UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

DHANUS RAJ KANAGA RAJ

DEVELOPMENT OF A MULTI-SENSOR IMPEDIMETRIC ELECTRONIC TONGUE DEVICE FOR DISCRIMINATION OF BLACK TEA BASED ON CHEMOMETRICS

DESENVOLVIMENTO DE UM DISPOSITIVO DE LÍNGUA ELETRÔNICA COM MULTI-SENSORES IMPEDIMÉTRICOS PARA DISCRIMINAÇÃO DO CHÁ PRETO COM BASE EM QUIMIOMETRIA

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Dissertation presented to the Faculty of Food Engineering of the University of Campinas in partial fulfilment of the requirements for the degree of Master in Food Engineering.

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Advisor: Prof. Dr. Douglas Fernandes Barbin

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DEDICATION

My biggest supporters and Blessings of my life: my family.
To my parents Vanaja and Kanaga Raj, my sister Priyanka
and her spouse Kannan, my grandmother Rathinam, beloved
friend Kaven, Catherine and to a late girlfriend of mine, Anshika.

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ABSTRACT

Tea (*Camellia sinensis*) is the second most globally consumed beverage being palatable and consumed for its therapeutical properties. India is one of the largest consumer and exporter of black tea with cultivation site spread at different location of the country. The parameters that influence tea quality include processing methodologies and geographical origin, and they impact the final chemical composition and are responsible for distinct tea quality. The black tea in market comes in various grades and are generally categorized based on their leaf size, namely whole leaf, broken leaf, fannings and dust. It is important to have a rapid assessment of tea quality, for economic purposes, regarding tea authentication and commercialization. Traditional methods of analysing tea are done through chemical and sensorial methods which requires costly equipment like HPLC (High-performance liquid chromatography) and GC (Gas chromatography), and can be time consuming. The objective of this study is to develop and utilize an impedimetric multi-sensor device (electronic tongue) for identification of black tea from Brazil and India and determine its major composition, using chemometrics. Electronic-tongue (e-tongue) is an analytical instrument that is highly reliable, cost efficient and less time-consuming when compared to sensory analysis and other analytical techniques. The signal from the sensors is acquired as complex data to the computer, and this data is processed using multivariate analyses such as Principal Component Analysis (PCA), Partial Least Square Discriminant analysis (PLS-DA) and Partial Least Square Regression (PLSR). PCA was applied to the data, and results showed separation of Brazilian and Indian samples in PC1 and PC2, in addition to good separation between whole leaf category and the fanning and dust categories. Classification model using PLS-DA enabled to distinguish the Brazilian and Indian samples with 100% sensitivity and accuracy. PLS-DA showed the classification based on grades was not possible due to the high degree of similarities between grades. The prediction models for the reference analysis i.e., total flavonoids, total polyphenols and caffeine showed R^2_p values of 0.75, and 0.67 respectively. The novel multi-sensor impedimetric device coupled to chemometrics can be effective in discriminating black tea samples based on their origin and could find potential applications in the tea and other beverages industry.

Keywords: impedimetric multi-sensors; e-tongue; chemometrics; black tea

RESUMO

O chá (Camellia sinensis) é a segunda bebida mais consumida globalmente, sendo palatável e consumida por suas propriedades terapêuticas. A Índia é um dos maiores consumidores e exportadores de chá preto com locais de cultivo espalhados em diferentes localidades do país. Os parâmetros que influenciam a qualidade do chá incluem metodologias de processamento e origem geográfica, impactam na composição química final e são responsáveis por distintas qualidades do chá. O chá preto no mercado vem em vários graus e geralmente são categorizados com base no tamanho da folha, ou seja, folha inteira, folha quebrada, leque e pó. É importante ter uma avaliação rápida da qualidade do chá, para fins econômicos, quanto à autenticação e comercialização do chá. Os métodos tradicionais de análise do chá são feitos através de métodos químicos e sensoriais que requerem equipamentos caros como HPLC (cromatografia líquida de alta performance) e GC (cromatografia gasosa) e podem ser demorados. O objetivo deste estudo é desenvolver e utilizar um dispositivo impedimétrico multissensor (língua eletrônica) para identificação de chá preto do Brasil e da Índia e determinar sua composição majoritária, usando quimiometria. A língua eletrônica (e-língua) é um instrumento analítico altamente confiável, econômico e que consome menos tempo quando comparado à análise sensorial e outras técnicas analíticas. O sinal dos sensores é adquirido como dados complexos para o computador, e esses dados são processados usando análises multivariadas, como Análise de Componente Principal (PCA), Análise Discriminante Parcial de Mínimos Quadrados (PLS-DA) e Regressão Parcial de Mínimos Quadrados (PLSR). A PCA foi aplicada aos dados e os resultados mostraram separação de amostras brasileiras e indianas em PC1 e PC2, além de uma boa separação entre a categoria folha inteira e as categorias leque e pó. O modelo de classificação usando PLS-DA permitiu distinguir as amostras brasileiras e indianas com 100% de sensibilidade e precisão. O PLS-DA mostrou que a classificação por notas não foi possível devido ao alto grau de similaridade entre as notas. Os modelos de previsão para a análise de referência, ou seja, flavonoides totais, polifenóis totais e cafeína, apresentaram valores de R2p de 0,75 e 0,67, respectivamente. O novo dispositivo impedimétrico multissensor acoplado à quimiometria pode ser eficaz na discriminação de amostras de chá preto com base em sua origem e pode encontrar aplicações potenciais na indústria de chá e outras bebidas.

Palavras-chave: multissensores impedimétricos; língua eletrônica; quimiometria; chá preto

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SUMMARY

CHAPTER 1 - INTRODUCTION

1.1 INTRODUCTION & JUSTIFICATION

Tea, apart from their refreshing and taste relishing ability, holds significant health benefits. Black tea helps control low-density lipoprotein (LDP) cholesterol (DAVIES et al., 2003), reduces risk of stroke in long term consumption (LARSSON; VIRTAMO; WOLK, 2013), while green tea catechins has shown antimicrobial properties by intervening with bacterial cell membranes inhibiting pathogens (REYGAERT, 2018a) and prevent cancer at early onsets (YIANNAKOPOULOU, 2014). Thereby tea is an integral part of diet and has good consumer awareness of its health benefits.

Black tea (*Camellia sinensis*) is commercially produced through the four stages namely: plucking, withering, fermentation and firing. Apart from the processing conditions like fermentation that brings in major chemical changes in the tea leaves, another key factor is the geographical condition. For instance, Darjeeling tea is harvested under favorable climatic conditions and natural conditions such as slow plucking. Black tea is categorised based on their grade, geographical condition and processing methods. Quality control is required at the processing and supply chain level. Along the chain of tea processing, the product may undergo intentional and unintentional adulteration to alter tea quality in the processing stages or end product (RAKESH et al., 2017) (VERMA; BHATTI; KUMARI, 2013). Adulteration in final products unnoticed can lead to human health complication (DEB PAL; DAS, 2018). It is very important to identify product adulteration and assure quality check at each processing stages to ensure safe and good tasting tea. Traditional chemical analyzes are utilized for qualitative and quantitative studies of the tea such as high-performance liquid chromatography (HPLC), gas chromatography (GC) and spectrophotometric analyzes correlated with human sensory value for determining final tea quality (LIANG et al., 2002). These methods come with demerits of being laborious and time consuming analyses. Hence, there is a need to research non-destructive instrumental analytical methods for food analysis (AOUADI et al., 2020).

Electronic tongue is extensively used in analyzing liquid food such as honey (CIURSA; OROIAN, 2021), milk (CIOSEK; BRUDZEWSKI; WRÓBLEWSKI, 2006) , wine (RUDNITSKAYA et al., 2007), olive oil (APETREI; APETREI, 2014), fruit taste (KANTOR et al., 2008) and tea (YAN et al., 2017). The major advantage of the impedimetric multi-sensor device is that it does not require reference electrode unlike the voltametric and potentiometric e-tongues (DEL VALLE, 2010). Quality of tea is proportional to its taste and studies have reported the efficiency of multi-sensing devices in qualitative, quantitative analysis and adulterant determination in tea (GHOSH et al., 2012) (REN et al., 2021a) (LI;

LEI; LIANG, 2015).

The complex data of unrecognized pattern is collected from the analytical instrument and transferred to the computer where they are processed using various multivariate analysis technique. Chemometrics, since its inception in the 1960's, went through extensive evolution and has widened the applications in current times, being an effective statistical and mathematical tool in extracting the chemical information from the analytes. They are known for their pattern recognition (qualitative purpose) and multivariate calibration (quantitative purpose) applications. The unsupervised pattern recognition method is principal Component analysis (PCA), which is employed for exploratory analyses. The supervised pattern recognition method includes partial least square discriminant analysis (PLS-DA) and SIMCA, that are extensively used for building classification models. The difference between the unsupervised and supervised methods is defining the samples prior to data treatment. The former is commonly used as a dimensionality reduction technique meanwhile the latter focuses more on bringing the samples under class labels to build classification models.

This work proposes an impedimetric multi-sensing device (formally known as electronic tongue) in discriminating Brazilian and Indian black tea composition. The reference analyses such as total polyphenols, total flavonoids and caffeine will be used in building prediction models employing partial least square regression (PLSR) technique for quantitative analyzes.

CHAPTER 2 - OBJECTIVES

2.1 GENERAL OBJECTIVE

Conceiving and evaluating an impedimetric multi-sensor device (electronic tongue) as a reliable tool for characterization of black tea based on its composition and discrimination of samples from Brazil and India.

2.2 SPECIFIC OBJECTIVE

- Designing and assessing a multi-sensor device (electronic tongue) for liquid food samples
- Characterizing black tea samples according to their composition.
- Applying supervised methods to characterize black tea composition and classify black tea based on their origin.

CHAPTER 3- LITERATURE REVIEW

3.1 Tea

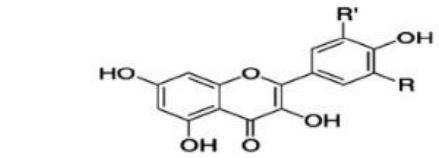
Tea (*Camellia sinensis*) consumption dates to ancient times, and are nowadays cultivated across the world, being China and India the largest producers of tea (KOTTAWA-ARACHCHI et al., 2014). The common tea types are black tea (fermented), green tea (unfermented) and oolong tea (semi-fermented), each accounting for 78%, 20% and 2% respectively, for the annual production (PRIY et al., 2015). Tea is consumed as an infusion of the dried leaves and provides health benefits to the consumer due to the antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic properties (KUMAR et al., 2007). Although green tea is arguably the healthier variety, as studies show it notably reduces risk of obesity, diabetes, and cardio-vascular issues, black tea has several benefits that makes it a highly marketed product.

3.2 Chemistry of black tea

The major components of fresh tea leaves are polyphenols, chlorophylls, carotenoids and essential constituents like lipids, carbohydrate, and vitamins (CHATURVEDULA; PRAKASH, 2013). Catechin is a polyphenolic compound that is abundantly found in green tea, making about 30% of their chemical constitution (REYGAERT, 2018b). However, the catechin fractions in black tea is only 9% because of the biochemical conversions to other compounds.

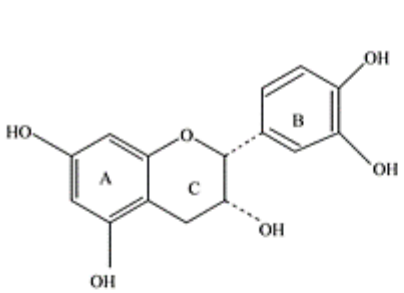
The principal catechin are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG) (SHRIVASTAVA; PATERIYA; SINGH, 2018). During the black tea processing stages, the secondary polyphenols in leaves undergo biochemical changes. Resulting in the combination of stored catechin with polyphenoloxidase (PPO) and peroxidase (PO) enzyme to form derivative polyphenolic compounds thearubigins (TR) and theaflavins (TF) (GREGORY; BENDALL, 1966). Upon completion of the tea leaves processing, the final chemical constituents of black tea include: thearubigins (12-18%), amino acids (13-15%), phenolic acids (10-12%), theaflavins (3-6%), caffeine (2.5-4.5%) and catechin (3-10%) (BUTT et al., 2014a). The key indicator of quality in black tea are the TR and TF compound and another 14 series of flavon-3-ol glycopyranosides. (SCHARBERT; HOFMANN, 2005).

The above discussion emphasises that polyphenols and flavonoids are important chemical parameters in deciding final tea quality. The chemical structure of polyphenols, flavanols and caffeine are illustrated in Figure 1, Figure 2 and Figure 3.

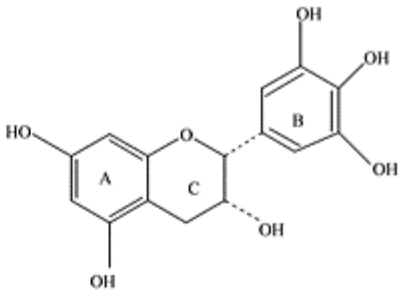


R= R'= H Kaempferol
R=H, R' = OH querecetin
R=R'= OH Myricetin

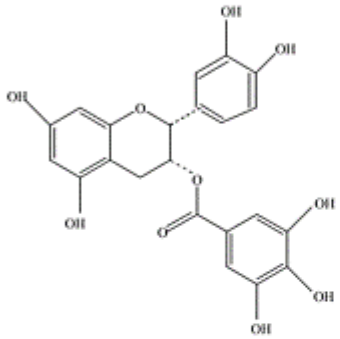
Figure 1.Structure of flavanol (Source: BUTT et al., 2014a)



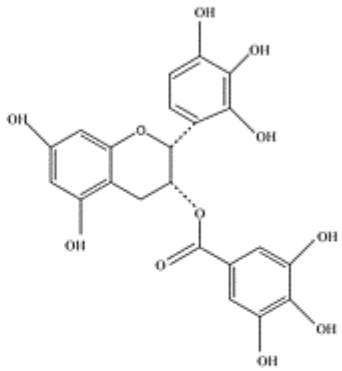
(-)-Epicatechin



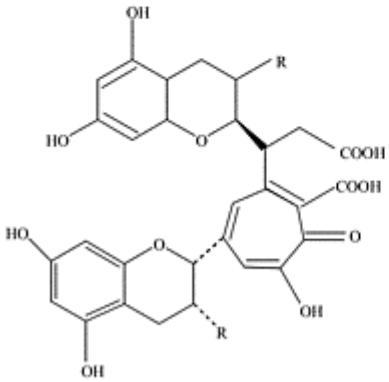
(-)-Epigallocatechin



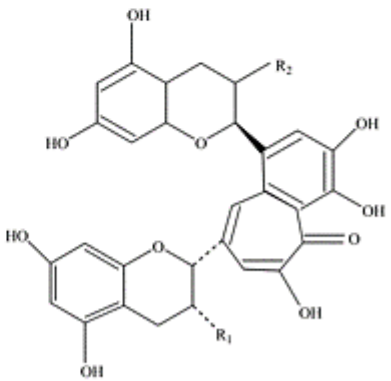
(-)-Epicatechin-3-gallate



(-)-Epigallocatechin-3-gallate



Thearubigins
(R= Gallate or other groups)



	R_1	R_2
Theaflavin	OH	OH
Theaflavin-3-gallate	Gallate	OH
Theaflavin-3'-gallate	OH	Gallate
Theaflavin-3,3'-gallate	Gallate	Gallate

Figure 2.Structure of some polyphenols in black tea (Source: SAJILATA; BAJAJ; SINGHAL, 2008)

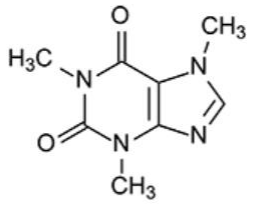


Figure 3. Structure of caffeine (Source: ABEBE BELAY, 2011)

3.3 Black tea processing

The black tea is industrially produced involving four steps: withering, maceration/rolling, fermentation and firing. Black tea is classified into orthodox teas and cut-tear-curl (CTC) teas based on their process after withering. The former uses orthodox rollers to produce large leaf teas whereas the later uses CTC machines to produce smaller particle teas (KOSIŃSKA; ANDLAUER, 2014). Depending on the geographical conditions, the plucking begins when the plant reaches optimal height and the most preferred are the fresh bud and the subsequent two youngest leaves. Mature tea leaves are also utilized to produce black tea, the final chemical composition in mature leaves varies from buds, which are not of primary importance (QHAIRUL; ABU BAKAR; MAMAT, 2013). The withering processes is performed by evenly spreading the harvested tea leaves in trays placed one above another in a closed or open chamber with monitored air circulations (BHAGAT; R.D.BARUAH; S.SAFIQUE, 2010). The withering process remains a constant pre-processing step for all types of black tea and it is important to reduce moisture and bringing important bio-chemical changes (DEB; JOLVIS POU, 2016). Then the withered leaves are passed through a rotorvane and undergo the CTC process, followed by the fermentation. The fermentation process involves the formation of oxidized polyphenolic compounds thearubigins (TR) and theaflavins (TF) from the enzymatic oxidative reaction of catechins. The green tea manufacturing excludes this step of oxidative fermentation to preserve catechins (POU, 2016b). The optimal temperature and time for fermentation based on studies suggest 25° C and 60 minutes respectively to produce good quality teas (ASIL; RABIEI; ANSARI, 2012). Drying terminates fermentation by reducing the moisture content, and helps preserve samples.

3.4 Black tea classification: Origin and grades

There exist over thirty species of tea plant, among them *Camellia sinensis* and *Camellia assamica* are preferred due to their abundant bioactive chemicals such as polyphenols, alkaloids, flavonoids, triterpenes and tannins. The genetic diversity in the tea

species is brought by their principal origin, mainly from China, India, and Cambodia (ROBB; BROWN, 2001). The phytochemical constituents of tea based on the geographical origin is reported by WONG; SIRISENA; NG, 2022. There are genetically modified plant species to improve productivity and increase beneficial bioactive compounds (SANDAL et al., 2007). The tea species within a country can vary to large extent and that forms the outline in this work to discriminate tea between two completely different origin- Brazilian and Indian.

The Indian teas are diverse and the key principle would be their geographical origination (GUHA, 1968) (LUTGENDORF, 2012). From the muscatel flavoring Darjeeling tea from north to the fragrant and brisky Niligiri tea in the south, there are specific factors responsible for the difference in tea quality between geographical regions of same country. The agricultural conditions, soil chemistry, climatic influence, harvesting methods play a crucial role (PATRA et al., 2013; RAHAMAN; ARUCHAMY, 2022). Darjeeling tea that accounts to 1% of global tea production has its recognition for unique flavor and non-reproducibility of taste. It is observed the agricultural parameters like soil, slow process of plucking and severe withering process and patterned rainfall in the Darjeeling hills gives its credential (SINGH et al., 2016). Similarly, the Niligiri tea in South India known for their non-reproducibility of their briskness and fragrance, is brought by the local geographical conditions. The other key tea producing regions in India are Assam, Dooars, Kangra, Anamallais, Munnar and Wayanad that have their own distinction in tea qualities (DAS, 2015). The Darjeeling, Niligiri and Assamese tea ranks the best amongst others. Thereby it is an important factor to consider the place of origin in evaluating the tea quality. E-tongue will be a robust and reliable tool for tea characterization from different origin and grades.

