Testing herbal medicines plants mixtures using a taste sensor "an electronic tongue" and multivariate data analysis

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This Thesis was submitted in partial fulfillment of the requirements for the Master's Degree of Science in:

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This thesis (Testing herbal medicines plants mixtures using a taste sensor “an electronic tongue” and multivariate data analysis) was successfully defended and approved on 14-Feb-2019.

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February, 2019 — شباط، 2019

Testing herbal medicines plants mixtures using a taste sensor “an electronic tongue” and multivariate data analysis

Dedication

To my parents who taught me to work hard for the things I aspire to achieve. To my husband, brothers and sisters for their spiritual love. To my little daughter. Finally, to everyone who helped me during this important stage in my life.

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List of Abbreviations

ANN	Artificial neural network
ANOVA	Analysis of variance
BODs	Biological oxygen demand
BPNN	Back propagation neural network
CE	Capillary electrophoresis
ChemFET	Chemically modified field effect transistors
CODs	Chemical oxygen demand
CV	Coefficient of variation
DF	Discriminant function
DFA	Discriminant function analysis
ET	Electronic tongue
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HCA	Hierarchical cluster analysis
HPLC	High performance liquid chromatography
LC-M	Liquid chromatography-mass
LDA	Linear discrimination analysis
MH-Agar	Mueller Hinton Agar
MVDA	Multivariate data analysis
P	Probability
PARC	Pattern recognition
PC	Principle component
PCA	Principle component analysis

PLS	Partial least squares
PLS-DA	Partial least squares-discrimination analysis
PVC	Poly vinyl chloride
R^2	Squared correlation coefficient
RMSE	Root-mean-square error
RPD	Ratio of standard error of performance to standard deviation
RSD	Relative standard deviation
SD	Standard deviation
SIMCA	Soft independent modeling of class analogy
TLC	Thin-layer chromatography
UV-Vis	Ultraviolet-Visible

Testing herbal medicines plants mixtures using a taste sensor 'an electronic tongue' and multivariate data analysis

Haneen Majed Taha

Dr. Nawaf Abu-Khalaf

Abstract

Herbal plants play important role in various health applications. Nowadays, scientists are emphasizing on analytical methods and chemometric applications for identifying many herbs from closely related species and other discrimination factors. Despite the presence of highly precise analytical methods, still there are some disadvantages for them, a multi-sensor called an electronic tongue (ET) system is an alternative, promising and compatible technology.

This study was carried out to present an emerging research field of sensors technology (*i.e.* ET). ET and multivariate data analysis (MVDA) were used in quality control of local herbal medicines, to identify various locally produced samples of herbal extracts tea and to test the ability of ET to quantify their taste. Firstly, six tea samples, including four herbal tea, black and green tea, were tested using ET. Data obtained by ET was analyzed using MVDA. Different validation methods were used including a human taste panel (*i.e.* consumer test) and spectrophotometric measurements of caffeine content in the tea brands. Secondly, two herbal tea samples (*i.e.* Nanus and Morning tea) were mixed in different concentrations and tested using ET. Finally, a local product called Relax (an herbal mixture used as laxative) was studied to detect the stability or benchmarking of the product, as well as its alternative products in the market.

The results revealed that ET could successfully discriminate three of the six herbal tea samples and predict several taste parameters and the caffeine content in each tea brand. Moreover, ET could detect a mixture of two tea brands efficiently. As for the Relax granules, results indicated little or no effect of the storage time (shelf-time) on the analyzed production batches. Moreover, the local Relax proved its ability to compete with foreign products.

The overall results of the present work provided baseline information for the possible use of ET, with the help of MVDA in data interpretation, for testing quality and taste of herbal medical plants mixtures. Furthermore, it opens a door for new possible applications in Palestine.

Keywords: electronic tongue; herbal medicines; PCA; DFA; PLS; taste panel; caffeine.

1. Introduction

1.1 Overview

Herbal plants were the only source of medications for years before any other chemical processes were applied. Even now, many traditional drugs relay mainly on herb's extracts, since the discovery of these pharmaceutical agents have been accomplished through the screening of active ingredients of plant's natural products. A wide use and an increase interest of herbal plants in Palestine for complementary and alternative medicine, was noticed due to the biodiversity of this area (Elkhair, Fadda, & Mohsen, 2010; Li *et al*., 2015; Rubio-Moraga *et al*., 2013).

The evaluation of medical plants quality derived the attention towards many modern analytical approaches (Jing, Deguang, Linfang, Shilin, & Minjian, 2011). A number of researchers have reported selective, sensitive and versatile analytical techniques including *e.g.* thin-layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass (LC-M) (Gavali, Pradhan, & Waghmare, 2016; Ramautar, Somsen, & de Jong, 2013; Zhang, Sun, Wang, Han, & Wang, 2012).

