Classification of Epigallocatechin and Catechin in Impedometric Mode using PCA

Mopsy Dhiman, Pawan Kapur, Madan Lal Singla, Abhijit Ganguli

Abstract*— *Due to the presence of innumerable compounds and their diverse contribution to tea quality an assessment of tea quality is a difficult task. As a result tea samples are assessed by experienced tea tasters and an instrumental evaluation of tea quality is not practiced in the industry. There had been a very few reports where instruments like electronic tongue/electronic nose has been used for the discrimination of taste of tea samples. In this paper, an Impedance study has been carried out at Epigallocatechin and Catechin levels present in black tea using Glassy Carbon electrode and its fingerprint mapping was done using Principal Component Analysis. Similar data has been generated from the known individual antioxidant compounds and the respective mixture. The antioxidant level has been also extracted from the complex structure of the other antioxidants present in black tea. It has been found that impedance data and their PCA have been able to clearly discriminate the presence of these two compounds. The reproducibility has been studied continuously for about month's time which lies within the + 2% of the output.

Index Terms* - *Fingerprint Mapping, Principal Component Analysis, Antioxidants, Impedance.

I. INTRODUCTION

Tea is one of the world's most popular beverages, second only to water, prepared by water infusion of dried leaves from Camellia sinensis. Consumption of teas may be associated with potential health benefits. The major groups of antioxidants in tea are flavonoids that appear to be digested, absorbed and metabolized by the body. Furthermore, as well as demonstrating antioxidant activity in vitro they also appear to have antioxidative potential in vivo.Tea is a complex mixture of components presenting both volatile and non volatile compounds, responsible for aroma and flavor. Various kinds of polyphenols present in tea are phenolic acids, flavonoids (catechin), tannins and other phenolics [1]. Catechins, a group belongs to flavonoids, have been reported to be the most effective antioxidants in tea [2-3]. However, polyphenol content is varied with factors, like tea varieties, origins, plantation area, tea types, infusion conditions and degree of fermentation [4-5]. (Evaluation of black tea quality is a complex task and is carried out by experienced tea tasters in the industry. However, human panel tasting is purely subjective and suffers from inconsistency and unpredictability due to various human factors like individual variability, decrease in sensitivity due to prolonged exposure, fatigue, and variable mental states. Traditionally, quality of beverage was measured by human sense, which includes vision, olfaction and gustation. In recent times, chemical, physical and analytical tools are being used for quality check and its control. Many researchers have worked in this field [6-12] to identify the pharmacological and physiological effects of various types of tea.

Tea quality is also assessed by many different analytical instruments like gas chromatography (GC) [13] high-performance liquid chromatography (HPLC) [14], and capillary electrophoresis (CE) [15]. However, all these methods are time consuming, laborious, and expensive. Moreover the innumerable compounds present in tea and their multidimensional contribution makes the objective estimation of tea quality extremely difficult [16]. This led to the necessity of instruments like electronic nose and tongue that can give fast, reliable, and repeatable results.

An electronic tongue is an array of sensors that work on the liquid samples. The response of the sensors is not specific, i.e., they do not respond to a particular constituent of the aqueous solution but to the whole for all the constituents of a particular solution [17]. The collective response of the tongue sensors varies from solution to solution due to the presence of different compounds and ions. Electronic tongue sensors, in general, make use of this collective response, and various electrochemical methods have been exploited for such analysis. Potentiometry, voltammetry, and conductometry are some of the measurement techniques that have been used successfully in electronic tongues for the classification of wine [18], fat content of milk [19] several beverages like tea, beer, and juice [20-22] water quality [23] and other food stuffs like tomatoes [24] .In the context of black tea, a pioneering work has been done by Ivarsson et al. [17], where discrimination of tea by means of a voltammetric electronic tongue using different applied waveforms has been established. An amperometric electronic tongue has been used for the quantification of tea astringency [25].Furthermore; a self-polishing electronic tongue using buffered tea samples has been described in [26]. Another interesting work has been reported using potentiometric electronic tongue to determine the taste of Korean green tea [27]. Impedance techniques have been used as one of the important tool [16] for quality assessment of various types of beverages. Due to complex analysis several chemo metric techniques are being applied to data treatment [29] like principal component analysis (PCA), soft independent modeling of class analogy (SIMCA), hierarchical cluster analysis (HCA), canonical analysis (CA), discriminant analysis (DA), principal component regression (PCR) and

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partial least square analysis (PLS). In the present study, Glassy Carbon (GC) electrode has been deployed to measure the impedance of individual antioxidant compound dissolved in water and their mixture. PCA was applied for classification purpose of these antioxidants. It was observed that EGC and CAT can be classified very clearly in the antioxidant mixture.