Grading is an important parameter considered for trade and commercial purposes. There are numerous types of grades available for black tea which is graded based on their size and appearance. Orange pekoe (OP) are basic medium grade types named to classify black teas. Fanning is smaller in size which are product of left-over whole leaf grade teas and so is for dust grades ("TEA STATION & TEA FOUNTAIN," 2004). The grades vary from whole leaf and broken leaf to dust type teas which as indicated for orthodox and ctc types of black tea in **Table 1.** Tea adulteration comes in all forms of grades in a motive to make profit leaving the ethics of consumer wellbeing behind. Adulterant can alter the usual composition and thereby affect final tea quality. E-tongue can be effective in identifying the quality degradation (WANG; XIAO, 2013).

Table 1. Standardized key Indian tea grades as acquired from ("INDIAN TEA ASSOCIATION", 2017)

Tea	Type of tea	Grades	Abbreviation
Orthodox	Orthodox Whole leaf	TGF OP	Tippy golden flowery orange pekoe
		GF OP	Golden flowery orange pekoe
		OP	Orange Pekoe
	Orthodox Broken	BOP	Broken Orange Pekoe
		GF BOP	Golden flowery Broken orange pekoe
		BPS	Broken Pekoe Soucheng
		FBOP	Flowery Broken orange pekoe
	Orthodox Fanning	GOF	Golden Orange Fanning
		FOF	Flowery Orange Fanning
	Orthodox Dust	OPD	Orthodox pekoe dust
		OCD	Orthodox Churamani dust
CTC	CTC Whole leaf	FP	Flowery Pekoe
		PEKOE	Pekoe
	CTC Broken	BOP	Broken Orange Pekoe
		BPS	Broken Pekoe Souchong
	CTC Fannings	OF	Orange Fannings
		PF	Pekoe Fannings
	CTC Dust	PD	Pekoe dust
		CD	Churamani dust
		GD	Golden Pekoe
		SRD	Super red dust

3.4 Economic importance of tea quality control

Black tea undergoes vigorous and lengthy process before reaching final consumer. There are chances of undergoing adulteration, either by intentional and unintentional means. In the intentional means of adulteration, teas are adulterated with remains of dust or mature leave samples. The mature leaves have reduced chemical composition and altered tea quality which is not desirable. Under the unintentional means, the change in processing conditions without producer's awareness can impact final quality. In both the cases, quality control is

necessary and is possible through analysing their chemical composition. Traditional chemical analysis is time consuming and laborious.

Non-destructive technologies have been used as alternative tools for effective tea quality control and monitoring (**Table 2).** E-tongue in specific has gained significant importance in the last two decades as a tool to analyse liquid samples. Multi sensing devices are built in a way to mimic human tongue in identifying taste profiles of food. Applications in wine (RODRÍGUEZ-MÉNDEZ et al., 2016), milk (DIAS et al., 2009), coffee (ALESSIO et al., 2016), tea (CHENG et al., 2013) have been previously reported.

Table 2.Application of non-destructive technologies in black tea quality assessment

Technology	Chemometrics	Description	Reference
E-tongue	Si-PLS, VCPA, Si-VCPA-PLS	Use of cyclic voltammetric e-tongue in identifying total free amino acid content in black tea	(OUYANG et al., 2020)
E-nose	PCA, LDA, ANN	Classification of tea grades using an e-nose	(LIU; YU; GU, 2019)
Hyperspectral imaging	SVM, RF	Application in discriminating Keemun black tea based on their grades	(REN et al., 2021b)
NIR Spectroscopy	PLS-R	Use of Near infrared spectroscopy at 2142 nm for total polyphenol identification.	(RANATUNGA et al., 2021)
Raman Spectroscopy	PLS-LDA, ResNet, PLS, LSSVM, 1D-CNN	Use of Raman spectroscopy in assessing black tea during fermentation process	(LUO et al., 2022)
FTIR spectroscopy	PCA, HCA	FTIR spectrum as a tool to discriminate black tea composition	(CAI et al., 2015)

3.5 Sensing devices

Sensors gained attention in the last two decades with rapid increase in research output including food systems. The sensors are defined as a device that converts the physical phenomena into electrical signals (WILSON J S, 2005). Their interface between biological systems and sensing units have made the extensive application for analysing chemicals and

food (TERRY; WHITE; TIGWELL, 2005). These biosensors are divided into sensing elements (bio-receptors) and transducers (ADLEY, 2014), where the former is based on antibiotics, enzymes, gene, DNA etc (VERMEEREN et al., 2009). The latter is based on the optical, mass, and electro-chemical principles (PRABOWO; PURWIDYANTRI; LIU, 2018). The transduction mechanism involved in electrochemical based bio-sensors include ameperometric, conductometric voltametric, potentiometric, impedimetric. The ameperometric functions by measuring the current of the flowing electrons generated through oxidation/reduction of the analyte in the biochemical reaction (DAVIS, 1985). The potentiometric functions by measuring the potential difference between the reference and working electrodes (KARYAKIN et al., 1996). The voltametric functions by measuring the current generated from oxidation/reduction of the analyte in reaction when a potential is applied. The conductometric and impedimetric functions by measuring conductance and impedance generated from the biochemical reaction of the analyte.

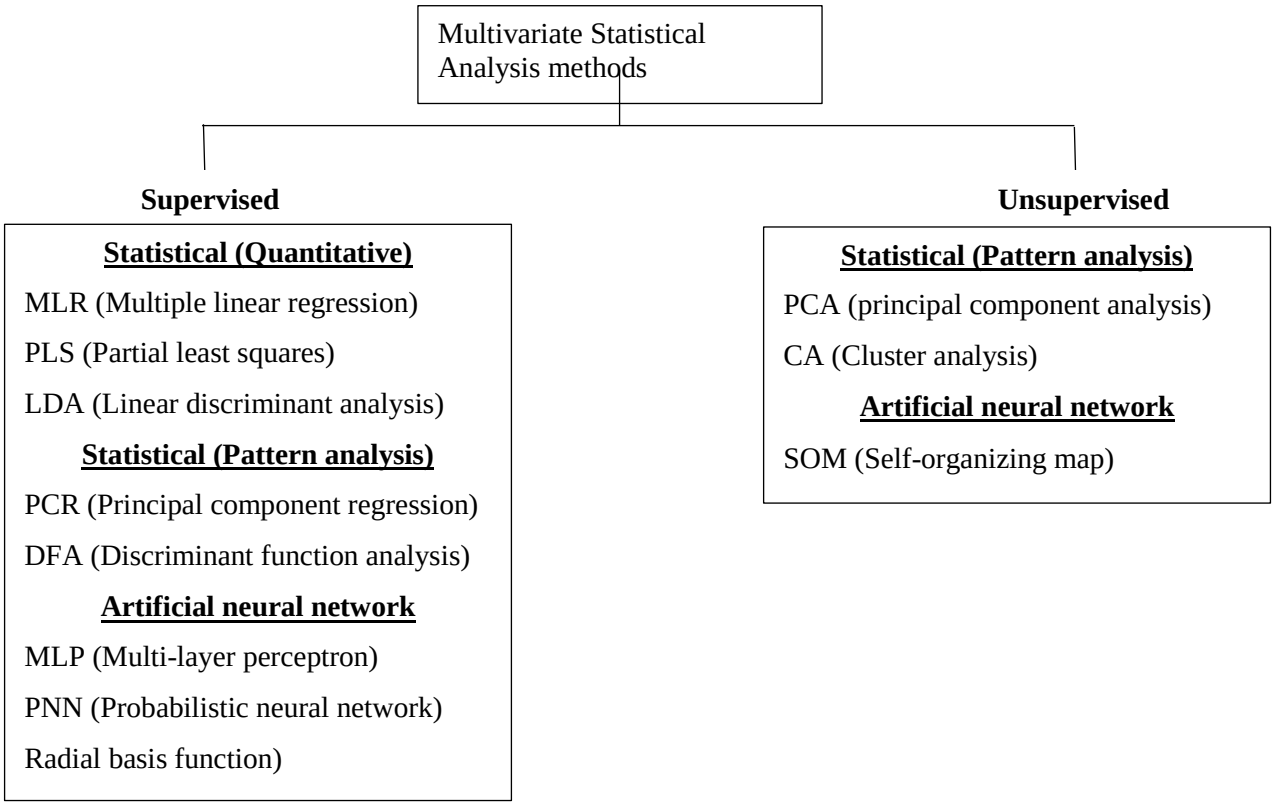
Applications of the various electrochemical transducers in food includes food safety, microbiological control, quality estimation, process control. In application of tea, they have shown effective results for geographical origin and grade classification(CHEN; ZHAO; VITTAYAPADUNG, 2008; PAN; YAN; CHEN, 2022), fermentation time monitoring(GHOSH et al., 2015).

3.6 Data treatment

Data acquisition is the transfer of signals from the sensors to the PC, following this proceeds the data treatment. Multi-sensor devices are complex and the generated pattern require multi variate analysis techniques. The various pattern recognition techniques can be of parametric and non-parametric. In parametric, the sensing unit's data are treated as a probability distribution function. The non-parametric deals with biologically inspired techniques (SHESKIN, 1997). The machine learning algorithms plays crucial role with specific techniques categorised as supervised, i.e., PLS (Partial least squares), MLP (Multilayer perceptron) and unsupervised i.e., PCA (Principal component analysis) (BRERETON, 2022), SOM (Self-organising map). The supervised technique is classified based on the existence of output attributes and the algorithm to predict/classify these attributes. Thereby supervised algorithms can be used as classification and prediction (regression) tools (BERRY M W, 2020).

Unsupervised learning techniques, lack the output attributes and are extensively used for clustering and association mining. Clustering is a technique used to group the unlabelled

data within the input attributes. Meanwhile associate mining tends to show the relationship between the input attributes (MARSLAND, 2014).



3.6.1 Principal component analysis

PCA is a tool for dimensionality reduction without losing much information. The multi-dimensionality factorial (descriptive) method, followed by many improvements since its inception in 1960s has been widely used for data exploration and qualitative observation (CORDELLA, 2010). It employs mathematical procedure to arrive at the principal components (PCs). The final PCs are linearly uncorrelated variables which are less comparing to the original variables. Pattern analysis is possible using the PCAs, which might be difficult to analysis with original data comprising higher dimensions (KUMAR, 2017).

Using equation 2, decomposition of the matrix (samples x variables) into array of T scores (samples x main components) and an orthogonal matrix of L weights (variable x main components) is carried out. The PCs are represented with scores which are a set of information from the spectrum of each sample (FERREIRA, 2015a) (JOLLIFFE IT; CORDELLA C, 2002).

$$X = TL \qquad \text{(Eq. 2)}$$

3.6.2 Partial least squares- Discriminant analysis

The classification models functions by classifying samples belonging to a set of experimental measures according to the reference properties. This way, sorting samples as good/bad, false/true, active/non-active etc (FERREIRA, 2015a). PLS-DA is pattern classification method which is modified from the traditional LDA (Linear discriminant analysis). LDA functions by reducing the variance within the class whose frequencies are unequal and their performance are examined based on randomly generated test data. There is a maximal ratio of between-class variance to the within-class variance in any particular data set, thereby assuring maximal separability (BALAKRISHNAMA; GANAPATHIRAJU, 1998) (THARWAT et al., 2017). The LDA is modified to a partial least squares version where there is maximum covariance between variables X and Y. It holds advantage over PCA where the variable X are unaware of class labels (PACK, 1993). The Y matrix is encoded to specific values showing their class in matrix X. The encoded values of Y can be between 0 to 1 which is possible using probability density function and Bayesian theory (FERREIRA, 2015a). Analysis of each class is carried out and the values are assigned, if the sample belongs to the class number, then number 1 is assigned. If not, number 0 is assigned as illustrated in **Figure 1**.

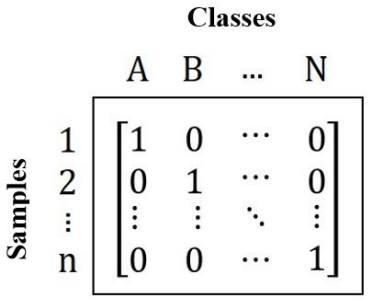


Figure 4: Matrix of dependent variables for N classes and n samples.

The performance of the model is evaluated by sensitivity (Eq.1), specificity (Eq.2) and accuracy (Eq.3).

$$\text{Sensitivity (\%)} = \frac{TP}{TP + FN} \quad \text{(Eq.12)}$$

$$\text{Specificity (\%)} = \frac{TN}{FP + TN} \quad \text{(Eq.13)}$$

$$\text{Accuracy (\%)} = \frac{TP + TN}{TP + TN + FP + FN} \quad \text{(Eq.14)}$$

where TP is true positive, TN is true negative, FN is false negative, and FP is false positive.

3.6.3 Partial least squares- Regression

The PSLR is a mathematical model(linear regression) built from the experimental measure is used for quantitative study. The regression is the ability to model for a response variable Y while using a predictor variable X. This method of relating two matrixes X and Y is utilized for producing quantitative multivariate models. The PLSR is always optional over other methods when handling data with multi collinearity (HÉBERGER, 2008) and can handle noise data (WOLD; SJOSTROM; ERIKSSON, 2001). PLSR finds application in finding the pattern for the chemical data in the spectral data acquired from analytical instruments (PRASADA RAO; BIJU, 2005). The calculation of linear regression is calculated using equations 15 and equation 16.

$$X = T_A L_A + E \quad \text{(Eq. 15)}$$

$$Y = T_A q + e \quad \text{(Eq. 16)}$$

Where T_A is the score matrix, L_A is the weight matrix (loadings), A represents how many latent variables (LVs) were chosen, E is the residue of X. Also, Y is the vector with the parameters to be analyzed, q is the vector of regression coefficients, e is a vector of residues of y (FERREIRA, 2015a).

The model performance is evaluated using root mean square error for cross validation (RMSEC), the root means square error for prediction (RMSEP) shown in equation 17 and equation18 respectively (BONA; MARÇO; VALDERRAMA, 2018; FERREIRA, 2015a). Other performance evaluators are residual predictive deviation (RPD) and range error ratio (RER) shown in equation 19 and 20 respectively

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{i=1}^{I}(y_i - \hat{y}_i)^2}{I}} \quad \text{(Eq.17)}$$

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{p=1}^{P}(y_p - \hat{y}_p)^2}{P}} \quad \text{(Eq.18)}$$

$$\mathrm{RPD} = \frac{\mathrm{SD}}{\mathrm{RMSEP\ ou\ RMSECV}} \quad \text{(Eq.19)}$$

$$\mathrm{RER} = \frac{\mathrm{Range}}{\mathrm{RMSEP\ ou\ RMSECV}} \quad \text{(Eq.20)}$$

Where Y is the predicted valuer of i^{th} observation, Yi is the measured valuer of i^{th} observation, I is the number of observations in calibration, cross validation and prediction set. P is the number of samples used in the prediction; SD is the standard deviation; Range is the range in variation in concentration of data.

CHAPTER 4- EXPLORATION OF AN IMPEDIMETRIC ELECTRONIC TONGUE AND CHEMOMETRICS FOR DISCRIMINATION OF BLACK TEA FROM DIFFERENT ORIGINS

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EXPLORATION OF AN IMPEDIMETRIC ELECTRONIC TONGUE AND CHEMOMETRICS FOR DISCRIMINATION OF BLACK TEA FROM DIFFERENT ORIGINS

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Abstract

Tea (*Camellia sinensis*) is the second most consumed beverage globally due to its flavour and therapeutical properties. The geographical location is a key indicator for distinct tea quality as there are various grades in the market, demanding a rapid assessment of tea quality grade for economic purposes. We have developed and applied a multi-sensing impedimetric device (electronic tongue) based on Layer-by-Layer (LbL) nanostructured materials as sensing units in the discrimination of black tea composition based on two geographical origins. A printed circuit board (PCB) comprising four different sensing units, coupled to an automated multiplexer and impedance analyzer measured the electrical response from the tea samples. PLS-DA and PLSR were applied to the data obtained from the electronic tongue to classify samples according to country of origin and to predict total flavonoids, total polyphenols and caffeine content. Discriminant analysis using PLS-DA was able to distinguish Brazilian from Indian tea samples. Total flavonoids and caffeine content were determined to characterize samples from different origin, and could be predicted using partial least squares regression (PLSR) with coefficients of determination (R^2_P) of 0.77 and 0.71, respectively. The results show the potential of impedimetric e-tongue along with chemometrics as an effective tool in discriminating teas based on the origin of the leaves.

Keywords: impedimetric multi-sensors; e-tongue; chemometrics; black tea

Highlights

- Identification of tea quality grade and origin is important for economic purposes
- Tea quality is related to its sensorial properties and chemical composition
- A novel impedimetric multi-sensor (e-tongue) device was proposed
- We used chemometrics for characterisation of tea samples
- Results demonstrate the potential of e-tongue and chemometrics for tea analysis

List of abbreviations and symbols

LV – latent variables

PCA – principal component analysis

PLS-R – partial least squares regression

PLS-DA – partial least square discriminant analysis

RMSEC – root mean square error of calibration

RMSECV – root mean square error of cross-validation

RMSEP – root mean square error of prediction

R^2_C *– coefficient of determination of calibration*

R^2_{CV} *– coefficient of determination of cross-validation*

R^2_P *– coefficient of determination of prediction*

RPD – residual predictive deviation

RER – range error ratio

ET- electronic tongue

IDE- gold-plated interdigitated electrodes

PDDA- poly (diallyl dimethylammonium chloride) solution

CuTsPc- copper phthalocyanine-3,4',4'',4'''-tetrasulfonic acid tetrasodium salt

MMt-K- montmorillonite clay

PDDA/PEDOT:PSS - poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate)

CTC- cut-tear-curl

TFC- total flavonoid content

TPC- total polyphenol content

4.1 Introduction

Black tea (*Camellia sinensis*) accounts for more than 70% of the globally tea production due to its therapeutical properties. India, one of the main players in the global black tea market, also known for the tea diversity within the country (Darjeeling, Nilgiris, and Assamese teas), globally recognized for their taste. Tea quality is attributed to its sensorial properties, as a fine quality tea tends to have a desirable aroma and taste. The four major predominant tastes in black tea are astringency, bitterness, sweetness, and umami formed by the combination or individual influence of various compounds like catechins, theaflavins, thearubigins, caffeine, and free amino acids to name a few (CHATURVEDULA, 2013; SHAO; ZHANG, 2019). The variation in tea quality is related to the processing methods, geographical conditions, and harvesting practices (WANG et al., 2019; ZHENG et al., 2016). The four key processing methods of traditionally produced black tea are withering, rolling, fermentation, and firing (DEB; JOLVIS POU, 2016; POU, 2016a). Black tea is mostly classified into two groups as orthodox and cut-tear-curl (CTC) based on the difference in their production process, predominantly at the second step, where rolling is applied only to orthodox teas, whereas they are replaced by ctc machine for the latter to produce smaller tea particles. The fermentation process brings oxidised polyphenolic compounds, such as theaflavins and thearubigins formed by the enzymatic oxidation of catechins.