Despite their long analytical success, these techniques have a number of limitations in use; including long analytical duration, high cost analysis, complex sample preparations and the need to have a skilled person (Papieva *et al.*, 2011). Recently, researchers have shown an increased interest in a new chemical sensor-array named electronic tongue (ET) (Yaroshenko *et al.*, 2015). It can be defined as: "An analytical instrument comprising an array of nonspecific, low selective, chemical sensors with high stability and cross-sensitivity to different species in solution, and an appropriate method of pattern recognition (PARC) and/or multivariate calibration for data processing" (Vlasov, Legin, Rudnitskaya, Di Natale, & D'Amico, 2005).

ET mimics the human gustatory system. Some research called it taste sensor. A primary concern of this taste sensing systems is its ability to analyze the total gustatory impression instead of analyzing each ingredient alone (Eckert *et al.*, 2013). In humans the taste sensations occur when molecules trigger signals in the mouth that are sent to the brain, where a specific taste is registered. In ET, an array of non-specific sensors is applied into dissolved ingredients providing a unique finger print for each sample depending on the difference in the electrical response of different materials (Gupta, Sharma, Kumar, & Roy, 2010; Kalit, Markovic, Kalit, Vahcic, & Havranek, 2014; Smyth & Cozzolino, 2012).

The role of ET in general has received increased attention across a number of domains compared to other analytical methods, due to its rapid, fast sample preparation, reliability, useful in identifying and quantifying of several liquid compounds, its acceptable accuracy and relatively low cost (Abu-Khalaf & Iversen, 2007a; Abu-Khalaf & Iversen, 2007b, Wang, Liu, Wu, & Hsia, 2015).

Identification and quantification of various aqueous samples can be done mainly through the correct interpretation of ET signals (Ramamoorthy, Mohamed, & Devi, 2014). A multivariate data analysis (MVDA) (also called chemometrics) has been used for that purpose. MVDA has several techniques e.g. principle component analysis (PCA) (Major *et al*., 2011), linear discrimination analysis (LDA) (Ciosek & Wroblewski, 2011), Hierarchical cluster analysis (HCA) (Kalit *et al*., 2014), discriminant function analysis (DFA) (Men, Ning, & Chen, 2013), soft independent modeling of class analogy (SIMCA) (Wang, Niu, Hui, & Jin, 2015), partial least squares (PLS) (Campbell *et al*., 2012) and artificial neural network (ANN) (Ramamoorthy *et al*., 2014).

In this work, we used ET to identify various local produced samples of herbal extracts tea and test the ability of ET to quantify their taste. The samples were provided by a local company named Bajjora (Tulkarm, Palestine).

To achieve this goal, four herbal tea samples (*i.e.* Lipo, Morning, Nanus and Gasobal) were tested. Both green and black tea were also used, with green tea as a reference. Data obtained by ET for these six samples was analyzed using MVDA. Different validation methods were used including a human taste panel (*i.e.* consumer test, in which a group of six persons were asked to taste tea samples) and spectrophotometric measurements. Moreover, the antimicrobial activity of these brands was also investigated against two intestinal bacterial strains (*i.e.* gram positive (*Staphylococcus aureus)* and gram-negative bacteria *(Escherichia coli)*) (Appendix A).

Finally, a local product called Relax (an herbal mixture) was studied using ET to detect the stability or benchmarking of the product, 11 batches were provided by the local factory Bajjora (Tulkarm, Palestine). Each batch was produced in a different month. Also, comparing signals of Relax with other products, used for the same medical purposes as laxatives in the local market, was carried out.

1.2 Aim of the study

The primary goal of this study is to use ET and MVDA, as they are emerging and promising research field, in quality control of local herbal medicines. This will contribute in helping and supporting the improvement of local herbal medicines market and open a door for further applications.

1.3 Objectives

The objectives of this research are:

- To get different fingerprints for each of the herbal tea brands using ET.
- To obtain a relation between ET and human taste panel scores.
- To relate the spectrophotometric measurements of caffeine in the tea samples to ET.
- To detect signals that represent different ratios resulted from mixing two of herbal tea brands.
- Use ET to discriminate between different production batches of the local medicine herbal extract called Relax, and to recognize the most closely related available products among its comparisons in the local market.

2. Literature review

2.1 Electronic tongue (ET)

A large and growing body of literature has investigated history and modifications of ET over the last decades. The idea of ET was firstly introduced to the world in 1900s (Tahara & Toko, 2013). Since then, ET was used mainly as a quality management tool in food and beverage. Several attempts later led to a taste sensor with a global selectivity that can quantify different chemical substances and mimics human gustatory system by discriminating sourness, saltiness, umami, bitterness and sweetness (Fig. 1) (Gutierrez-Capitan *et al*., 2014; Kobayashi *et al*., 2010; Ramamoorthy *et al*., 2014;Vlasov, Legin, & Rudnitskaya, 2008;).