II. EXPERIMENTAL SECTION

A. *Materials and method (Electrodes and standards used)*

Glassy Carbon (GC) working electrode, Platinum (Pt) wire counter electrode and Ag/AgCl (4M KCl) reference electrode were supplied by CH Instruments (Austin, USA) whereas antioxidants used, viz. CAF, GA, (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC) and (+)-catechin (C) were purchased from Sigma Chemicals Co. St. Louis, MO,USA. De-ionized (DI) water was used otherwise.

B. *Experimental*

An experimental set up of Impedance Spectroscopy is shown in Fig.1. All impedance measurements were carried out at room temperature. GC working electrode was polished with alumina powder [30] of particle size of 1.0, 0.3, and 0.05 µm stepwise to get smooth surface. Each reading was taken after washing the electrode well with DI water. The data was recorded and displayed by the software provided with the electrochemical workstation which communicates with a personal computer using a RS232 link. Pt wire and Ag/AgCl (4MKCl) were used as counter and reference electrodes, respectively. All samples were of the 1mM concentration were used in this study. Five observations per day were recorded for each sample continuously for 30 days. The analysis was carried out after generating the mean data.

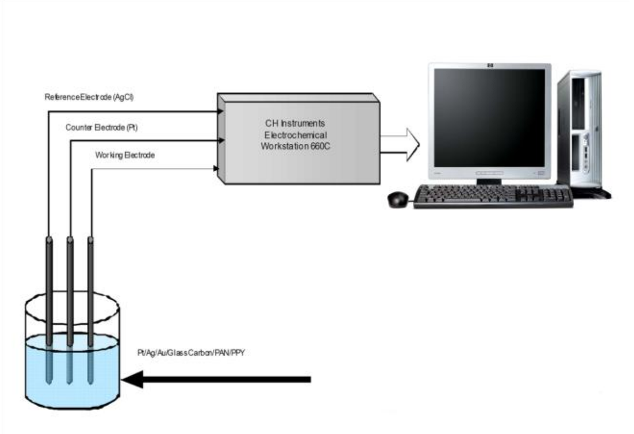

Fig.1: Set up of Electrochemical Workstation

C. *Data analysis and statistics*

Statistical Analysis software, PAST (Paleontological Statistics) was used for performing PCA. The PCA is a common pattern recognition algorithm used to analyze data obtained from impedance system [31].

In particular, PCA is used to reduce the complexity of data by computing a new, much smaller set of uncorrelated variables which best represent the original data. The application of PCA on the obtained impedance data transforms it into two or three coordinates, which yields an information-rich representative data set. The transformation actually projects the multidimensional data into coordinates that maximize the variance while minimizing the correlation in the data set.

III. RESULTS AND DISCUSSION

A. *Impedance response*

The impedance study was carried out with the open circuit potential (Eocp). Eocp of every sample was observed for 10 minutes over a frequency range of 1Hz to 100 kHz. The electrical behavior of each antioxidant solution was measured repeatedly in the above frequency range at room temperature using AC technique of complex impedance spectroscopy.

Fig.2 shows the complex impedance plots (Nyquist plots of Caffiene (CAF), Gallic Acid (GA), Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), Epigallocatechin (EGC), Epicatechin (EC) and Catechin (C) and their mixture using GC electrode. Two compounds EGC and CAT showed only semicircle formation while for other compounds straight line at an angle of 45° to the real axis has been observed.

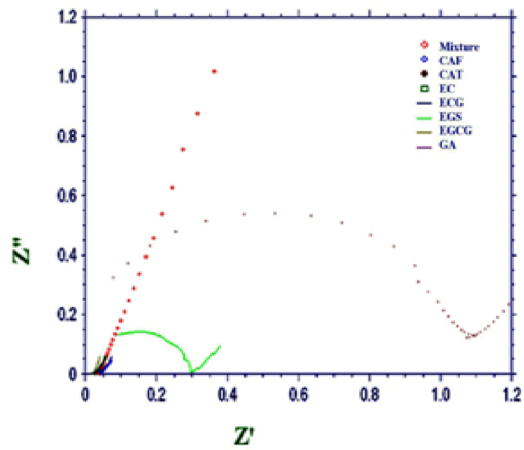

Fig.2: Nyquist plots for standard compounds used

The values of resistance, R and capacitance, C, can be obtained by an equivalent circuit of one parallel resistance-capacitance (RC) element. On the complex plot one semicircular arc arises due to RC element, representing the grain effect. For grain the equivalent electrical equations are