As much as the emphasis on various processing steps impacts tea quality(TESHOME, 2019), other key factors impacting tea quality are geographical conditions and harvesting methodology. The tea plant species mostly grow in high-altitude places with periodic rainfall and supporting soil parameters (BHAGAT; R.D.BARUAH; S.SAFIQUE, 2010; RAVICHANDRAN; RAMASWAMY, 1998) Darjeeling tea for instance is very popular for its non-reproducibility of taste and is mainly characterised because of its altered processing conditions, such as slowed plucking and severe withering process supported by climatic conditions e.g., patterned rainfall and supportive soil chemistry (BHUTIA, 2016). The geographical conditions and distinct processing methods are non-replicable and differs across geographical locations. The principal methods of tea classifications come by sensory and chemical analysis (Y et al., 2018), both having their drawbacks of requiring trained panels, laborious processes and non-cost effective. Within this context, the non-specific electronic tongue (ET) technology can aid tea classification.

The development of artificial taste sensors such as electronic tongues has gained importance in recent years as an analytical instrument. The most widely used traditional ET systems are classified based on their working principles as voltammetric, potentiometric and

impedimetric(SHIMIZU; BRAUNGER; RIUL JR, 2021). They find extensive application in classifying several samples, including tea classification based on grades and geographical location (HE et al., 2009); classification of red wines, coffee and milk (COSTA et al., 2014; DIAKO; EDWARDS; ROSS, 2013; PAN et al., 2019). Impedimetric ET works on measuring a change in the impedance signal from variations in the analysed samples. One major advantage of impedimetric ETs is their ability to operate without reference electrodes (SHIMIZU; BRAUNGER; RIUL JR, 2021), simplifying measurements and data acquisition. Moreover, most ET systems encompasses sophisticated instrumentation and prolonged experimental time.

To overcome this, a miniaturized impedimetric multi-sensor device operating without a reference electrode has been proposed to obtain a chemical fingerprint from tea samples. Briefly, the variation in the electrical properties of the materials chosen as sensing units, deposited onto identical gold interdigitated electrodes, facilitates the fingerprint formation(RIUL et al., 2004). Nanostructured films have proven to be more effective in analysing complex samples with increased sensitivity and deduction of taste even at lower levels (FERREIRA et al., 2003).

The objective of this work is to develop and apply a prototype multi-sensing device to discriminate black tea based on their geographical origin (Brazilian and Indian), while predicting the major components responsible for tea quality. Data obtained from an impedimetric e-tongue was analysed by applying chemometric tools such as principal component analysis (PCA), partial least square- regression (PLSR) and partial least square-discriminant analysis (PLS-DA) for classification and prediction models. The proposed ET device could be further applied for tea quality inspection as well as evaluation of other types of beverages.

4.2 Materials & methods

4.2.1 Sample collection and preparation

Fifteen Brazilian black tea samples numbered 1-15 (**Table 4)** were collected from Sitio Shimada Black tea factory located in Registro-SP, Brazil. Twenty Indian samples numbered 16-35 **(Table 4)** were purchased from a wholesaler in Assam, India.

4.2.2 Impedance measurement and data collection

4.2.3 Sample preparation

Black tea samples at 10 mg/mL were prepared using an infusion of 1 g of tea in 100 mL of distilled water. Then, it was cooled to room temperature and filtered using Whatman filter paper, the final extract was taken for ET measurements.

4.2.4 ET setup and measurement

The device and instrumentation setup, along with the system control were built as reported by Braunger et al. 2020 (BRAUNGER et al., 2020) with a minor modification by removing the microfluidic system. The materials deposited onto the collinear systems for sensing units are: sensing unit 1 (bare IDE), sensing unit 2 (PDDA/CuTsPc), sensing unit 3 (PDDA/MMt-K), sensing unit 4 (PDDA/PEDOT:PSS). Tea infusion was dropped over the sensors and the impedance was measured at amplitude of 25 mV where frequencies ranged from 1-10^6 Hz. Each sample was measured in triplicates.

The change in impedance by varying frequencies was measured using the impedance analyser and data was scrutinized using chemometrics, such as principal component analysis (PCA) (screening), Partial least squares-regression (PLSR) (prediction of chemical parameters) and Partial least squares-discriminant analysis (PLS-DA) (classification of samples according to quality grades and origin).

4.2.5 Data pre-processing

Auto-scaling was used to pre-process the dataset, considering that the signal acquired had little noise and was the prominent source of variation among samples. The pre-processing and data analysis were performed using PLS Toolbox 8.9.1 from Eigenvector Research, Inc. (Manson, WA, USA) for Matlab R2019a (Mathworks, Natick, USA).

4.2.6 Reference analysis

4.2.6.1 Extract preparation

Tea extract was performed as described by the international organization for standardization (ISO) 14502-1. An amount of 0.20 grams of each sample was weighed and taken in a volumetric flask. Then, 5 mL of 70% methanol at 70 °C was added and the extract was mixed and heated using a vortex at 70 °C for 10 minutes followed by cooling at room temperature. The extract was centrifuged at 200 g for 10 minutes and the supernatant was collected in a centrifuge tube. The extraction step was repeated twice and both extracts were combined to fill a 10 mL flask.

4.2.6.2 Total flavonoid content (TFC)

The total flavonoid content (TFC) was determined by the aluminium chloride assay using quercetin as standard as described by Veggi, Cavalcanti and Meireles, 2014 (VEGGI; CAVALCANTI; MEIRELES, 2014). An amount of 1.0 mL of the prepared extract was diluted with water to 10 mL. Then, 1.0 mL (in triplicates) from this diluted volume was taken in a 10 mL volumetric flask containing 4 mL of distilled water. Additionally, 0.3 mL of 5 % $NaNO_2$ was added, and 0.3 mL of 10 % $AlCl_3$ and 2 mL of 1 M NaOH were added after 5

and 6 min, respectively. Then, 2.4 mL of distilled water was added to the flask to fill a 10 mL flask followed by vortexing. The absorbance was measured at 510 nm using a UV/Vis spectrophotometer. The TFC was expressed in mg of quercetin equivalents (QE)/g. The concentration of flavonoids in the samples was derived from a standard curve of quercetin ranging from 20 to 140 μg/mL.

4.2.6.3 Total polyphenol Content (TPC)

The total polyphenol content was determined by spectrophotometer using gallic acid as standard as described by the international organization for standardization (ISO) 14502-1. An amount of 1.0 mL of the prepared extract was diluted with water to 100 mL. Then, 1.0 mL (in triplicates) from this diluted volume was taken in separate tubes containing 5 mL of 10% folin-ciocalteu's reagent, and 4 mL of 7.5% (w/v) sodium carbonate solution was added to each tube that were then kept at room temperature for 60 minutes. The absorbance was measured at 765 nm using a UV/Vis spectrophotometer. The TFC was expressed as mg of gallic acid equivalents (GAE)/g. The concentration of polyphenols in the samples was derived from a standard curve of gallic acid ranging from 10 to 100 μg/mL.

4.2.6.4 Caffeine content

The high-performance liquid chromatography (HPLC) analysis for the caffeine content was performed as described by Sanches et al. 2022 (SANCHES et al., 2022) with slight modifications.

The modifications consisted in replacing the phase A & B with 1% formic acid and acetonitrile respectively. The gradient variation was as follows: 97% to 94% A (3 minutes), 94% to 90% A (3 minutes), 90% to 87% A (8 minutes), 87% to 85% A (2 minutes), 85% to 80% A (2 minutes), 80% to 75% A (1 minute), 75% to 70% A (2 minutes), 70% to 50% A (5 minutes), 50% to 70% A (3 minutes), 70% to 97% A (4 minutes) at a flow rate of 1 mL/min. Before each injection, the system was re-equilibrated for 2 minutes (97% A). The column oven temperature was set to 29° C. The selected chromatograms for the analysis were 350 nm, 325 nm and 260 nm. The experiment was performed in duplicates. Caffeine was predominantly absorbed in UV at 260 nm, which is considered a common wavelength for the caffeine.

4.2.7 Statistical data analysis

Statistical analysis to compare the chemical data (TFC, TPC & caffeine) differences seen among black tea samples based on their origin and grades were carried out using analysis of variance (ANOVA) and Turkey multiple-comparison test ($p < 0.05$) using Minitab® version 19 (Minitab LLC, Pennsylvania, USA).

4.2.8 Multivariate analyses

4.2.8.1 Principal component analysis (PCA)

PCA is an unsupervised multivariate analysis tool to explore the qualitative attributes of the data, and it works by reducing the initial data to lower dimensions while still holding vital information. In this work, PCA was applied to the pre-processed data to visualize the clustering of black tea according to their origin and grades. PCA was built with a 95% confidence level and outliers (anomalous samples) were detected and eliminated using residual Q and Hotelling T^2 values.

4.2.8.2 Classification analysis

Partial least squares discriminant analysis (PLS-DA) is a supervised dimension reduction tool that is also capable of feature selection and classification (BARKER; RAYENS, 2003; CHRISTIN et al., 2013; NGUYEN; ROCKE, 2002). It is a prominent tool in building classification models for the ET data, helping discriminate black tea samples based on their origin and grade. Kennard-Stone (KS) algorithm was applied to split the samples into two sets as training (70%) and external validation (independent test) (30%) (KENNARD; STONE, 1969). To ensure model validation and avoid bias, permutation was carried out (LINDGREN et al., 1996; VAN DER VOET, 1994). The lowest value of root means square error of cross-validation (venetian blinds) (RMSECV) was used to choose the optimal number of latent variables (LV), which was evaluated by model performance according of sensitivity (Eq.1), specificity (Eq.2) and accuracy (Eq.3). Sensitivity is a measure of how often the model correctly identified a positive sample as positive; specificity is a measure of how accurate the model was against false positives; accuracy is the ability of the model to identify actually true and false samples out from all samples including the false positive and negative samples.

$$\text{Sensitivity (\%)} = \frac{TP}{TP + FN} \quad \text{(Eq.21)}$$

$$\text{Specificity (\%)} = \frac{TN}{FP + TN} \quad \text{(Eq2.2)}$$

$$\text{Accuracy (\%)} = \frac{TP + TN}{TP + TN + FP + FN} \quad \text{(Eq.23)}$$

where TP is true positive, TN is true negative, FN is false negative, and FP is false positive.

4.2.8.3 PLSR for prediction of TFC, TPC & Caffeine content

Partial least squares regression (PLSR) is a statistical method used to build quantitative models with predictors (X) and responses (Y) (SHARIFI, 2016). In PLSR, the

variables are reduced and the smaller set of predictors are used for regression. PLSR is used to predict a set of dependent variables from those of independent predictors (ABDI, 2010). PLSR was applied in calibration set to assess the ability of ET data to predict the TFC, TPC & caffeine content in black tea from external validation set (FERREIRA, 2015b). Similar to classification analysis, the samples were divided into two sets as training (70%) and external validation (30%) (KENNARD; STONE, 1969). Also, the optimal LV was chosen based on the lowest value of root means square error of cross-validation (venetian blinds) (RMSECV).

The performance of the calibration models was assessed by the coefficient of determination (R^2) and root mean square error for calibration (R_c^2, RMSEC) and cross validation (R_{CV}^2, RMSECV). Predictive capacity of the models was assessed using the coefficient of determination (R_P^2), root mean square error for prediction (RMSEP), where the general formula is presented in Eq.5 and Eq.6. (SKIBSTED et al., 2004), and also residual predictive deviation (RPD) and range error ratio (RER), where the general formula is presented in Eq.7 and Eq.8 (RAMBO; AMORIM; FERREIRA, 2013; SAEYS; MOUAZEN; RAMON, 2005).

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{i=1}^{I}(y_i - \hat{y}_i)^2}{I}} \quad \text{(Eq.24)}$$

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{p=1}^{P}(y_p - \hat{y}_p)^2}{P}} \quad \text{(Eq.25)}$$

$$\mathrm{RPD} = \frac{\mathrm{SD}}{\mathrm{RMSEP\ ou\ RMSECV}} \quad \text{(Eq.26)}$$

$$RER = \frac{Range}{RMSEP\ ou\ RMSECV} \quad \textit{(Eq.27)}$$

Where Y is the predicted valuer of ith observation; Yi is the measured valuer of ith observation; I is the number of observations in calibration, cross validation and prediction set; P is the number of samples used in the prediction; SD is the standard deviation; Range is the range in variation in concentration of data.

4.3 Results & Discussion

4.3.1 TFC, TPC & caffeine content in black tea

Black tea samples presented total flavonoids ranging from 6.81 to 33.70 mg QE/g in which there was a significant difference between Brazilian and Indian samples with average values of 11.38 and 22.34 mg QE/g, respectively (Turkey's test, $p < 0.05$) (**Table 3**). Similar values were reported by Pereira et al. 2014 [29] (6.35 - 8.92 mg QE/g) in Brazilian tea samples and Qhairul, Abu Bakar, and Mamat 2013 [30] (19.07 - 33.70 mg QE/g) in Asian tea samples.

Table 3.Reference analysis for black tea samples based on the origin

Classification	Total flavonoids (mg QE/g)		Caffeine (mg/L)		Total Polyphenols (% GAE)	
	Mean	Range	Mean	Range	Mean	Range
Brazilian	11.38^{B}±2.37	6.66-33.75	37.40^{B}±10.26	20.32-68.07	12.44^{B}±2.86	8.69-17.98
Indian	22.34^{A}±4.96	11.02-33.75	60.03^{A}±7.98	47.91-77.69	15.09^{A}±3.2	11.29-26.99

* The data correspond to the mean ± SD of three repetitions. Different letters for the same parameter analyzed indicate significant differences between origin. by ANOVA ($P< 0.05$). TFC (Total flavonoid content), caffeine content, TPC (Total polyphenol content) for Brazilian and Indian black tea samples.

The total polyphenols ranged from 8.69 to 26.99 % GAE, with an average value of 12.44 and 15.09 % GAE in Brazilian and Indian tea samples, respectively, which were similar to previous reports [31] where TPC ranged from 8.42 - 17.62 % GAE in black tea. Samples 8, 15, 26 presented total polyphenol content higher than the average values of samples from respective geographical origin. There was no significant difference in total polyphenols between tea samples from different quality grades. PCA scores for TPC (Figure 10.c in supplementary material) shows that lower range values clearly separate from the medium and higher range values. This validates the insignificant differences seen between grades.

On the other hand, caffeine in black tea ranged from 22.25 to 75.26 mg/L, with significant difference between Brazilian and Indian samples. Previous work done by Shao and Zhang 2019 [8] showed caffeine in black tea ranging from 40 to 95 mg/L. It was observed that caffeine values for Brazilian tea are lower than for Indian tea, similarly to the trend seen for flavonoids.

Table 4.Reference analysis for black tea samples

Classification	Number	Grade	Total flavonoids (mg QE/g)		Caffeine (mg/L)		Total Polyphenols (% GAE)	
			Mean	Range	Mean	Range	Mean	Range
	1	A	11.2±0.08	11.1-11.25	36.05±10.06	28.94-43.16	11.88±0.07	11.8-11.93
	2	A	14.26±0.2	14.02-14.37	30.94±7.56	25.60-36.28	12.37±0.36	12.02-12.74
	3	B	12.64±0.3	12.43-12.98	39.92±3.08	37.74-42.10	15.96±0.28	15.63-16.13
	4	A	11.29±0.05	11.25-11.34	30.16±4.55	26.95-33.38	10.42±0.13	10.33-10.57
	5	A	10.01±0.01	10-10.16	36.7±3.27	34.40-39.01	12.07±0.24	11.85-12.32
	6	B	10.06±0.09	10-10.16	35.81±1.15	35.03-36.62	12.59±0.42	12.35-13.08
Brazilian	7	A	15.54±0.47	15-15.87	36.93±6.1	32.63-41.25	17.25±0.48	16.89-17.8
	8	A	12.58±0.08	12.5-12.65	42.35±7.76	36.87-47.84	17.33±0.32	17.12-17.7
	9	A	6.81±0.13	6.66-6.89	31.43±5.56	27.50-35.36	8.59±0.14	8.43-8.69
	10	A	6.84±0.05	6.78-6.88	38.25±0.29	38.04-38.46	8.9±0.05	8.84-8.95
	11	B	10.1±0.1	10-10.2	25.67±1.55	26.77-27.80	9.24±0.24	8.99-9.45
	12	A	13.11±0.01	13.1-13.13	25.25±6.97	20.32-30.19	11.32±0.58	10.65-11.71
	13	A	11.25±0.05	11.2-11.3	40.63±0.26	40.45-40.81	11.14±0.44	10.64-11.46
	14	B	13.11±0.01	13.11-13.13	56.7±7.37	51.49-61.92	12.09±0.82	11.26-12.9
	15	B	10.33±0.33	10-10.66	54.2±19.6	40.36-68.07	15.52±2.45	13.08-17.98
	16	B	20.6±0.07	20.53-20.65	59.22±7.06	54.23-64.21	13.54±0.84	12.58-14.09
Indian	17	B	23.77±0.02	23.27-23.79	72.83±3.95	70.03-75.63	12.82±0.93	11.89-13.74

Classification	Number	Grade	Total flavonoids (mg QE/g)		Caffeine (mg/L)		Total Polyphenols (% GAE)	
	18	B	21.59±0.51	21-21.88	60.33±6	56.09-64.57	11.38±0.1	11.29-11.48
	19	C	28.75±0.05	28.7-28.8	62.02±0.01	62.01-62.02	11.73±0.2	11.49-11.87
	20	D	18.79±0.1	18.71-18.9	55.35±2.13	53.84-56.85	13.06±0.44	12.63-13.5
	21	B	20.53±0.18	20.63-20.33	54.31±6.5	49.71-58.90	18.6±0.34	18.2-18.8
	22	B	18.58±0.26	18.28-18.75	50.69±3.93	47.91-53.47	16.05±1.16	14.71-16.74
	23	B	20.64±0.03	20.62-20.68	66.51±7.84	60.97-72.05	14.65±0.44	14.19-15.07
	24	C	28.72±0.04	28.68-28.75	55.77±2.7	53.86-57.68	16.46±1.4	15.5-18.07
	25	D	18.77±0.07	18.71-18.85	49.16±1.71	47.95-50.37	14.77±0.6	14.14-15.33
Indian	26	B	25.62±0.02	25.6-25.63	55.26±2.02	53.83-56.70	26.67±0.28	26.51-26.99
	27	B	19.35±0.05	19.3-19.38	61.8±6.45	57.24-66.37	14.44±0.38	14.03-14.76
	28	B	33.7±0.05	33.65-33.75	75.26±0.13	75.17-75.35	15±0.09	14.92-15.1
	29	C	20.62±0.01	20.61-20.63	67.07±1.36	66.10-68.03	14.22±0.15	14.09-14.38
	30	D	18.67±0.12	18.54-18.75	71.74±8.41	65.79-77.69	14.44±0.33	14.06-14.68
	31	B	11.17±0.13	11.02-11.25	57.51±1.18	56.68-58.34	14.21±0.22	13.99-14.43
	32	B	26.25±0.05	26.19-26.3	53.8±0.92	53.15-54.45	16.96±1.01	15.8-17.59
	33	B	28.71±0.06	28.64-28.75	56.04±2.61	54.19-57.88	14.94±0.98	14.11-16.02
	34	C	20.08±0.07	20-20.12	53.29±4.05	50.43-56.15	14.04±1.06	12.84-14.83
	35	D	21.88±0.01	21.88-21.89	62.58±3.53	60.08-65.08	13.83±0.95	12.74-14.39

A- whole leaf, B- broken leaf, C- dust, D- fannings.

