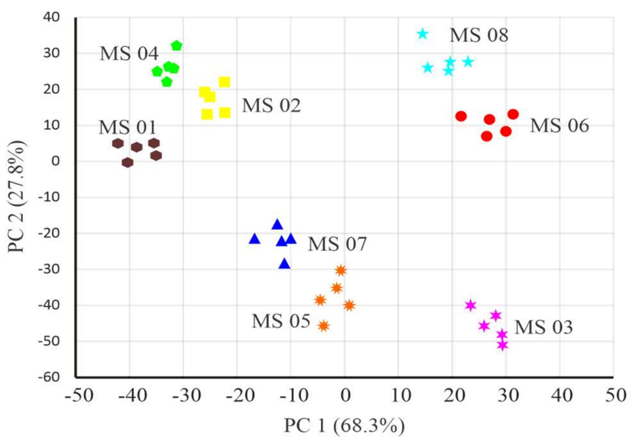

Fig.-4: Principal Component Analysis Graph of Milk Samples of Different Geographic Origin

In order to check the smart electronic tongue's ability to discriminate different geographical origin milk samples, a cluster analysis was carried out. The Euclidean distance was used to estimate the coefficient of similarity of the samples. The resulting dendrogram or similarity tree is presented in Figure-5.

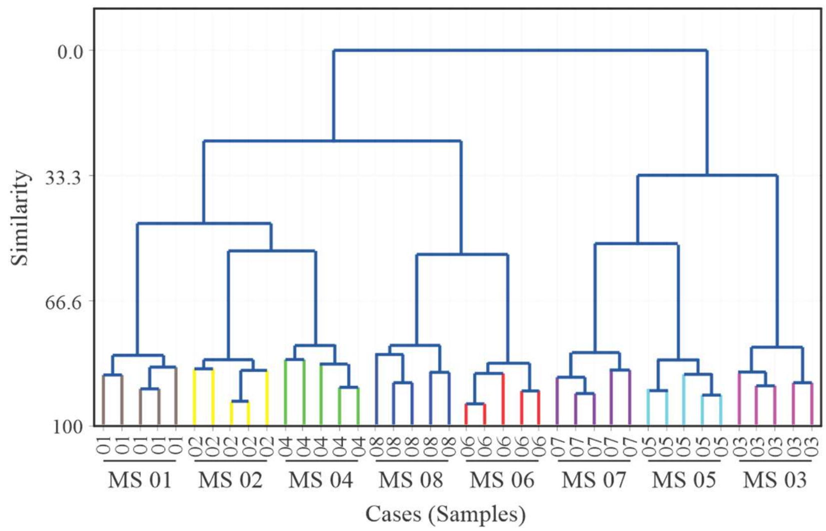

Fig.-5: Cluster Analysis Graph of Milk Samples of Different Geographic Origin

It could be observed that the eight samples of different geographical origin form well-defined groupings with levels of similarity between 15.9 and 21.6, clearly differentiating one from the other. On the other hand, some macro-groups were observed; a macro-group formed by the samples MS-04 and MS-02 with a similarity of 45, 7 which at the same time was grouped with the samples MS-01 with 53.5 of similarity. Additionally, two macro-groups formed by MS-08 with MS-06 (45.6 similarities) and MS-07 with MS-05 (47.8 similarities) were observed. The macro-group formed by the samples MS-07 and MS-05 presented a similarity of 66.1 with the sample MS-03. The results obtained from the cluster analysis were consistent with those obtained with the principal components analysis, where clear differentiations of the samples were observed by geographical origin and three macro-groups that correlate with samples collected in sites with certain agroclimatic similarities.

CONCLUSION

A smart electronic tongue integrated with a sensor array composed of 7 polypyrrole sensors doped with different doping agents showed cross sensitivity against different geographical origin milk samples. The signals registered by the smart electronic tongue allowed the discrimination of different geographical origin milk samples from Cordoba (Colombia), through a pattern recognition statistical analysis using artificial neural networks for the analysis of principal components. In addition, the spatial distribution of the samples in the principal components graph showed a certain relationship with the areas of origin of the samples, presenting macro-groups that corresponded to the agroclimatic and environmental subregions.

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