$Z' = R + Rg/1 + (\omega RgCg)^2$

$Z'' = Rg[\omega RgCg/1 + (\omega RgCg)^2]$ where Rg and Cg are the grain resistance and grain capacitance respectively. The CAT showed a semicircle both in high and low frequency corresponding to electron transfer limiting process. There is a short linear part at low frequency which may be attributed to diffusion limiting step of electrochemical process.

EGC Showed the first semicircle occurring in the high frequency region corresponds to the behavior of bulk EGC compound while the second in low frequency region corresponds to electrode polarization or interfacial interactions. The exact cause for this behavior is not known, however it may be due to the difference in chemical structures of these compounds. This response of CAT and EGC can be attributed to Bauerle's equivalent circuit (Fig.3).

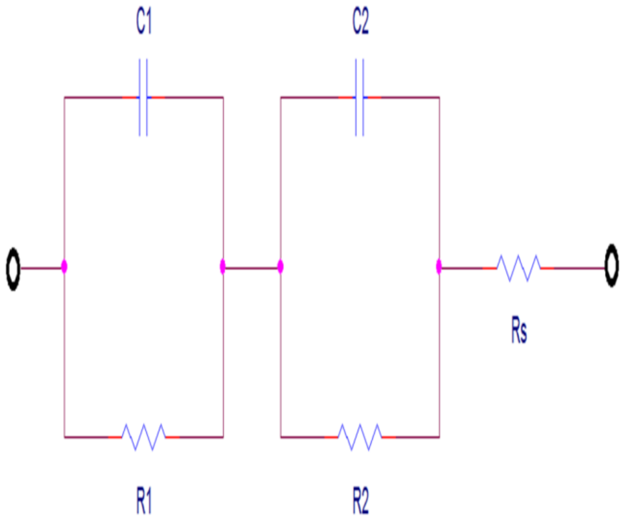

Fig.3: Bauerle's equivalent circuit

It has been found that there are short linear parts at low frequencies (for CAF, GA, EGCG, ECG, EC and their mixture) resulting from the diffusion limiting step of the electrochemical process. The respective semicircles diameters at the high frequencies correspond to the charge transfer resistance at the electrode surface. The mixture of these compounds showed a straight line at an angle of 45°, whereas the semicircular behavior has disappeared in this case which may be due to the domination of other (CAF, GA, EGCG, ECG, EC) antioxidant compounds over, CAT and EGC.

When the impedance is plotted in the complex plane, we obtained a semicircle combined with a straight line at an angle of 45° to the real axis, which may be due to Warburg impedance. The physical model explaining this phenomenon is Randle's equivalent electrical circuit (Fig.4) and the behavior can be attributed to diffusion phenomenon occurring at the solution–electrode interface. The impedance data were simulated using the Randle's equivalent circuit consisting of a parallel combination of the capacitance (C) and charge transfer resistance by redox reactions (Rct) in series with the supporting electrolyte resistance (Rs). The fitting of spectras to the equivalent circuit has indicated a good agreement between the circuit model and the real experimental data, especially at the high frequency values.

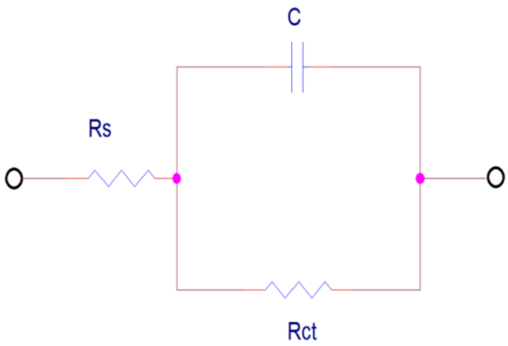

Fig.4: Randle's equivalent electrical circuit

IV. DATA ANALYSIS USING PCA

Fig.5 shows the projections of normalized PCA results on the analysis of EGC, CAF, GA, EGCG and EC, CAT, ECG and their mixture. The PCA shows the corresponding scores plot obtained after normalizing the impedance values. The data were plotted in a two dimensional plane formed by the first two PCs, that is PC1 and PC2, which captured 99.96% of the data variance. They appear to provide the best discrimination of the samples. The scatter plot of PC1 (variability; 98.94%) vs. PC2 (variability; 1.02%) is shown in Fig.5. The plot gives information about the patterns of species of antioxidant compounds present in black tea and their mixture which shows totally different patterns from the one obtained by CAT and EGC.