* The data correspond to the mean ± SD of three repetitions in TFC and TPC, while two repetitions in caffeine.

Significant differences were seen for most individual samples by ANOVA ($P < 0.05$), (please see table 10 in supplementary material). TFC (Total flavonoid content), caffeine content, TPC (Total polyphenol content) for individual black tea samples.

4.3.2 Principal component analysis

PCA was performed using the data obtained from the whole data set including the four sensors (**Figure 2**). However individual contributions of each sensor are reported and discussed separately, to provide a deeper understanding of the results.

From the score plot, PC1 and PC2 explained 89.72% of the total variation and displayed the trend where Brazilian tea predominantly fell on the negative scores in PC1 (**Figure 2a**) while Indian samples are on the positive scores. The same could be observed in PC2 scores where Brazilian tea are predominantly on positive scores, while Indian samples are on negative scores. Both PCs allowed to differentiate the two classes of tea based on their origin. It is possible to observe a few samples that overlaps between the two classes in the mid-region that might indicate similar set of parameters between these classes. The overlapping Brazilian samples include tea label 13 (**Table 4**). The remaining samples were just one of the triplicates, which might be outliers, and this applies to all the overlapping Indian samples.

The PC scores for tea from different grades (**Figure 2b**) showed that the whole leaf category fell on the positive scores of PC2, meanwhile fanning and dust fell on the negative scores of PC2. It is noteworthy to observe the broken leaf spread on both scores, mainly because this grade had samples from both Brazilian and Indian classes. It is observable that the samples were overlapping with each other and may indicate higher degree of similarity between grades. Analysing the PCA for each reference analysis (Figure 13 in supplementary material), the Brazilian samples and Indian samples were differentiated based on these reference values, however, it was not possible to observe a separation trend between grades. A similar work carried out by Chen et al. 2020 [32] using a voltametric e-tongue to identify tea grade showed similar PCA results where the e-tongue signal could not clearly separate samples based on grades because of similarities between the classes.

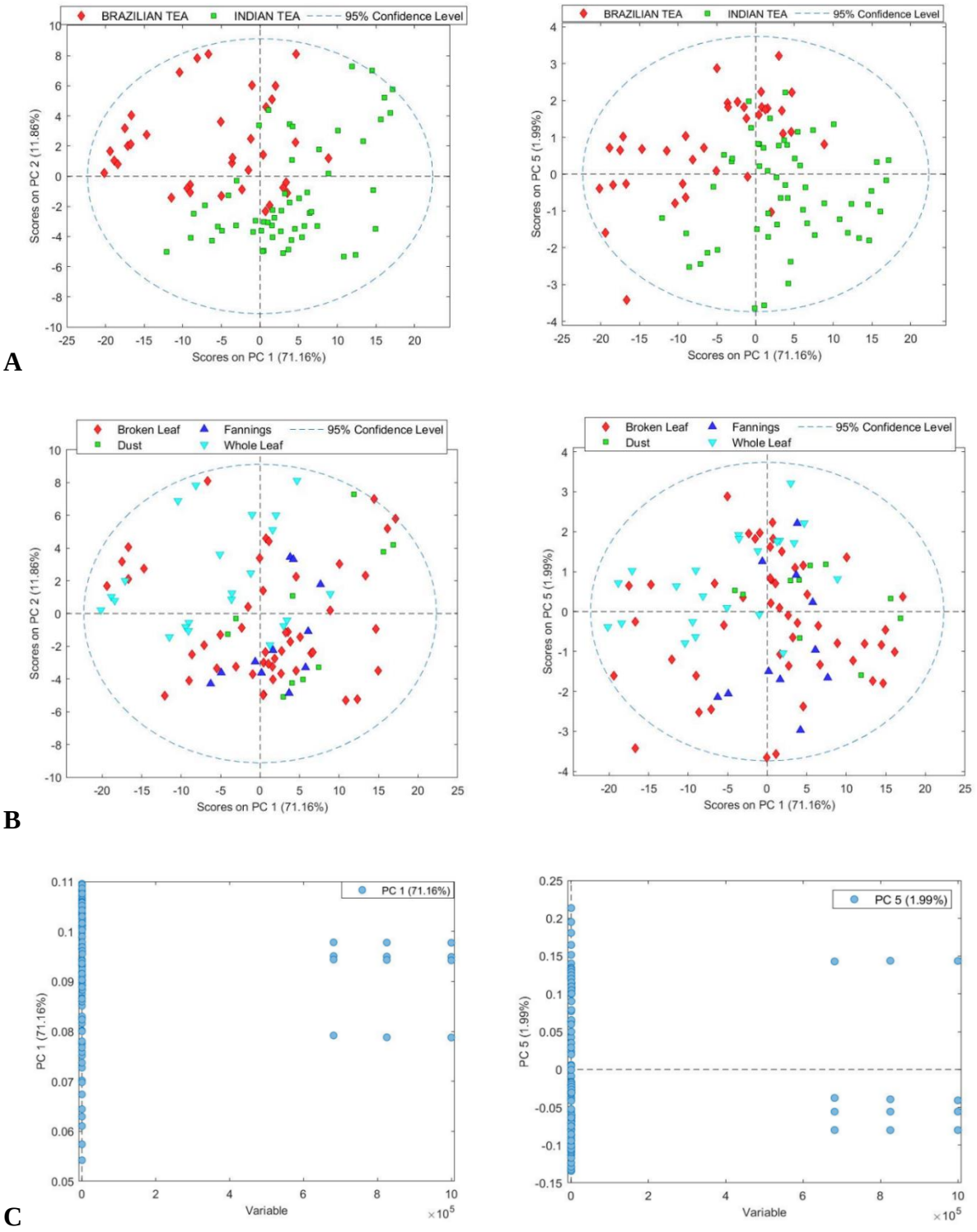


Figure 5.PCA showing scores a) origin and b) grades; and c) loadings PC1 and PC2 for the ET data with all frequencies and sensors included

Auto-scaling was applied to the raw data. This pre-processing is based on the principle of giving equal weight to each sensing unit, which is crucial to perform multivariate analysis methods. The loadings showed the frequencies in the beginning of variables i.e., 10-

100 Hz contributed to the majority of variation seen in the PC1 and PC2. That is an expected result since at this frequency range the observed electrical characteristics is mainly ruled by the electric double-layer formed at the electrode/electrolyte interface (FERREIRA et al., 2003).

The frequency of 1000 Hz is ideal and holds the information needed, which was also examined in the study reported by Braunger et al. 2020 [19] (**Figure 3)**. The scores of PC1 and PC2 explained 98.03% of the total variation, which is higher than variation explained by PC1 and PC2 using the full range of frequencies, with Brazilian samples predominately on negative scores while the Indian samples were on the positive side of PC1. It was observed that PC1 was related to the total flavonoid contents, where samples in the negative side of PC1 had low TFC content, and samples in the positive side of PC1 had high TFC content.

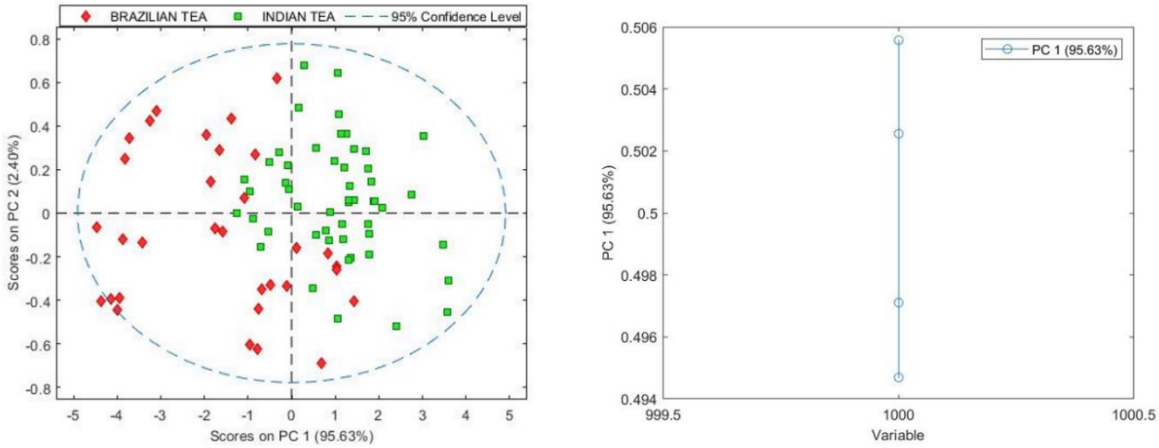
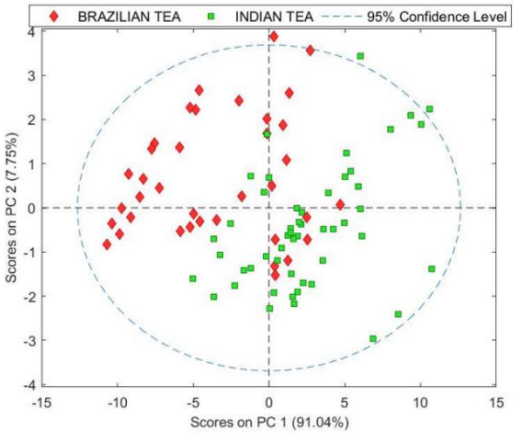
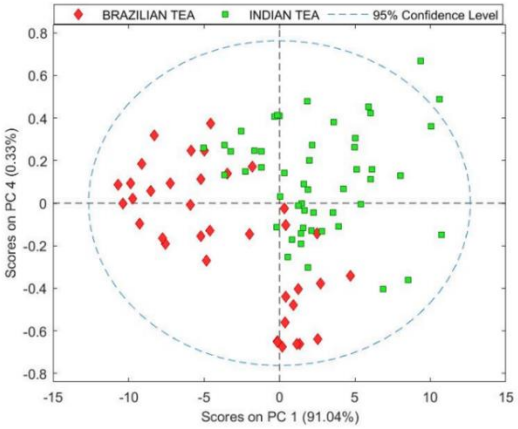


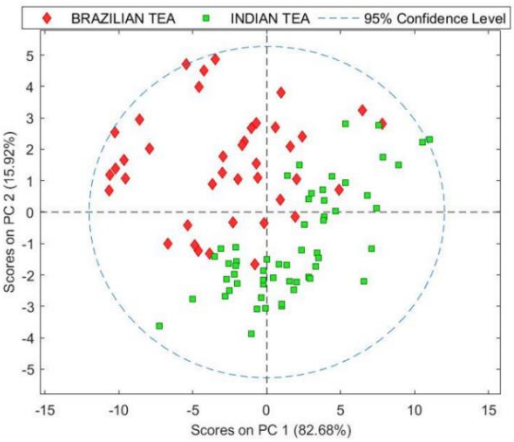
Figure 6.PCA showing scores and loadings a) origin for the ET data at the frequency of 1000 Hz

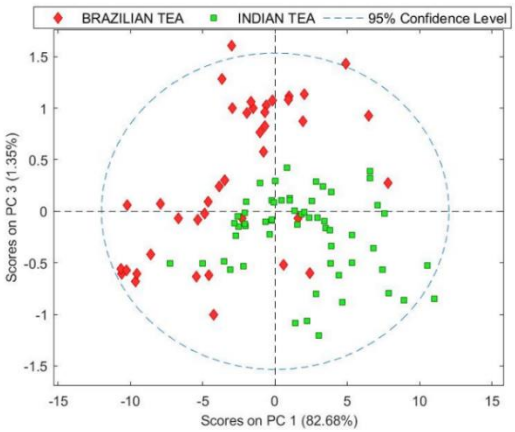
PCA scores plot for individual sensors with full range of frequencies is shown in **Figure 4**. The score plot PC1 and PC2 for sensing unit 1, shown in **Figure 4.a** explains 98.00% of the total variations, with Brazilian samples predominantly on the negative scores of PC1. Indian samples were predominantly on the negative scores of the PC2. Thereby sensing unit 1 contributes for the separation of Indian samples from the Brazilians samples. Similar trends were seen for sensing unit 2 and 3, shown in **Figure 4.b and Figure 4.c,** where it was possible to observer a separation of samples from different origin. The score plot for the sensing unit 4 as shown in **Figure 4.d** indicates the Brazilian sample predominately fell on the positive scores of PC2, while Indian samples fell predominantly on the positive scores of PC1.


BRAZILIAN TEA
INDIAN TEA
95% Confidence Level
Scores on PC 2 (7.75%)
Scores on PC 1 (91.04%)

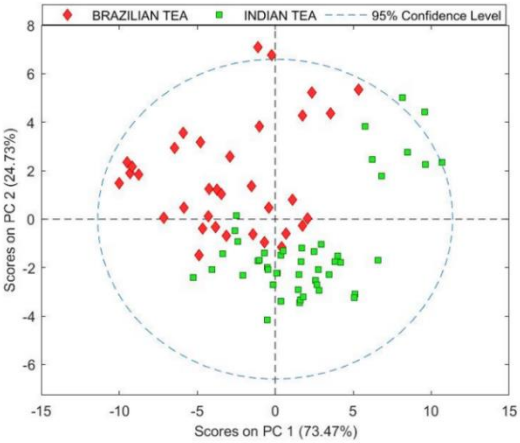

BRAZILIAN TEA
INDIAN TEA
95% Confidence Level
Scores on PC 4 (0.33%)
Scores on PC 1 (91.04%)

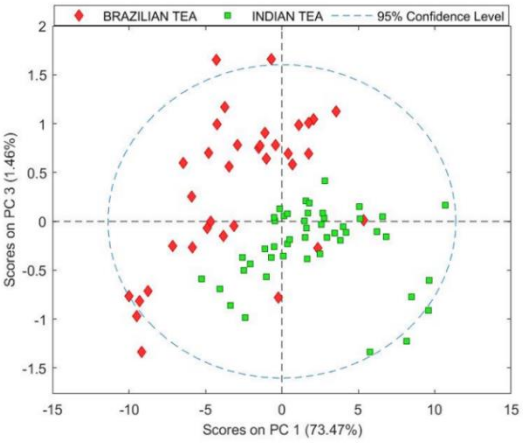
A


BRAZILIAN TEA
INDIAN TEA
95% Confidence Level
Scores on PC 2 (15.92%)
Scores on PC 1 (82.68%)

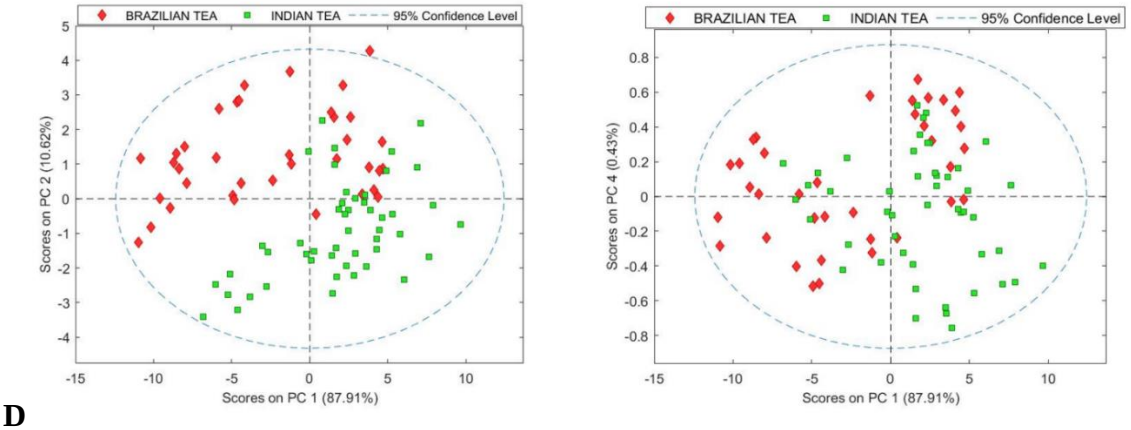

BRAZILIAN TEA
INDIAN TEA
95% Confidence Level
Scores on PC 3 (1.35%)
Scores on PC 1 (82.68%)

B


BRAZILIAN TEA
INDIAN TEA
95% Confidence Level
Scores on PC 2 (24.73%)
Scores on PC 1 (73.47%)


BRAZILIAN TEA
INDIAN TEA
95% Confidence Level
Scores on PC 3 (1.46%)
Scores on PC 1 (73.47%)

C



D

Figure 7.PCA showing scores for samples based on origin for a) sensing unit 1 b) sensing unit 2 c) sensing unit 3 and d) sensing unit 4 from the ET data with all frequencies

4.3.3 Classification models using PLS-DA

The PLS-DA model for the black tea using ET data is shown in **Table 5** displays the values for sensitivity, selectivity, accuracy. The classification based on origin with the two classes (Brazilian and Indian) can be seen from the confusion matrix with the numbers on the diagonal of the matrix which correctly classified samples. This indicates that PLS-DA model was able to discriminate the black tea samples upon origin with 100% accuracy and sensitivity. A similar work involving a different ET by Yan et al. 2017 [33] ,was an effective tool in classification of Anji-white tea based on geographical origin reaching sensitivity of 91.7%. The work required additional chemicals to calibrate the instrument and comparatively larger time for analysis, whereas our proposed novel impedimetric multi-sensing device could distinctively differentiate tea based on geographical origin with lesser instrumentation setup and 100% sensitivity.

Table 5.Sensitivity, specificity, accuracy values for classification of black tea according to origin using electronic tongue.

Sample	LV	Calibration			Prediction		
		sensitivity	specificity	accuracy	sensitivity	specificity	accuracy
Brazilian	4	0.94	0.95	0.95	1.0	1.0	1.0
Indian	4	0.95	0.94	0.95	1.0	1.0	1.0

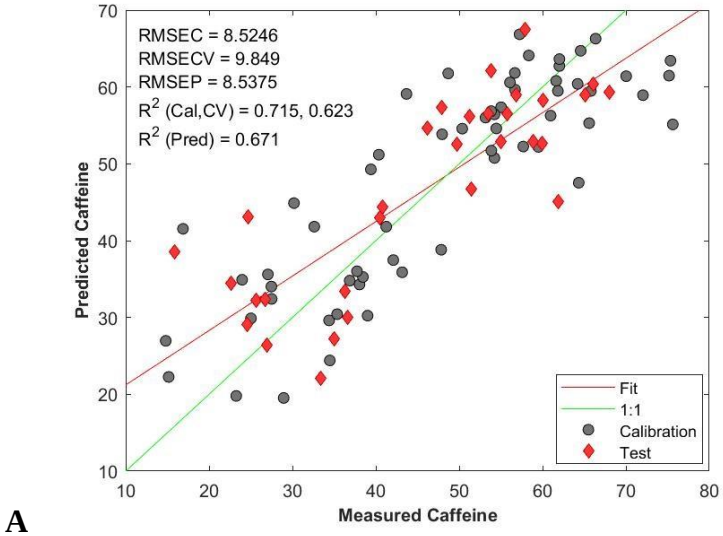
*Significant classification model with random t-test < 0.005 at 95% confidence level.

The classification model based on grades did not yield good classification models, which can be explained by work carried out by Alasalvar et al. 2012 [34], which presented

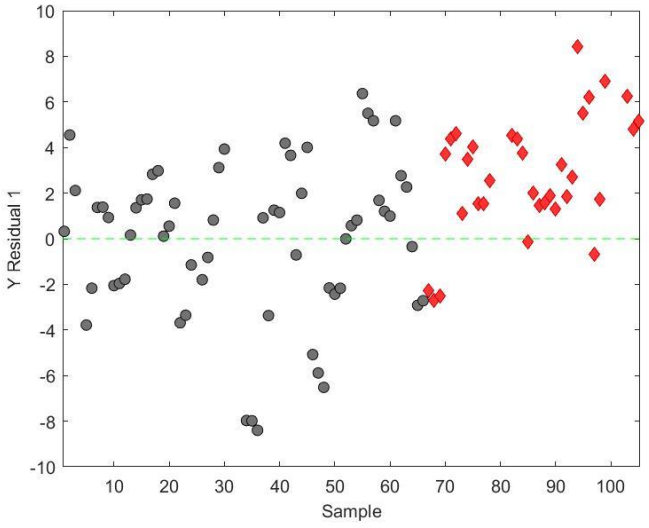
that the main difference seen in grade is the flavor characteristics bought by combinations of non-volatile and volatile active compounds. The loss of volatile active compounds over time could have also rendered the tea quality, making it difficult to note the minor variations seen between grades. Considering the importance of the volatiles, as a previous study by Ruichong Zhi et al. 2017 (ZHI; ZHAO; ZHANG, 2017) showing the confusion matrix with some misclassified tea grades, with higher rate for e-tongue than e-nose. Despite this, the misclassification rates were reduced and the accuracy improved with their proposed fusion method. Thereby, coupling the ET to e-nose might help improve classifying tea based on grades with higher accuracy, but coupling two different techniques would make data analysis complex.

4.3.4 PLSR prediction of reference analysis

The partial least square regression (PLSR) model helps to overview the relationship seen between the ET data and the reference analysis (TFC, TPC & caffeine). **Figure 5** and **Figure 6** shows the RMSEC, RMSEP and coefficient of determination (R^2) for the ET data. The pre-processing method of auto-scaling was employed for building the models.



A



B

Figure 8.PLSR models accounting the best performance for (a) Total flavonoids by using the ET data; (b) Residual versus Sample

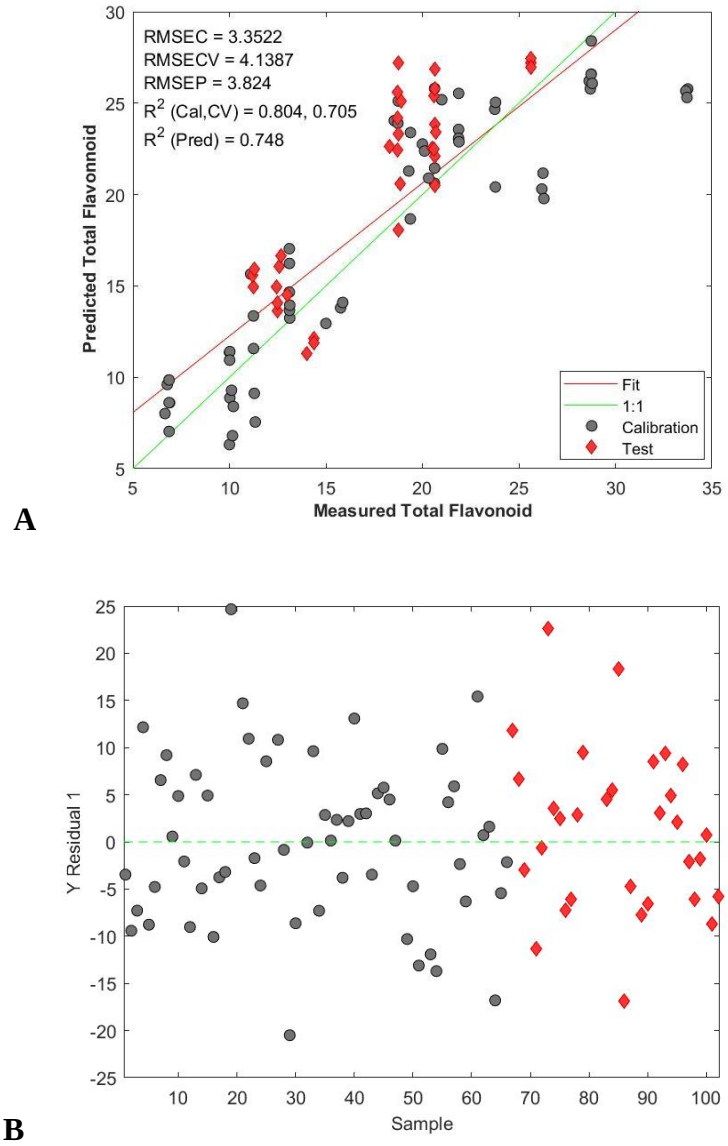


Figure 9.PLSR models accounting the best performance for (a) Caffeine by using the ET data; (b) Residual versus Sample

The PLSR model's parameters for the ET are shown in **Table 6**. TPC calibration and prediction models showed lower R^2. From the reference analysis we could notice that there was no significant difference between the classes for TPC and, consequently, it was not an efficient parameter in building prediction models due to the lack of variation. A possible reason is the compound non-specificity of the sensors, a hypothesis is that ET have recognized some of the polymeric forms individually and not whole, because polyphenols as a whole form a complex group of molecules (BUTT et al., 2014) . The total flavonoids and caffeine have reliable calibration and prediction models, even considering the non-specificity of the sensing units used here.

Table 6.Parameters for the calibration and prediction sets for reference analysis in black tea with PLSR

Chemical parameter	LV	Calibration		Prediction			
		R^2	RMSCEV	R^2	RMSEP	RPD	RER
Total flavonoids (TFC)	5	0.80	4.14	0.75	3.82	1.94	7.08
Total Polyphenols (TPC)	6	0.41	1.24	0.02	0.87	2.03	9.94
Caffeine	6	0.72	9.85	0.67	8.54	1.87	7.37

The models for total flavonoids showed an R^2 of 0.80 for calibration models and R^2 of 0.75 for prediction models with 5 LV. However, the absolute error indicated by RMSE for calibration/predictions were too high, which is also observable from the model with data's spread wide away from the baseline (1:1). The same can be noted for caffeine with the models showing R^2 values of 0.72 and 0.67 for calibration and prediction, respectively. The high RMSE values for both models indicate a lowered accuracy in predicting caffeine levels. To confirm the same, model performance was evaluated using RPD and RER values. For the TFC, and caffeine, the RPD vales were 1.94 and 1.87 respectively. The RPD values between 1.5 and 2.0 indicates the model is able to distinguish between low and high values. With values between 2.0 and 2.5 is ideal for quantitative purposes, values between 2.5 and 3.0 is considered a good model and any values above 3.0 is considered excellent model (SAEYS; MOUAZEN; RAMON, 2005). For the TFC, and caffeine, the RER vales were 7.08 and 7.37 respectively. The RER values of the model, according to American cereal chemicals association (AACC), if between 4.0 and 10.0 is qualified for screening calibration. The values between 10.0 and 15.0 is acceptable for quality control and any values above 15.0 is good for quantification (RAMBO; AMORIM; FERREIRA, 2013).

There are few reports of building prediction models for black tea in the literature to compare with the models built using the ET data. The impedimetric e-tongue does not use materials having specificity with complex compounds in tea, nonetheless, a 100% accuracy can be achieved despite the difficulty to get a better performance model.

4.4 Conclusion

Electronic tongue is an emergent non-destructive analytical technique with applications in several types of samples (EL-MESERY; MAO; ABOMOHRA, 2019). The novel impedimetric ET used in this work to discriminate the tea quality based on origin showed promising results. PLS-DA applied to the data acquired by the ET device classified

samples with 100% accuracy according to their origin. This work presents an ET functioning by lower set-up time, minimal time of analysis and chemical-free that can be an effective tool to classify black tea sample based on their origin with aid of chemometrics. Future work may integrate the microfluidic channel to the ET setup, to improve efficiency where it requires much lesser quantity of samples than the existing setup. Also, to improve on the existing sensing units, by experimenting selective sensing materials for a particular compound that impact taste (example: caffeine for bitterness) to increase efficiency in classifying the tea quality based on their chemical compositions.

4.5 References

ABDI, H. Partial least squares regression and projection on latent structure regression (PLS Regression). **Wiley Interdisciplinary Reviews: Computational Statistics**, v. 2, p. 97–106, ago. 2010.

ADLEY, C. Past, Present and Future of Sensors in Food Production. **Foods**, v. 3, n. 3, p. 491–510, 19 ago. 2014.

ALASALVAR, C. et al. Flavor Characteristics of Seven Grades of Black Tea Produced in Turkey. **Journal of Agricultural and Food Chemistry**, v. 60, n. 25, p. 6323–6332, 27 jun. 2012.

ALESSIO, P. et al. Analysis of Coffees Using Electronic Tongues. Em: **Electronic Noses and Tongues in Food Science**. [s.l.] Elsevier, 2016. p. 171–177.

AOUADI, B. et al. Historical evolution and food control achievements of near infrared spectroscopy, electronic nose, and electronic tongue—critical overview. **Sensors (Switzerland)**, v. 20, n. 19, p. 1–42, 1 out. 2020.

APETREI, I. M.; APETREI, C. Detection of virgin olive oil adulteration using a voltammetric e-tongue. **Computers and Electronics in Agriculture**, v. 108, p. 148–154, 2014.

ASIL, M.; RABIEI, B.; ANSARI, R. Optimal fermentation time and temperature to improve biochemical composition and sensory characteristics of black tea. **Australian Journal of Crop Science**, v. 6, out. 2012.

BALAKRISHNAMA, S.; GANAPATHIRAJU, A. **INSTITUTE FOR SIGNAL AND INFORMATION PROCESSING LINEAR DISCRIMINANT ANALYSIS-A BRIEF TUTORIAL**. [s.l: s.n.].

BARKER, M.; RAYENS, W. Partial least squares for discrimination. **Journal of Chemometrics**, v. 17, n. 3, p. 166–173, 24 mar. 2003.

BERRY M W. **Supervised and Unsupervised Learning for Data Science**. Cham: Springer International Publishing, 2020.

BHAGAT, R.; R.D.BARUAH; S.SAFIQUE. Bhagat, R.M. R. Deb Baruah and S. Safique

(2010). Climate and tea [Camellia sinensis (L.) O. Kuntze] production with special reference to North Eastern India: a review. J. Environ. Res. Develop. 4(4):1017-1028. **Journal oF Environmental Research & Development**, v. 4, p. 1017–1028, ago. 2010.

BHUTIA, S. Darjeeling Tea Industry: A Geographical Perspective on Production, Development, Promotion and Prospects. **Khoj:An International Peer Reviewed Journal of Geography**, v. 3, p. 84, ago. 2016.

BONA, E.; MARÇO, P. H.; VALDERRAMA, P. Chemometrics Applied to Food Control. Em: **Food Control and Biosecurity**. [s.l.] Elsevier, 2018. p. 105–133.

BRAUNGER, M. L. et al. Influence of the Flow Rate in an Automated Microfluidic Electronic Tongue Tested for Sucralose Differentiation. **Sensors**, v. 20, n. 21, p. 6194, 30 out. 2020.

BRERETON, R. G. Introduction to statistical, algorithmic and theoretical basis of principal components analysis. **Journal of Chemometrics**, v. 36, n. 9, 12 set. 2022.

BUTT, M. S. et al. Black Tea Polyphenols: A Mechanistic Treatise. **Critical Reviews in Food Science and Nutrition**, v. 54, n. 8, p. 1002–1011, 5 jan. 2014a.

BUTT, M. S. et al. Black Tea Polyphenols: A Mechanistic Treatise. **Critical Reviews in Food Science and Nutrition**, v. 54, n. 8, p. 1002–1011, 5 jan. 2014b.

CAI, J. et al. Using FTIR spectra and pattern recognition for discrimination of tea varieties. **International Journal of Biological Macromolecules**, v. 78, p. 439–446, jul. 2015.

CHATURVEDULA, V. Tea-Aroma, Taste, Color and Bioactive Constituents. **Journal of medicinal plant research**, v. 5, p. 2110, ago. 2013.

CHATURVEDULA, V.; PRAKASH, I. Tea-Aroma, Taste, Color and Bioactive Constituents. **Journal of medicinal plant research**, v. 5, p. 2110, out. 2013.

CHEN, Q.; ZHAO, J.; VITTAYAPADUNG, S. Identification of the green tea grade level using electronic tongue and pattern recognition. **Food Research International**, v. 41, p. 500–504, 31 dez. 2008.

CHEN, X. et al. Non-parametric partial least squares–discriminant analysis model based on sum of ranking difference algorithm for tea grade identification using electronic tongue data. **Sensors and Actuators B: Chemical**, v. 311, p. 127924, maio 2020.

CHENG, T.-J. et al. Electronic Tongue for Tea Tasting. Em: **Tea in Health and Disease Prevention**. [s.l.] Elsevier, 2013. p. 469–481.

CHRISTIN, C. et al. A Critical Assessment of Feature Selection Methods for Biomarker Discovery in Clinical Proteomics. **Molecular & Cellular Proteomics**, v. 12, n. 1, p. 263–276, jan. 2013.

CIOSEK, P.; BRUDZEWSKI, K.; WRÓBLEWSKI, W. Milk classification by means of an electronic tongue and Support Vector Machine neural network. **Measurement Science and Technology**, v. 17, p. 1379, out. 2006.

CIURSA, P.; OROIAN, M. Voltammetric e-tongue for honey adulteration detection. **Sensors**, v. 21, n. 15, 1 ago. 2021.

CORDELLA, C. B. Y. Principal component analysis: The basic building block of chemometrics. **Actualite Chimique**, 1 out. 2010.

COSTA, A. et al. Electronic tongue as a rapid tool for the assessment of coffee flavour and chemical composition. **Proceedings of IEEE Sensors**, v. 2014, p. 2126–2129, 12 dez. 2014.

DAS, A. Chai: The Experience of Indian Tea [Book Review]. **NEHU Journal**, v. 13, p. 98–100, 1 dez. 2015.

DAVIES, M. J. et al. **Role of Flavonoids in the Diet Black Tea Consumption Reduces Total and LDL Cholesterol in Mildly Hypercholesterolemic Adults 1J. Nutr**. [s.l: s.n.]. Disponível em: <https://academic.oup.com/jn/article/133/10/3298S/4687625>.

DAVIS, G. Electrochemical techniques for the development of amperometric biosensors. **Biosensors**, v. 1, n. 2, p. 161–178, jan. 1985.

DEB PAL, A.; DAS, T. Analysis of adulteration in black tea. **International Journal of Biology Research 253 International Journal of Biology Research**, v. 3, p. 253–257, 2018.

DEB, S.; JOLVIS POU, K. R. A Review of Withering in the Processing of Black Tea. **Journal of Biosystems Engineering**, v. 41, n. 4, p. 365–372, 1 dez. 2016.

DEL VALLE, M. Electronic Tongues Employing Electrochemical Sensors. **Electroanalysis**, v. 22, n. 14, p. 1539–1555, 2010.

DIAKO, C.; EDWARDS, C.; ROSS, C. **Red Wine Quality Assessment using the Electronic Tongue**. [s.l: s.n.].

DIAS, L. A. et al. An electronic tongue taste evaluation: Identification of goat milk adulteration with bovine milk. **Sensors and Actuators B: Chemical**, v. 136, n. 1, p. 209–217, fev. 2009.

EL-MESERY, H.; MAO, H.; ABOMOHRA, A. Applications of Non-destructive Technologies for Agricultural and Food Products Quality Inspection. **Sensors**, v. 19, n. 4, p. 846, 18 fev. 2019.

FERREIRA, M. et al. High-Performance Taste Sensor Made from Langmuir−Blodgett Films of Conducting Polymers and a Ruthenium Complex. **Analytical Chemistry**, v. 75, n. 4, p. 953–955, 1 fev. 2003.

FERREIRA, M. M. C. **Quimiometria: conceitos, métodos e aplicações**. 1. ed. Campinas: Editora da UNICAMP, 2015a.

FERREIRA, M. M. C. **Quimiometria: conceitos, métodos e aplicações**. [s.l.] Editora da UNICAMP, 2015b.

GHOSH, A. et al. Prediction of theaflavin and thearubigin content in black tea using a voltammetric electronic tongue. **Chemometrics and Intelligent Laboratory Systems**, v. 116, p. 57–66, jul. 2012.

GHOSH, A. et al. Monitoring the Fermentation Process and Detection of Optimum Fermentation Time of Black Tea Using an Electronic Tongue. **IEEE Sensors Journal**, v. 15, n. 11, p. 6255–6262, nov. 2015.

GREGORY, R. P. F.; BENDALL, D. S. The purification and some properties of the polyphenol oxidase from tea (Camellia sinensis L.). **The Biochemical journal**, v. 101 3, p. 569–81, 1966.

GUHA, A. Sir Percival Griffiths: The History of the Indian Tea Industry (Weidenfield and Nicolson, 5 Winsby Street, London W., 1967) Pp. 730; Price 8 gns. **The Indian Economic & Social History Review**, v. 5, n. 2, p. 195–197, 1 jun. 1968.

HE, W. et al. Evaluation of Chinese tea by the electronic tongue: Correlation with sensory properties and classification according to geographical origin and grade level. **Food Research International**, v. 42, n. 10, p. 1462–1467, dez. 2009.

HÉBERGER, K. Chapter 7 - Chemoinformatics—multivariate mathematical–statistical methods for data evaluation. Em: VÉKEY, K.; TELEKES, A.; VERTES, A. (Eds.). **Medical Applications of Mass Spectrometry**. Amsterdam: Elsevier, 2008. p. 141–169.

INDIAN TEA ASSOCIATION. Disponível em: <https://www.indiatea.org/>. Acesso em: 24 out. 2021.