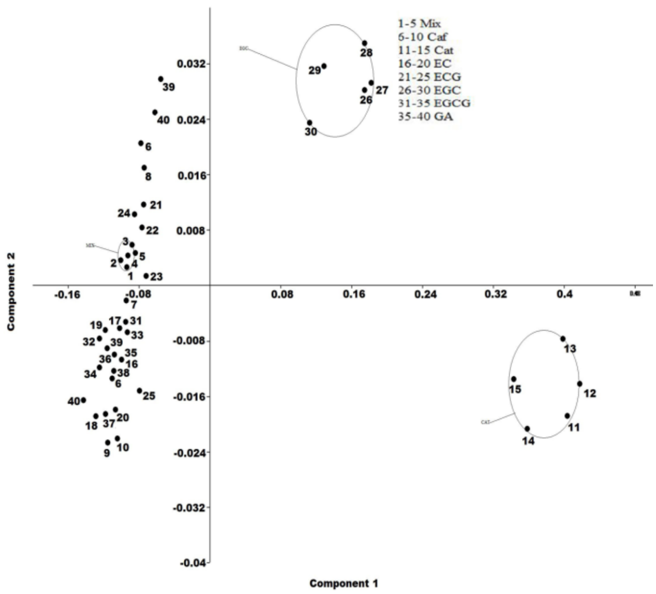

Fig.5: PCA of standard compounds and its mixture

A single PCA model including the four training sets has been constructed. The score plot showed there is an overlapping of the different antioxidant compounds even when only two PC's were used. Besides, a good discrimination between the classes was observed and the different types of antioxidants were located in different quadrants on the score plot.

Analyzing Fig.5, EGC lies in 1st quadrant and lies at positive axis of PC1 and negative axis of PC2. In 2nd and 3rd quadrant lies five of antioxidant compounds (CAF, GA, ECG, EGCG, EC and their mixture) while CAT and EGC lies separately. This indicates that these antioxidant compounds are positively correlated to one another. This can be attributed to structural similarity between these compounds, whereas CAT lies in 4th quadrant.

Samples were grouped together into different clusters; EGC and CAT are clearly separated which shows that EGC and CAT share relatively similar patterns. Using PCA model five of antioxidant compounds lie together (CAF, GA, ECG, EGCG, EC and mixture of antioxidant compounds) suggesting that they are similar and the other antioxidants (EGC and CAT) are separated from these compounds. PCA as shown has successfully been able to distinguish samples into different regions based on its unique characteristics as classified by PC1 and PC2 axis. PCA has become indispensible to multivariate analysis and was used on the impedance data obtained on scanning seven antioxidant compounds present in black tea from the known compounds along with their mixture. While in both the PCA and impedance plots the clusters corresponding to CAT and EGC are distinctly visible.

V. CONCLUSION

An Impedance study has been carried out in this paper at Epigallocatechin (EGC) and Catechin (CAT) levels present in known individual antioxidant compounds and the respective mixture. The electrochemical impedance study was carried out using CHI 660C with the open circuit potential (Eocp). The CAT showed a semicircle both in high and low frequency corresponding to electron transfer limiting process. EGC Showed the semicircle occurring in the high frequency region corresponds to the behavior of bulk EGC compound while the low frequency region corresponds to electrode polarization. The mixture of these compounds showed a straight line at an angle of 45°, whereas the semicircular behavior has disappeared in this case. Using PCA samples were grouped together into different clusters; EGC and CAT are clearly separated which indicates that EGC and CAT share relatively similar patterns. The Impedance and PCA plots show that EGC and CAT were clearly classified from other standard tea compounds. Using both the PCA and impedance plots the clusters corresponding to CAT and EGC are distinctly visible. An Exact phenomenon for these antioxidants is not yet clear. This work proposes study of antioxidant compounds using impedance mode and PCA. However, the data set is used only for few standard compounds and does not possess wide variability. The system, however, can be made versatile by incorporating the knowledge of standard compounds/tea samples from multiple gardens spread across various agro-climatic zones of India and other tea-producing countries. Nevertheless, the impedance technique described in this paper, being low cost and portable, is affordable by the tea industry and has the potential to be a useful taste-measuring instrument for day-to-day use in the black tea industry.

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