KANTOR, D. B. et al. Electronic tongue for sensing taste changes with apricots during storage. **Sensors and Actuators, B: Chemical**, v. 131, n. 1, p. 43–47, 14 abr. 2008.

KARYAKIN, A. A. et al. Potentiometric biosensors based on polyaniline semiconductor films. **Sensors and Actuators B: Chemical**, v. 33, n. 1–3, p. 34–38, jul. 1996.

KENNARD, R. W.; STONE, L. A. Computer Aided Design of Experiments. **Technometrics**, v. 11, n. 1, p. 137–148, fev. 1969.

KOSIŃSKA, A.; ANDLAUER, W. Antioxidant Capacity of Tea. Effect of Processing and Storage. Em: **Processing and Impact on Antioxidants in Beverages**. [s.l.] Elsevier Inc., 2014. p. 109–120.

KOTTAWA-ARACHCHI, J. et al. Biochemical Characteristics of Tea (Camellia L. spp.) Germplasm Accessions in Sri Lanka: Correlation between Black Tea Quality Parameters and Organoleptic Evaluation. **International Journal of Tea Science**, v. 10, p. 3–13, out. 2014.

KUMAR, K. Principal component analysis: Most favourite tool in chemometrics. **Resonance**, v. 22, n. 8, p. 747–759, 5 ago. 2017.

KUMAR, V. et al. Health benefits of tea consumption. **Tropical Journal of Pharmaceutical Research**, v. 6, p. 785–792, out. 2007.

LARSSON, S. C.; VIRTAMO, J.; WOLK, A. Black tea consumption and risk of stroke in women and men. **Annals of Epidemiology**, v. 23, n. 3, p. 157–160, mar. 2013.

LI, Y.; LEI, J.; LIANG, D. Identification of fake green tea by sensory assessment and electronic tongue. **Food Science and Technology Research**, v. 21, n. 2, p. 207–212, 2015.

LIANG, Y. et al. **Analytical, Nutritional and Clinical Methods Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions**. [s.l: s.n.]. Disponível em: <www.elsevier.com/locate/foodchem>.

LINDGREN, F. et al. Model validation by permutation tests: Applications to variable selection. **Journal of Chemometrics**, v. 10, n. 5–6, p. 521–532, 1 set. 1996.

LIU, H.; YU, D.; GU, Y. Classification and Evaluation of Quality Grades of Organic Green Teas Using an Electronic Nose Based on Machine Learning Algorithms. **IEEE Access**, v. 7, p. 172965–172973, 2019.

LUO, X. et al. Using surface-enhanced Raman spectroscopy combined with chemometrics for black tea quality assessment during its fermentation process. **Sensors and Actuators B: Chemical**, v. 373, p. 132680, dez. 2022.

LUTGENDORF, P. Making tea in India: Chai, capitalism, culture. **Thesis Eleven**, v. 113, n. 1, p. 11–31, 1 dez. 2012.

MACDONALD, J. R. et al. **Impedance Spectroscopy: Theory, Experiment, and Applications, Third Edition**. [s.l: s.n.].

MARSLAND, S. R. **Machine Learning: An Algorithmic Perspective, Second Edition**. 2014.

NGUYEN, D. V.; ROCKE, D. M. Tumor classification by partial least squares using microarray gene expression data. **Bioinformatics**, v. 18, n. 1, p. 39–50, 1 jan. 2002.

OUYANG, Q. et al. Measurement of total free amino acids content in black tea using electronic tongue technology coupled with chemometrics. **LWT**, v. 118, p. 108768, jan. 2020.

PACK, S. Chemometrics for analytical chemistry - Volume I: PC-aided statistical data analysis, M. Meloun, J. Militky and M. Forina, Ellis Horwood (Series in Analytical Chemistry), Chichester, 1992, ISBN 0-13-126376-5, No. of pages: 330. Price: £65.00. **Journal of Chemometrics**, v. 7, n. 3, p. 225, 1993.

PAN, M. et al. Comparison of Sensory and Electronic Tongue Analysis Combined with HS-SPME-GC-MS in the Evaluation of Skim Milk Processed with Different Preheating Treatments. **Molecules**, v. 24, n. 9, p. 1650, 26 abr. 2019.

PAN, T.; YAN, R.; CHEN, Q. Geographical origin of green tea identification using LASSO and ANOVA. **Food Science and Technology**, v. 42, 2022.

PEREIRA, V. P. et al. Determination of phenolic compounds and antioxidant activity of green, black and white teas of Camellia sinensis (L.) Kuntze, Theaceae. **Revista Brasileira de Plantas Medicinais**, v. 16, n. 3, p. 490–498, set. 2014.

POU, K. R. J. Fermentation: The Key Step in the Processing of Black Tea. **Journal of Biosystems Engineering**, v. 41, p. 85–92, ago. 2016a.

POU, K. R. J. Fermentation: The Key Step in the Processing of Black Tea. **Journal of Biosystems Engineering**, v. 41, p. 85–92, out. 2016b.

PRABOWO, B.; PURWIDYANTRI, A.; LIU, K.-C. Surface Plasmon Resonance Optical Sensor: A Review on Light Source Technology. **Biosensors**, v. 8, n. 3, p. 80, 26 ago. 2018.

PRASADA RAO, T.; BIJU, V. M. SPECTROPHOTOMETRY | Organic Compounds. Em: WORSFOLD, P.; TOWNSHEND, A.; POOLE, C. (Eds.). **Encyclopedia of Analytical Science (Second Edition)**. Second Edition ed. Oxford: Elsevier, 2005. p. 358–366.

PRIY, R. et al. Tea: Production, Composition, Consumption and its Potential as an Antioxidant and Antimicrobial Agent. **International Journal of Food and Fermentation Technology**, v. 5, p. 95, out. 2015.

QHAIRUL, N.; ABU BAKAR, M. F.; MAMAT, H. Phytochemicals and antioxidant properties of different parts of Camellia sinensis leaves from Sabah Tea Plantation in Sabah, Malaysia. **International Food Research Journal**, v. 20, p. 307–312, 1 jan. 2013.

RAHAMAN, S. A.; ARUCHAMY, S. Land Suitability Evaluation of Tea (Camellia sinensis L.) Plantation in Kallar Watershed of Nilgiri Bioreserve, India. **Geographies**, v. 2, n. 4, p. 701–723, 11 nov. 2022.

RAKESH, * et al. Analysis of tea powder for adulterant. **IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS**, v. 12, n. 4, p. 37–42, [s.d.].

RAMBO, M. K. D.; AMORIM, E. P.; FERREIRA, M. M. C. Potential of visible-near infrared spectroscopy combined with chemometrics for analysis of some constituents of coffee and banana residues. **Analytica Chimica Acta**, v. 775, p. 41–49, maio 2013.

RANATUNGA, M. et al. NIR Spectroscopic Determination of Polyphenol Content in Teas and Tea Extract at 2142 nm. **Sensors and Materials**, v. 33, n. 12, p. 4135, 16 dez. 2021.

RAVICHANDRAN, R.; RAMASWAMY, P. The impact of mechanization of tea harvesting on the quality of south Indian CTC teas. **Food Chemistry**, v. 63, p. 61–64, ago. 1998.

REN, G. et al. Estimation of Congou black tea quality by an electronic tongue technology combined with multivariate analysis. **Microchemical Journal**, v. 163, 1 abr. 2021a.

REN, G. et al. Hyperspectral imaging for discrimination of Keemun black tea quality categories: Multivariate calibration analysis and data fusion. **International Journal of Food Science & Technology**, v. 56, n. 6, p. 2580–2587, 3 jun. 2021b.

REYGAERT, W. C. **Green tea catechins: Their use in treating and preventing infectious diseases**. **BioMed Research International**Hindawi Limited, , 2018a.

REYGAERT, W. C. Green Tea Catechins: Their Use in Treating and Preventing Infectious Diseases. **BioMed research international**, v. 2018, p. 9105261, 17 jul. 2018b.

RIUL, A. et al. Wine classification by taste sensors made from ultra-thin films and using neural networks. **Sensors and Actuators B: Chemical**, v. 98, n. 1, p. 77–82, mar. 2004.

ROBB, C.; BROWN, P. Catechins in tea: Chemistry and analysis. **Advances in chromatography**, v. 41, p. 379–410, 1 fev. 2001.

RODRÍGUEZ-MÉNDEZ, M. L. et al. Electronic Noses and Tongues in Wine Industry.

Frontiers in Bioengineering and Biotechnology, v. 4, 25 out. 2016.

RUDNITSKAYA, A. et al. Prediction of the Port wine age using an electronic tongue. **Chemometrics and Intelligent Laboratory Systems**, v. 87, n. 1, p. 125–131, 15 maio 2007.

SAEYS, W.; MOUAZEN, A. M.; RAMON, H. Potential for Onsite and Online Analysis of Pig Manure using Visible and Near Infrared Reflectance Spectroscopy. **Biosystems Engineering**, v. 91, n. 4, p. 393–402, ago. 2005.

SANCHES, V. L. et al. Comprehensive analysis of phenolics compounds in citrus fruits peels by UPLC-PDA and UPLC-Q/TOF MS using a fused-core column. **Food Chemistry: X**, v. 14, p. 100262, jun. 2022.

SANDAL, I. et al. Agrobacterium-mediated genetic transformation of tea leaf explants: effects of counteracting bactericidity of leaf polyphenols without loss of bacterial virulence. **Plant Cell Reports**, v. 26, n. 2, p. 169–176, 2007.

SCHARBERT, S.; HOFMANN, T. Molecular Definition of Black Tea Taste by Means of Quantitative Studies, Taste Reconstitution, and Omission Experiments. **Journal of Agricultural and Food Chemistry**, v. 53, n. 13, p. 5377–5384, 1 jun. 2005.

SHAO, J.; ZHANG, Y. Determination of caffeine content in tea beverages. **IOP Conference Series: Earth and Environmental Science**, v. 330, n. 4, p. 042056, 1 out. 2019.

SHARIFI, A. **Partial Least Squares-regression (PLS-regression) In Chemometrics**. ago. 2016.

SHESKIN, D. J. Handbook of Parametric and Nonparametric Statistical Procedures. **Technometrics**, v. 46, p. 369–370, 1997.

SHIMIZU, F.; BRAUNGER, M.; RIUL JR, A. **Electronic Tongues: Fundamentals and recent advances**. [s.l: s.n.].

SHRIVASTAVA, R. R.; PATERIYA, P.; SINGH, M. **Green tea-A short reviewInt J Ind Herbs Drugs**. [s.l: s.n.].

SINGH, A. et al. Tea Research for Darjeeling Tea Industry: Various Aspects. Em: [s.l: s.n.]. p. 195–239.

SKIBSTED, E. T. S. et al. New Indicator for Optimal Preprocessing and Wavelength Selection of Near-Infrared Spectra. **Applied Spectroscopy**, v. 58, n. 3, p. 264–271, 31 mar. 2004.

TEA STATION & TEA FOUNTAIN . Disponível em: <http://www.teafountain.com/teagrades>. Acesso em: 25 out. 2021.

TERRY, L. A.; WHITE, S. F.; TIGWELL, L. J. The Application of Biosensors to Fresh Produce and the Wider Food Industry. **Journal of Agricultural and Food Chemistry**, v. 53, n. 5, p. 1309–1316, 1 mar. 2005.

TESHOME, K. Effect of tea processing methods on biochemical composition and sensory quality of black tea (Camellia sinensis (L.) O. Kuntze): A review. **Journal of Horticulture**

and Forestry, v. 11, p. 84–95, 2019.

THARWAT, A. et al. Linear discriminant analysis: A detailed tutorial. **AI Communications**, v. 30, p. 169–190, 2017.

VAN DER VOET, H. Comparing the predictive accuracy of models using a simple randomization test. **Chemometrics and Intelligent Laboratory Systems**, v. 25, n. 2, p. 313–323, nov. 1994.

VEGGI, P. C.; CAVALCANTI, R. N.; MEIRELES, M. A. A. Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation. **Journal of Food Engineering**, v. 131, p. 96–109, jun. 2014.

VERMA, R.; BHATTI, V.; KUMARI, R. Analysis of Various Tea Samples. **International Journal of Phytopharmacy**, v. 3, out. 2013.

VERMEEREN, V. et al. DNA Sensors with Diamond as a Promising Alternative Transducer Material. **Sensors**, v. 9, n. 7, p. 5600–5636, 14 jul. 2009.

WANG, J.; XIAO, H. Discrimination of different white chrysanthemum by electronic tongue. **Journal of food science and technology**, v. 50, n. 5, p. 986–992, out. 2013.

WANG, Y. et al. Impact of Six Typical Processing Methods on the Chemical Composition of Tea Leaves Using a Single *Camellia sinensis* Cultivar, Longjing 43. **Journal of Agricultural and Food Chemistry**, v. 67, n. 19, p. 5423–5436, 15 maio 2019.

WILSON J S. CHAPTER 1 - Sensor Fundamentals. Em: WILSON, J. S. (Ed.). **Sensor Technology Handbook**. Burlington: Newnes, 2005. p. 1–20.

WOLD, S.; SJOSTROM, M.; ERIKSSON, L. PLS-regression: A Basic Tool of Chemometrics. **Chemometrics and Intelligent Laboratory Systems**, v. 58, p. 109–130, out. 2001.

WONG, M.; SIRISENA, S.; NG, K. Phytochemical profile of differently processed tea: A review. **Journal of Food Science**, v. 87, n. 5, p. 1925–1942, 2 maio 2022.

Y, D. et al. Correlation between Sensory Evaluation Scores and Flavor Attributes of Black Teas from Around the World. **Journal of Food Processing & Technology**, v. 09, n. 04, 2018.

YAN, S. M. et al. Electronic tongue combined with chemometrics to provenance discrimination for a green tea (Anji-White tea). **Journal of Food Quality**, v. 2017, 2017a.

YAN, S.-M. et al. Electronic Tongue Combined with Chemometrics to Provenance Discrimination for a Green Tea (Anji-White Tea). **Journal of Food Quality**, v. 2017, p. 1–6, 2017b.

YIANNAKOPOULOU, E. C. **Interaction of green tea catechins with breast cancer endocrine treatment: a systematic review**. **Pharmacology**, 2014.

ZHENG, X.-Q. et al. Recent Advances in Volatiles of Teas. **Molecules**, v. 21, n. 3, p. 338, 11

mar. 2016.

ZHI, R.; ZHAO, L.; ZHANG, D. A Framework for the Multi-Level Fusion of Electronic Nose and Electronic Tongue for Tea Quality Assessment. **Sensors**, v. 17, n. 5, p. 1007, 3 maio 2017.

CHAPTER 5-GENERAL CONCLUSION

Chapter 5- General conclusion

A new multi-sensor device was conceived and tested for black tea analysis. A bare sensor and three different material were tested to enhance sensing capacity. The proposed novel impedimetric electronic tongue in combination with chemometrics is an effective tool to discriminate black tea based on their geographical origin. PCA scores helped visualize the separation between samples from different origin and grades. PLS-DA indicated good performance, separating the Brazilian and Indian black tea samples. Meanwhile, PLS-R indicated a reliable model with ability limited to only identifying the higher/lower values of flavonoids and caffeine. As a fast, analytical tool, the multi-sensing device could be an effective equipment to classify black tea from different origins. Future work may focus on use of microfluidic channels to improve the device sensitivity; also, different sensing materials may be tested to provide better results.

REFERENCES

References

ABDI, H. Partial least squares regression and projection on latent structure regression (PLS Regression). **Wiley Interdisciplinary Reviews: Computational Statistics**, v. 2, p. 97–106, ago. 2010.

ADLEY, C. Past, Present and Future of Sensors in Food Production. **Foods**, v. 3, n. 3, p. 491–510, 19 ago. 2014.

ALASALVAR, C. et al. Flavor Characteristics of Seven Grades of Black Tea Produced in Turkey. **Journal of Agricultural and Food Chemistry**, v. 60, n. 25, p. 6323–6332, 27 jun. 2012.

ALESSIO, P. et al. Analysis of Coffees Using Electronic Tongues. Em: **Electronic Noses and Tongues in Food Science**. [s.l.] Elsevier, 2016. p. 171–177.

AOUADI, B. et al. Historical evolution and food control achievements of near infrared spectroscopy, electronic nose, and electronic tongue—critical overview. **Sensors (Switzerland)**, v. 20, n. 19, p. 1–42, 1 out. 2020.

APETREI, I. M.; APETREI, C. Detection of virgin olive oil adulteration using a voltammetric e-tongue. **Computers and Electronics in Agriculture**, v. 108, p. 148–154, 2014.

ASIL, M.; RABIEI, B.; ANSARI, R. Optimal fermentation time and temperature to improve biochemical composition and sensory characteristics of black tea. **Australian Journal of Crop Science**, v. 6, out. 2012.

BALAKRISHNAMA, S.; GANAPATHIRAJU, A. **INSTITUTE FOR SIGNAL AND INFORMATION PROCESSING LINEAR DISCRIMINANT ANALYSIS-A BRIEF TUTORIAL**. [s.l: s.n.].

BARKER, M.; RAYENS, W. Partial least squares for discrimination. **Journal of Chemometrics**, v. 17, n. 3, p. 166–173, 24 mar. 2003.

BERRY M W. **Supervised and Unsupervised Learning for Data Science**. Cham: Springer International Publishing, 2020.

BHAGAT, R.; R.D.BARUAH; S.SAFIQUE. Bhagat, R.M. R. Deb Baruah and S. Safique (2010). Climate and tea [Camellia sinensis (L.) O. Kuntze] production with special reference to North Eastern India: a review. J. Environ. Res. Develop. 4(4):1017-1028. **Journal oF Environmental Research & Development**, v. 4, p. 1017–1028, ago. 2010.

BHUTIA, S. Darjeeling Tea Industry: A Geographical Perspective on Production, Development, Promotion and Prospects. **Khoj:An International Peer Reviewed Journal of Geography**, v. 3, p. 84, ago. 2016.

BONA, E.; MARÇO, P. H.; VALDERRAMA, P. Chemometrics Applied to Food Control. Em: **Food Control and Biosecurity**. [s.l.] Elsevier, 2018. p. 105–133.

BRAUNGER, M. L. et al. Influence of the Flow Rate in an Automated Microfluidic Electronic Tongue Tested for Sucralose Differentiation. **Sensors**, v. 20, n. 21, p. 6194, 30 out. 2020.

References

BRERETON, R. G. Introduction to statistical, algorithmic and theoretical basis of principal components analysis. **Journal of Chemometrics**, v. 36, n. 9, 12 set. 2022.

BUTT, M. S. et al. Black Tea Polyphenols: A Mechanistic Treatise. **Critical Reviews in Food Science and Nutrition**, v. 54, n. 8, p. 1002–1011, 5 jan. 2014a.

BUTT, M. S. et al. Black Tea Polyphenols: A Mechanistic Treatise. **Critical Reviews in Food Science and Nutrition**, v. 54, n. 8, p. 1002–1011, 5 jan. 2014b.

CAI, J. et al. Using FTIR spectra and pattern recognition for discrimination of tea varieties. **International Journal of Biological Macromolecules**, v. 78, p. 439–446, jul. 2015.

CHATURVEDULA, V. Tea-Aroma, Taste, Color and Bioactive Constituents. **Journal of medicinal plant research**, v. 5, p. 2110, ago. 2013.

CHATURVEDULA, V.; PRAKASH, I. Tea-Aroma, Taste, Color and Bioactive Constituents. **Journal of medicinal plant research**, v. 5, p. 2110, out. 2013.

CHEN, Q.; ZHAO, J.; VITTAYAPADUNG, S. Identification of the green tea grade level using electronic tongue and pattern recognition. **Food Research International**, v. 41, p. 500–504, 31 dez. 2008.

CHEN, X. et al. Non-parametric partial least squares–discriminant analysis model based on sum of ranking difference algorithm for tea grade identification using electronic tongue data. **Sensors and Actuators B: Chemical**, v. 311, p. 127924, maio 2020.

CHENG, T.-J. et al. Electronic Tongue for Tea Tasting. Em: **Tea in Health and Disease Prevention**. [s.l.] Elsevier, 2013. p. 469–481.

CHRISTIN, C. et al. A Critical Assessment of Feature Selection Methods for Biomarker Discovery in Clinical Proteomics. **Molecular & Cellular Proteomics**, v. 12, n. 1, p. 263–276, jan. 2013.

CIOSEK, P.; BRUDZEWSKI, K.; WRÓBLEWSKI, W. Milk classification by means of an electronic tongue and Support Vector Machine neural network. **Measurement Science and Technology**, v. 17, p. 1379, out. 2006.

CIURSA, P.; OROIAN, M. Voltammetric e-tongue for honey adulteration detection. **Sensors**, v. 21, n. 15, 1 ago. 2021.

CORDELLA, C. B. Y. Principal component analysis: The basic building block of chemometrics. **Actualite Chimique**, 1 out. 2010.

COSTA, A. et al. Electronic tongue as a rapid tool for the assessment of coffee flavour and chemical composition. **Proceedings of IEEE Sensors**, v. 2014, p. 2126–2129, 12 dez. 2014.

DAS, A. Chai: The Experience of Indian Tea [Book Review]. **NEHU Journal**, v. 13, p. 98–100, 1 dez. 2015.

DAVIES, M. J. et al. **Role of Flavonoids in the Diet Black Tea Consumption Reduces Total and LDL Cholesterol in Mildly Hypercholesterolemic Adults 1J. Nutr**. [s.l: s.n.]. Disponível em: <https://academic.oup.com/jn/article/133/10/3298S/4687625>.

References

DAVIS, G. Electrochemical techniques for the development of amperometric biosensors. **Biosensors**, v. 1, n. 2, p. 161–178, jan. 1985.

DEB PAL, A.; DAS, T. Analysis of adulteration in black tea. **International Journal of Biology Research 253 International Journal of Biology Research**, v. 3, p. 253–257, 2018.

DEB, S.; JOLVIS POU, K. R. A Review of Withering in the Processing of Black Tea. **Journal of Biosystems Engineering**, v. 41, n. 4, p. 365–372, 1 dez. 2016.

DEL VALLE, M. Electronic Tongues Employing Electrochemical Sensors. **Electroanalysis**, v. 22, n. 14, p. 1539–1555, 2010.

DIAKO, C.; EDWARDS, C.; ROSS, C. **Red Wine Quality Assessment using the Electronic Tongue**. [s.l: s.n.].

DIAS, L. A. et al. An electronic tongue taste evaluation: Identification of goat milk adulteration with bovine milk. **Sensors and Actuators B: Chemical**, v. 136, n. 1, p. 209–217, fev. 2009.

EL-MESERY, H.; MAO, H.; ABOMOHRA, A. Applications of Non-destructive Technologies for Agricultural and Food Products Quality Inspection. **Sensors**, v. 19, n. 4, p. 846, 18 fev. 2019.

FERREIRA, M. et al. High-Performance Taste Sensor Made from Langmuir−Blodgett Films of Conducting Polymers and a Ruthenium Complex. **Analytical Chemistry**, v. 75, n. 4, p. 953–955, 1 fev. 2003.

FERREIRA, M. M. C. **Quimiometria: conceitos, métodos e aplicações**. 1. ed. Campinas: Editora da UNICAMP, 2015a.

FERREIRA, M. M. C. **Quimiometria: conceitos, métodos e aplicações**. [s.l.] Editora da UNICAMP, 2015b.

GHOSH, A. et al. Prediction of theaflavin and thearubigin content in black tea using a voltammetric electronic tongue. **Chemometrics and Intelligent Laboratory Systems**, v. 116, p. 57–66, jul. 2012.

GHOSH, A. et al. Monitoring the Fermentation Process and Detection of Optimum Fermentation Time of Black Tea Using an Electronic Tongue. **IEEE Sensors Journal**, v. 15, n. 11, p. 6255–6262, nov. 2015.

GREGORY, R. P. F.; BENDALL, D. S. The purification and some properties of the polyphenol oxidase from tea (Camellia sinensis L.). **The Biochemical journal**, v. 101 3, p. 569–81, 1966.

GUHA, A. Sir Percival Griffiths: The History of the Indian Tea Industry (Weidenfield and Nicolson, 5 Winsby Street, London W., 1967) Pp. 730; Price 8 gns. **The Indian Economic & Social History Review**, v. 5, n. 2, p. 195–197, 1 jun. 1968.

HE, W. et al. Evaluation of Chinese tea by the electronic tongue: Correlation with sensory properties and classification according to geographical origin and grade level. **Food Research International**, v. 42, n. 10, p. 1462–1467, dez. 2009.

References

HÉBERGER, K. Chapter 7 - Chemoinformatics—multivariate mathematical–statistical methods for data evaluation. Em: VÉKEY, K.; TELEKES, A.; VERTES, A. (Eds.). **Medical Applications of Mass Spectrometry**. Amsterdam: Elsevier, 2008. p. 141–169.

INDIAN TEA ASSOCIATION. Disponível em: <https://www.indiatea.org/>. Acesso em: 24 out. 2021.

KANTOR, D. B. et al. Electronic tongue for sensing taste changes with apricots during storage. **Sensors and Actuators, B: Chemical**, v. 131, n. 1, p. 43–47, 14 abr. 2008.

KARYAKIN, A. A. et al. Potentiometric biosensors based on polyaniline semiconductor films. **Sensors and Actuators B: Chemical**, v. 33, n. 1–3, p. 34–38, jul. 1996.

KENNARD, R. W.; STONE, L. A. Computer Aided Design of Experiments. **Technometrics**, v. 11, n. 1, p. 137–148, fev. 1969.

KOSIŃSKA, A.; ANDLAUER, W. Antioxidant Capacity of Tea. Effect of Processing and Storage. Em: **Processing and Impact on Antioxidants in Beverages**. [s.l.] Elsevier Inc., 2014. p. 109–120.

KOTTAWA-ARACHCHI, J. et al. Biochemical Characteristics of Tea (Camellia L. spp.) Germplasm Accessions in Sri Lanka: Correlation between Black Tea Quality Parameters and Organoleptic Evaluation. **International Journal of Tea Science**, v. 10, p. 3–13, out. 2014.

KUMAR, K. Principal component analysis: Most favourite tool in chemometrics. **Resonance**, v. 22, n. 8, p. 747–759, 5 ago. 2017.

KUMAR, V. et al. Health benefits of tea consumption. **Tropical Journal of Pharmaceutical Research**, v. 6, p. 785–792, out. 2007.

LARSSON, S. C.; VIRTAMO, J.; WOLK, A. Black tea consumption and risk of stroke in women and men. **Annals of Epidemiology**, v. 23, n. 3, p. 157–160, mar. 2013.

LI, Y.; LEI, J.; LIANG, D. Identification of fake green tea by sensory assessment and electronic tongue. **Food Science and Technology Research**, v. 21, n. 2, p. 207–212, 2015.

LIANG, Y. et al. **Analytical, Nutritional and Clinical Methods Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions**. [s.l: s.n.]. Disponível em: <www.elsevier.com/locate/foodchem>.

LINDGREN, F. et al. Model validation by permutation tests: Applications to variable selection. **Journal of Chemometrics**, v. 10, n. 5–6, p. 521–532, 1 set. 1996.

LIU, H.; YU, D.; GU, Y. Classification and Evaluation of Quality Grades of Organic Green Teas Using an Electronic Nose Based on Machine Learning Algorithms. **IEEE Access**, v. 7, p. 172965–172973, 2019.

LUO, X. et al. Using surface-enhanced Raman spectroscopy combined with chemometrics for black tea quality assessment during its fermentation process. **Sensors and Actuators B: Chemical**, v. 373, p. 132680, dez. 2022.

LUTGENDORF, P. Making tea in India: Chai, capitalism, culture. **Thesis Eleven**, v. 113, n.

1, p. 11–31, 1 dez. 2012.

MACDONALD, J. R. et al. **Impedance Spectroscopy: Theory, Experiment, and Applications, Third Edition**. [s.l: s.n.].

MARSLAND, S. R. **Machine Learning: An Algorithmic Perspective, Second Edition**. 2014.

NGUYEN, D. V.; ROCKE, D. M. Tumor classification by partial least squares using microarray gene expression data. **Bioinformatics**, v. 18, n. 1, p. 39–50, 1 jan. 2002.

OUYANG, Q. et al. Measurement of total free amino acids content in black tea using electronic tongue technology coupled with chemometrics. **LWT**, v. 118, p. 108768, jan. 2020.

PACK, S. Chemometrics for analytical chemistry - Volume I : PC-aided statistical data analysis, M. Meloun, J. Militky and M. Forina, Ellis Horwood (Series in Analytical Chemistry), Chichester, 1992, ISBN 0-13-126376-5, No. of pages: 330. Price: £65.00. **Journal of Chemometrics**, v. 7, n. 3, p. 225, 1993.

PAN, M. et al. Comparison of Sensory and Electronic Tongue Analysis Combined with HS-SPME-GC-MS in the Evaluation of Skim Milk Processed with Different Preheating Treatments. **Molecules**, v. 24, n. 9, p. 1650, 26 abr. 2019.

PAN, T.; YAN, R.; CHEN, Q. Geographical origin of green tea identification using LASSO and ANOVA. **Food Science and Technology**, v. 42, 2022.

PATRA, P. et al. **EFFECT OF CLIMATE CHANGE ON PRODUCTION OF DARJEELING TEA: A CASE STUDY IN DARJEELING TEA RESEARCH and DEVELOPMENT CENTRE, TEA BOARD, KURSEONG**. 2013.

PEREIRA, V. P. et al. Determination of phenolic compounds and antioxidant activity of green, black and white teas of Camellia sinensis (L.) Kuntze, Theaceae. **Revista Brasileira de Plantas Medicinais**, v. 16, n. 3, p. 490–498, set. 2014.

POU, K. R. J. Fermentation: The Key Step in the Processing of Black Tea. **Journal of Biosystems Engineering**, v. 41, p. 85–92, ago. 2016a.

POU, K. R. J. Fermentation: The Key Step in the Processing of Black Tea. **Journal of Biosystems Engineering**, v. 41, p. 85–92, out. 2016b.

PRABOWO, B.; PURWIDYANTRI, A.; LIU, K.-C. Surface Plasmon Resonance Optical Sensor: A Review on Light Source Technology. **Biosensors**, v. 8, n. 3, p. 80, 26 ago. 2018.

PRASADA RAO, T.; BIJU, V. M. SPECTROPHOTOMETRY | Organic Compounds. Em: WORSFOLD, P.; TOWNSHEND, A.; POOLE, C. (Eds.). **Encyclopedia of Analytical Science (Second Edition)**. Second Edition ed. Oxford: Elsevier, 2005. p. 358–366.

PRIY, R. et al. Tea: Production, Composition, Consumption and its Potential as an Antioxidant and Antimicrobial Agent. **International Journal of Food and Fermentation Technology**, v. 5, p. 95, out. 2015.

References

QHAIRUL, N.; ABU BAKAR, M. F.; MAMAT, H. Phytochemicals and antioxidant properties of different parts of Camellia sinensis leaves from Sabah Tea Plantation in Sabah, Malaysia. **International Food Research Journal**, v. 20, p. 307–312, 1 jan. 2013.

RAHAMAN, S. A.; ARUCHAMY, S. Land Suitability Evaluation of Tea (Camellia sinensis L.) Plantation in Kallar Watershed of Nilgiri Bioreserve, India. **Geographies**, v. 2, n. 4, p. 701–723, 11 nov. 2022.

RAKESH, * et al. Analysis of tea powder for adulterant. **IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS**, v. 12, n. 4, p. 37–42, [s.d.].

RAMBO, M. K. D.; AMORIM, E. P.; FERREIRA, M. M. C. Potential of visible-near infrared spectroscopy combined with chemometrics for analysis of some constituents of coffee and banana residues. **Analytica Chimica Acta**, v. 775, p. 41–49, maio 2013.

RANATUNGA, M. et al. NIR Spectroscopic Determination of Polyphenol Content in Teas and Tea Extract at 2142 nm. **Sensors and Materials**, v. 33, n. 12, p. 4135, 16 dez. 2021.

RAVICHANDRAN, R.; RAMASWAMY, P. The impact of mechanization of tea harvesting on the quality of south Indian CTC teas. **Food Chemistry**, v. 63, p. 61–64, ago. 1998.

REN, G. et al. Estimation of Congou black tea quality by an electronic tongue technology combined with multivariate analysis. **Microchemical Journal**, v. 163, 1 abr. 2021a.

REN, G. et al. Hyperspectral imaging for discrimination of Keemun black tea quality categories: Multivariate calibration analysis and data fusion. **International Journal of Food Science & Technology**, v. 56, n. 6, p. 2580–2587, 3 jun. 2021b.

REYGAERT, W. C. **Green tea catechins: Their use in treating and preventing infectious diseases**. **BioMed Research International**Hindawi Limited, , 2018a.

REYGAERT, W. C. Green Tea Catechins: Their Use in Treating and Preventing Infectious Diseases. **BioMed research international**, v. 2018, p. 9105261, 17 jul. 2018b.

RIUL, A. et al. Wine classification by taste sensors made from ultra-thin films and using neural networks. **Sensors and Actuators B: Chemical**, v. 98, n. 1, p. 77–82, mar. 2004.

ROBB, C.; BROWN, P. Catechins in tea: Chemistry and analysis. **Advances in chromatography**, v. 41, p. 379–410, 1 fev. 2001.

RODRÍGUEZ-MÉNDEZ, M. L. et al. Electronic Noses and Tongues in Wine Industry. **Frontiers in Bioengineering and Biotechnology**, v. 4, 25 out. 2016.

RUDNITSKAYA, A. et al. Prediction of the Port wine age using an electronic tongue. **Chemometrics and Intelligent Laboratory Systems**, v. 87, n. 1, p. 125–131, 15 maio 2007.

SAEYS, W.; MOUAZEN, A. M.; RAMON, H. Potential for Onsite and Online Analysis of Pig Manure using Visible and Near Infrared Reflectance Spectroscopy. **Biosystems Engineering**, v. 91, n. 4, p. 393–402, ago. 2005.

SANCHES, V. L. et al. Comprehensive analysis of phenolics compounds in citrus fruits peels by UPLC-PDA and UPLC-Q/TOF MS using a fused-core column. **Food Chemistry: X**, v.

14, p. 100262, jun. 2022.

SANDAL, I. et al. Agrobacterium-mediated genetic transformation of tea leaf explants: effects of counteracting bactericidity of leaf polyphenols without loss of bacterial virulence. **Plant Cell Reports**, v. 26, n. 2, p. 169–176, 2007.

SCHARBERT, S.; HOFMANN, T. Molecular Definition of Black Tea Taste by Means of Quantitative Studies, Taste Reconstitution, and Omission Experiments. **Journal of Agricultural and Food Chemistry**, v. 53, n. 13, p. 5377–5384, 1 jun. 2005.

SHAO, J.; ZHANG, Y. Determination of caffeine content in tea beverages. **IOP Conference Series: Earth and Environmental Science**, v. 330, n. 4, p. 042056, 1 out. 2019.

SHARIFI, A. **Partial Least Squares-regression (PLS-regression) In Chemometrics**. ago. 2016.

SHESKIN, D. J. Handbook of Parametric and Nonparametric Statistical Procedures. **Technometrics**, v. 46, p. 369–370, 1997.

SHIMIZU, F.; BRAUNGER, M.; RIUL JR, A. **Electronic Tongues: Fundamentals and recent advances**. [s.l: s.n.].

SHRIVASTAVA, R. R.; PATERIYA, P.; SINGH, M. **Green tea-A short reviewInt J Ind Herbs Drugs**. [s.l: s.n.].

SINGH, A. et al. Tea Research for Darjeeling Tea Industry: Various Aspects. Em: [s.l: s.n.]. p. 195–239.

SKIBSTED, E. T. S. et al. New Indicator for Optimal Preprocessing and Wavelength Selection of Near-Infrared Spectra. **Applied Spectroscopy**, v. 58, n. 3, p. 264–271, 31 mar. 2004.

TEA STATION & TEA FOUNTAIN . Disponível em: <http://www.teafountain.com/teagrades>. Acesso em: 25 out. 2021.

TERRY, L. A.; WHITE, S. F.; TIGWELL, L. J. The Application of Biosensors to Fresh Produce and the Wider Food Industry. **Journal of Agricultural and Food Chemistry**, v. 53, n. 5, p. 1309–1316, 1 mar. 2005.

TESHOME, K. Effect of tea processing methods on biochemical composition and sensory quality of black tea (Camellia sinensis (L.) O. Kuntze): A review. **Journal of Horticulture and Forestry**, v. 11, p. 84–95, 2019.

THARWAT, A. et al. Linear discriminant analysis: A detailed tutorial. **AI Communications**, v. 30, p. 169–190, 2017.

VAN DER VOET, H. Comparing the predictive accuracy of models using a simple randomization test. **Chemometrics and Intelligent Laboratory Systems**, v. 25, n. 2, p. 313–323, nov. 1994.

VEGGI, P. C.; CAVALCANTI, R. N.; MEIRELES, M. A. A. Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction:

Experimental data and economic evaluation. **Journal of Food Engineering**, v. 131, p. 96–109, jun. 2014.

VERMA, R.; BHATTI, V.; KUMARI, R. Analysis of Various Tea Samples. **International Journal of Phytopharmacy**, v. 3, out. 2013.

VERMEEREN, V. et al. DNA Sensors with Diamond as a Promising Alternative Transducer Material. **Sensors**, v. 9, n. 7, p. 5600–5636, 14 jul. 2009.

WANG, J.; XIAO, H. Discrimination of different white chrysanthemum by electronic tongue. **Journal of food science and technology**, v. 50, n. 5, p. 986–992, out. 2013.

WANG, Y. et al. Impact of Six Typical Processing Methods on the Chemical Composition of Tea Leaves Using a Single *Camellia sinensis* Cultivar, Longjing 43. **Journal of Agricultural and Food Chemistry**, v. 67, n. 19, p. 5423–5436, 15 maio 2019.

WILSON J S. CHAPTER 1 - Sensor Fundamentals. Em: WILSON, J. S. (Ed.). **Sensor Technology Handbook**. Burlington: Newnes, 2005. p. 1–20.

WOLD, S.; SJOSTROM, M.; ERIKSSON, L. PLS-regression: A Basic Tool of Chemometrics. **Chemometrics and Intelligent Laboratory Systems**, v. 58, p. 109–130, out. 2001.

WONG, M.; SIRISENA, S.; NG, K. Phytochemical profile of differently processed tea: A review. **Journal of Food Science**, v. 87, n. 5, p. 1925–1942, 2 maio 2022.

Y, D. et al. Correlation between Sensory Evaluation Scores and Flavor Attributes of Black Teas from Around the World. **Journal of Food Processing & Technology**, v. 09, n. 04, 2018.

YAN, S. M. et al. Electronic tongue combined with chemometrics to provenance discrimination for a green tea (Anji-White tea). **Journal of Food Quality**, v. 2017, 2017a.

YAN, S.-M. et al. Electronic Tongue Combined with Chemometrics to Provenance Discrimination for a Green Tea (Anji-White Tea). **Journal of Food Quality**, v. 2017, p. 1–6, 2017b.

YIANNAKOPOULOU, E. C. **Interaction of green tea catechins with breast cancer endocrine treatment: a systematic review**. **Pharmacology**, 2014.

ZHENG, X.-Q. et al. Recent Advances in Volatiles of Teas. **Molecules**, v. 21, n. 3, p. 338, 11 mar. 2016.

ZHI, R.; ZHAO, L.; ZHANG, D. A Framework for the Multi-Level Fusion of Electronic Nose and Electronic Tongue for Tea Quality Assessment. **Sensors**, v. 17, n. 5, p. 1007, 3 maio 2017.

APPENDICE A- IMPEDIMETRIC E-TONGUE CONSTRUCTION AND MEASUREMENT

Appendices

A.1Construction

The four sensing units are embedded onto a gold-plated interdigitated electrode (IDEs). Each of the individual IDEs signal will be routed through analog multiplexer which is controlled using the software in computer. The multiplexer comprises of an Arduino® with an ability to prevent cross-talking of routed signals. A typical instrumentation set-up including the micro-fluidic channel is shown in **Figure 7.** It should be noted we did not use the microfluidic channel for this study.

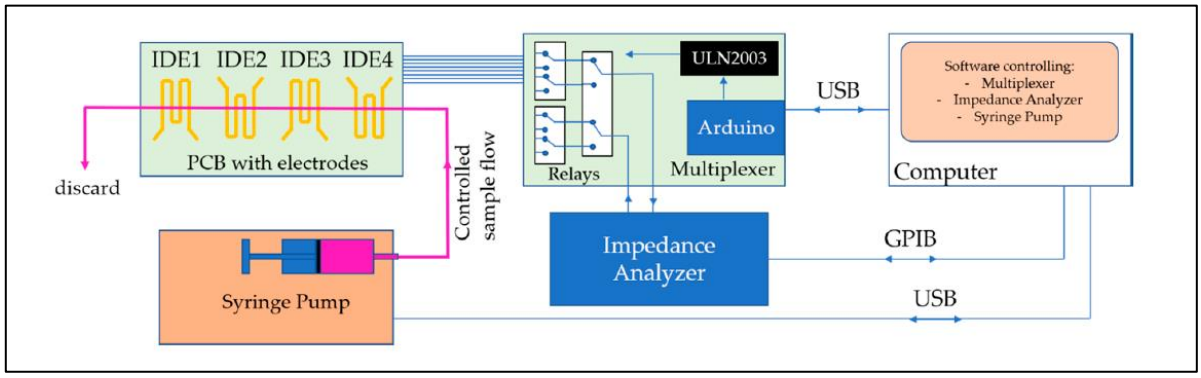


Figure 10.Construction of the multi-sensing impedimetric system with a microfluidic channel (Source: BRAUNGER et al., 2020).

A.2Sensing units

The four sensing units on the PCB board (IDE to IDE4) is deposited with varying chemical composition and electrical response to analyse different analytes, invariably replicating human tongue in recognizing diverse taste patterns. Employing the layer-by-layer technique the films assembly was carried out and among the four sensing units, one was left uncovered without modifications by nano structured materials. Thereby we have four distinct sensing units 1 to 4.

The materials deposited onto the sensing units after the nano-structured materials modifications is enlisted in **Table 7.** The poly (diallyl dimethylammonium chloride) solution, known as PDDA, forms the base for all the IDEs barring sensing unit 1.

Table 7.Sensing units after formation of bilayers using Layer by layer (LbL) fabrication

Sensing units	Composition	Suffix chemical abbreviation
1	bare IDE (none)	-
2	PDDA/CuTsPc	Copper phthalocyanine-3,4',4'',4'''-tetrasulfonic acid tetrasodium salt
3	PDDA/MMt-K	Montmorillonite clay
4	PDDA/PEDOT:PSS	Poly (3,4-ethylenedioxythiophene)- poly (styrenesulfonate)

A.3Impedance measurement

The transduction mechanism of an impedance analyser is illustrated in **Figure 8**. Applying both AC and DC signals, impedance is measured by letting the constraint current flow through unknown impedance and pass to the range resistor R_r (expressed in equation 1).

An impedance analyzer finds less application in electrochemical systems, and operates by the auto-balance bridge which is shown in **Figure 9** (MACDONALD et al., 2018).

$$Z_{unknown} = R_r \frac{ei}{ei} \quad \text{(Eq. 1)}$$

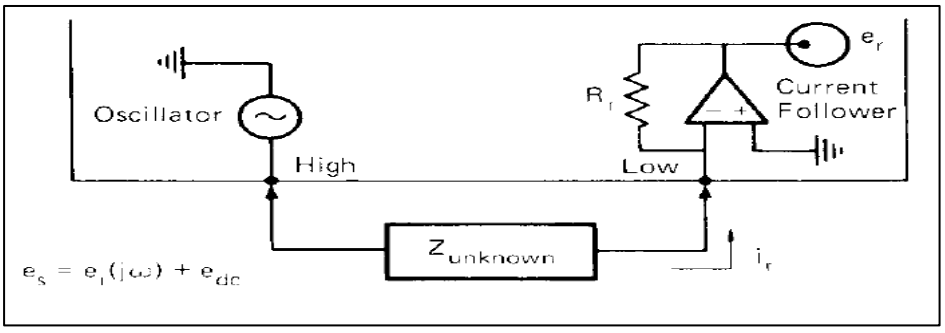


Figure 11.Impedance analyzer

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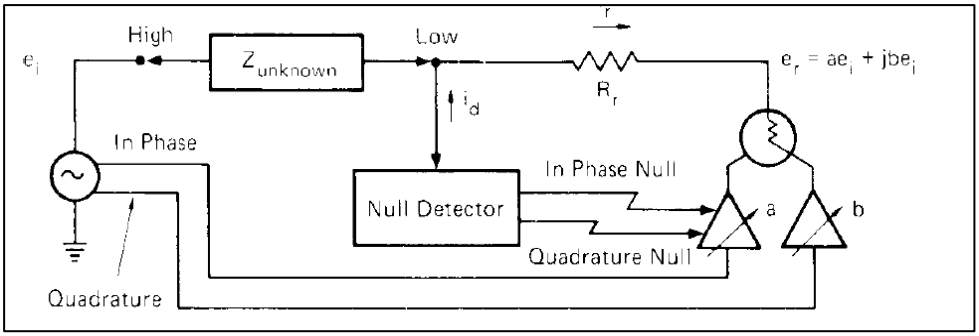

Figure 12.Diagram showing auto-balance bridge operation (Source: MACDONALD et al., 2018).

APPENDICE B - SUPPLEMENTARY MATERIAL OF CHAPTER 4

Appendices

Table 8.Expression of Total Polyphenols in mg GAE/g

Label	Label	Grade	Total Polyphenols (mg GAE/g)	
			Mean	Range
1	I	A	124±0.72	123.18-124.53
2	I	A	125.43±3.72	121.82-129.26
3	II	B	168.09±2.93	164.71-169.89
4	I	A	102.7±1.24	101.87-104.12
5	I	A	123.72±2.37	121.46-126.19
6	II	B	128.7±4.34	126.19-133.72
7	I	A	154.44±4.35	151.19-159.38
8	I	A	163.58±3.07	161.59-167.12
9	I	A	88.66±1.42	87.04-89.69
10	I	A	90.72±0.56	90.13-91.24
11	II	B	95.88±2.46	93.23-98.1
12	I	A	117.47±2.04	115.13-118.86
13	I	A	115.5±4.93	111.62-121.05
14	II	B	138.08±4.98	133.55-143.42
15	II	B	150.29±8.49	145.18-160.09
16	III	B	116.49±6.94	108.51-121.11
17	IV	B	112.85±7.88	104.94-120.69
18	V	B	99.46±0.83	98.71-100.35
19	VI	C	97.75±1.67	95.84-98.91
20	VII	D	111.03±3.57	107.46-114.6
21	III	B	121.95±2.18	119.43-123.21
22	IV	B	113.62±7.89	104.52-118.38
23	V	B	112.71±3.26	109.35-115.86
24	VI	C	126.58±10.43	119.43-138.55
25	VII	D	124.12±4.86	119.01-128.68
26	III	B	119.26±1.35	118.48-120.82
27	IV	B	132.58±3.7	128.53-135.77
28	V	B	131.8±0.84	131.1-132.73
29	VI	C	117.08±1.3	115.91-118.48
30	VII	D	126.74±3.14	123.15-129
31	III	B	120.9±1.99	118.95-122.92
32	IV	B	123.84±7.1	115.65-128.26
33	V	B	122.44±7.75	115.86-130.99
34	VI	C	120.83±8.81	110.82-127.42
35	VII	D	117.96±7.82	108.93-122.58

I- orange pekoe, II-flowery broken orange pekoe, III- broken pekoe, IV- broken orange pekoe, V-broken orange

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pekoe small, VI- pekoe dust C, VII- orange fannings; A- whole leaf, B- broken leaf, C- dust, D- fannings.

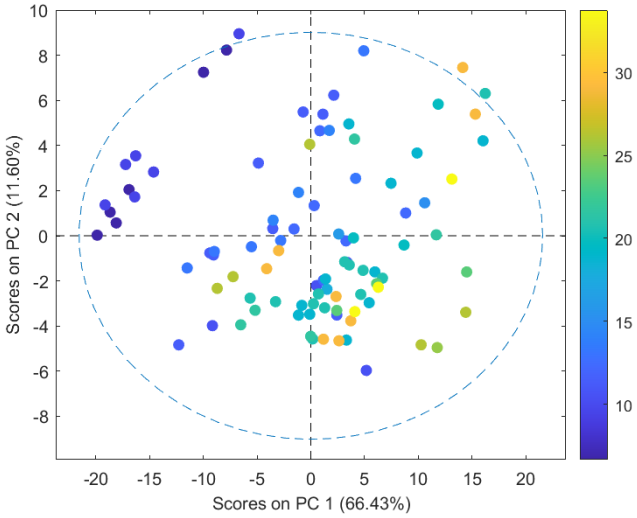
* The data correspond to the mean ± SD of three repetitions TPC, expressed in mg GAE/g. TPC (Total polyphenol content) for individual black tea samples.

Appendices

Table 9.Significant differences for black tea samples by ANOVA(P<0.05)

Sample number	Total Flavonoids (mg QE/g)	Sample number	Caffeine (mg/L)	Sample number	Total Polyphenols (% GAE)
28	33.71±0.05^{A}	28	75.26±0.13^{A}	26	26.67±0.28^{A}
19	28.75±0.05^{B}	17	72.83±3.95^{A,B}	21	18.6±0.34^{B}
24	28.72±0.04^{B}	30	71.74±8.41^{A,B}	8	17.33±0.32^{B,C}
33	28.72±0.06^{B}	29	67.07±1.36^{A,B}	7	17.25±0.48^{B,C,D}
32	26.25±0.05^{C}	23	66.51±7.84^{A,B,C}	32	16.97±1.01^{B,C,D,E}
26	25.62±0.02^{D}	35	62.58±3.53^{A,B,C,D}	24	16.46±1.4^{B,C,D,E,F}
17	23.78±0.02^{E}	19	62.02±0.01^{A,B,C,D}	22	16.05±1.16^{C,D,E,F,G}
35	21.89±0.01^{F}	27	61.8±6.45^{A,B,C,D}	3	15.96±0.28^{C,D,E,F,G}
18	21.59±0.51^{F}	18	60.33±6^{A,B,C,D,E}	15	15.52±2.45^{C,D,E,F,G,H}
23	20.64±0.03^{G}	16	59.22±7.06^{A,B,C,D,E,F}	28	15±0.09^{C,D,E,F,G,H,I}
29	20.63±0.01^{G}	31	57.51±1.18^{A,B,C,D,E,F}	33	14.95±0.98^{D,E,F,G,H,I,J}
16	20.61±0.07^{G,H}	14	56.7±7.37^{A,B,C,D,E,F}	25	14.78±0.6^{E,F,G,H,I,J}
21	20.53±0.18^{G,H}	33	56.04±2.61^{A,B,C,D,E,F}	18	14.65±0.1^{E,F,G,H,I,J,K}
34	20.08±0.07^{H}	24	55.77±2.7^{A,B,C,D,E,F,G}	27	14.44±0.38^{F,G,H,I,J,K,L}
27	19.36±0.05^{I}	20	55.35±2.13^{A,B,C,D,E,F,G,H}	30	14.44±0.33^{F,G,H,I,J,K,L}
20	18.79±0.1^{J}	26	55.26±2.02^{A,B,C,D,E,F,G,H}	29	14.23±0.15^{F,G,H,I,J,K,L,M}
25	18.77±0.07^{J}	21	54.31±6.5^{A,B,C,D,E,F,G,H,I}	31	14.21±0.22^{F,G,H,I,J,K,L,M}
30	18.68±0.12^{J}	15	54.2±19.6^{A,B,C,D,E,F,G,H,I}	34	14.04±1.06^{G,H,I,J,K,L,M,N}
22	18.58±0.26^{J}	32	53.81±0.92^{A,B,C,D,E,F,G,H,I}	35	13.84±0.95^{G,H,I,J,K,L,M,N}
7	15.55±0.47^{K}	34	53.29±4.05^{A,B,C,D,E,F,G,H,I}	16	13.54±0.84^{H,I,J,K,L,M,N,O}
2	14.26±0.2^{L}	22	50.69±3.93^{B,C,D,E,F,G,H,I}	20	13.07±0.44^{I,J,K,L,M,N,O,P}
14	13.12±0.01^{M}	25	49.16±1.71^{B,C,D,E,F,G,H,I,J}	17	12.82±0.93^{I,J,K,L,M,N,O,P}
12	13.12±0.01^{M,N}	8	42.35±7.76^{C,D,E,F,G,H,I,J}	6	12.59±0.42^{J,K,L,M,N,O,P,Q}
3	12.64±0.3^{M,N}	13	40.63±0.26^{D,E,F,G,H,I,J}	2	12.38±0.36^{K,L,M,N,O,P,Q}
8	12.58±0.08^{N}	3	39.92±3.08^{D,E,F,G,H,I,J}	14	12.09±0.82^{L,M,N,O,P,Q}
4	11.3±0.05^{O}	10	38.25±0.29^{D,E,F,G,H,I,J}	5	12.08±0.24^{M,N,O,P,Q}
13	11.25±0.05^{O}	7	36.93±6.1^{E,F,G,H,I,J}	1	11.88±0.07^{M,N,O,P,Q}
1	11.2±0.08^{O}	5	36.7±3.27^{E,F,G,H,I,J}	19	11.73±0.2^{N,O,P,Q}
31	11.17±0.13^{O}	1	36.05±10.06^{E,F,G,H,I,J}	18	11.39±0.1^{O,P,Q,R}
15	10.33±0.33^{P}	6	35.81±1.15^{F,G,H,I,J}	12	11.32±0.58^{O,P,Q,R}
11	10.11±0.1^{P}	9	31.43±5.56^{G,H,I,J}	13	11.15±0.44^{P,Q,R,S}
6	10.06±0.09^{P}	2	30.94±7.56^{H,I,J}	4	10.42±0.13^{Q,R,S,T}
5	10.01±0.01^{P}	4	30.16±4.55^{I,J}	11	9.25±0.24^{R,S,T}
10	6.85±0.05^{Q}	11	25.67±1.55^{J}	10	8.91±0.05^{S,T}
9	6.82±0.13^{Q}	12	25.25±6.97^{J}	9	8.59±0.14^{T}

*Means that do not share a letter are significantly different.



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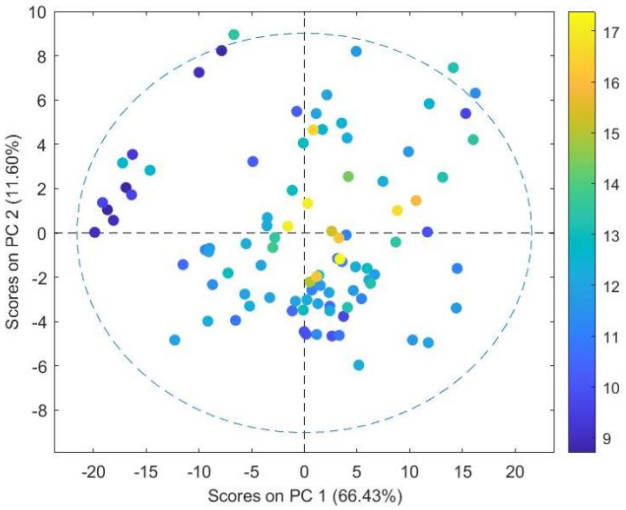

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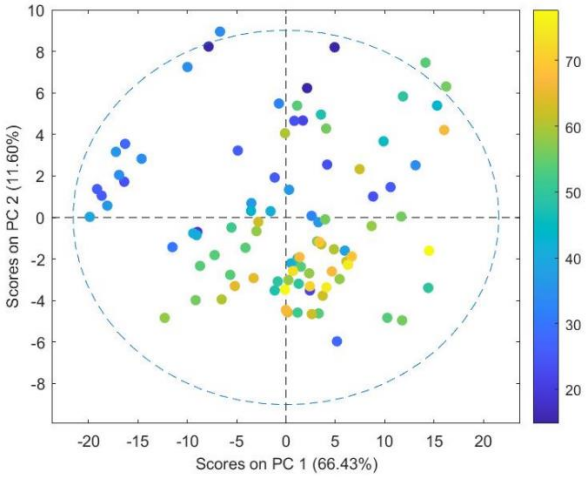

C

Figure 13.PCA for reference analysis based on the concentrations: a) Total flavonoids (mg QE/g) b) Caffeine (mg/L) c) Total polyphenols (% GAE)