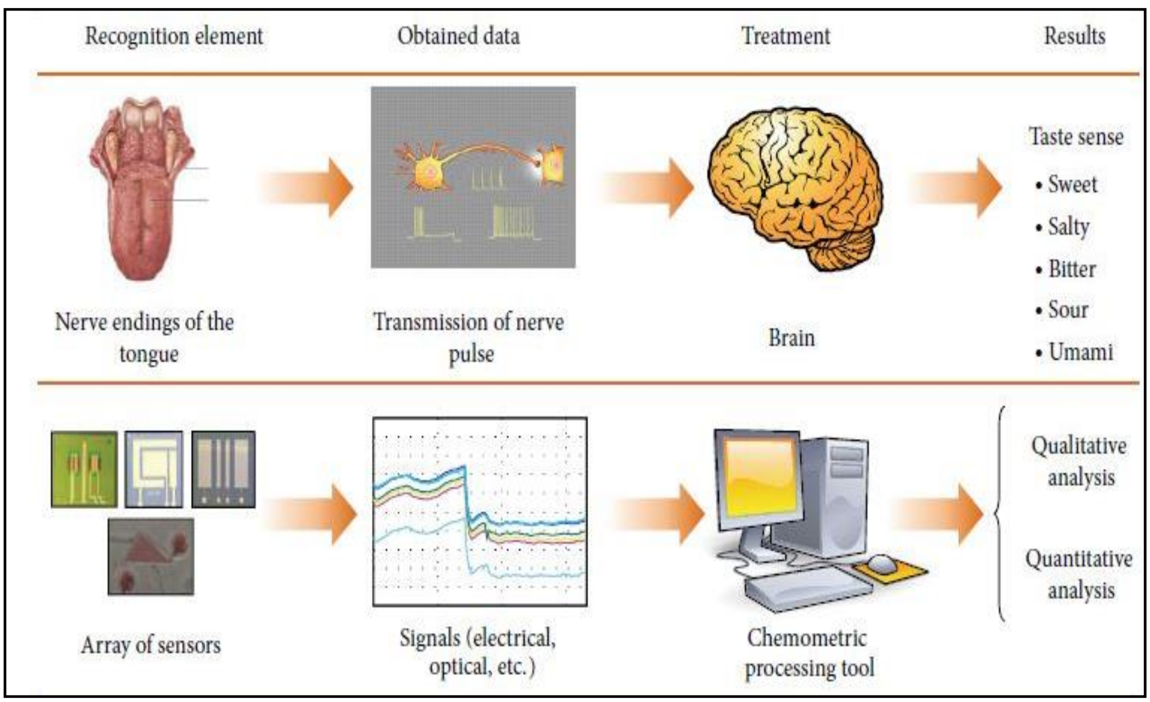

Fig. 1. Electronic tongue mimics human gustatory system (Gutierrez-Capitan et al., 2014).

It is now well established from a variety of studies that ET can be defined as: a simple analytical instrument consists of an array of non-specific sensors that has poor-selectivity and cross-sensitivity to chemical compounds in a mixture, with an appropriate chemometric method for data interpretation (Adnan *et al.*, 2013; Nunez, Ceto, Pividori, Zanoni, & Del Valle, 2013; Wadehra *et al.*, 2016). The probe membranes, transducer and statistical analysis in the ET have the same function as taste buds, neural transmission and cognition in the thalamus in humans, respectively (Raman, Stopfer, & Semancik, 2011).

2.1.1 Types of ET

There are several types of ET including potentiometric, voltammetric, taste sensor, resistive, gravimetric and optical. The most widely used are taste sensor, voltammetric and potentiometric. There are several types of sensors each with specific design depending on the type of ET and the application being used in.

2.1.1.1 Taste sensor

In 1990, a nonspecific sensory array for liquid sample analysis was introduced by Kiyoshi Toko and co-workers from Kyushu University /Japan who proposed a transducer with different lipid/ polymer membrane for each electrode, this device was named a taste sensor then later they named it as ET. Fig. 2 shows schematic representation of taste sensor (Toko, Tahara, Habara, Kobayashi, & Ikezaki, 2016; Wang, Liu, Wu, & Hsia, 2015). Different purposes of research require the use of different lipid/ polymer membrane. Eight electrodes were used to sense the five tastes, which human gustatory system can detect. The potential difference between the eight electrodes and the silver/silver chloride reference electrode is measured (Woertz, Tissen, Kleinebudde, & Breitkreutz, 2010; Woertz, Tissen, Kleinebudde, & Breitkreutz, 2011).

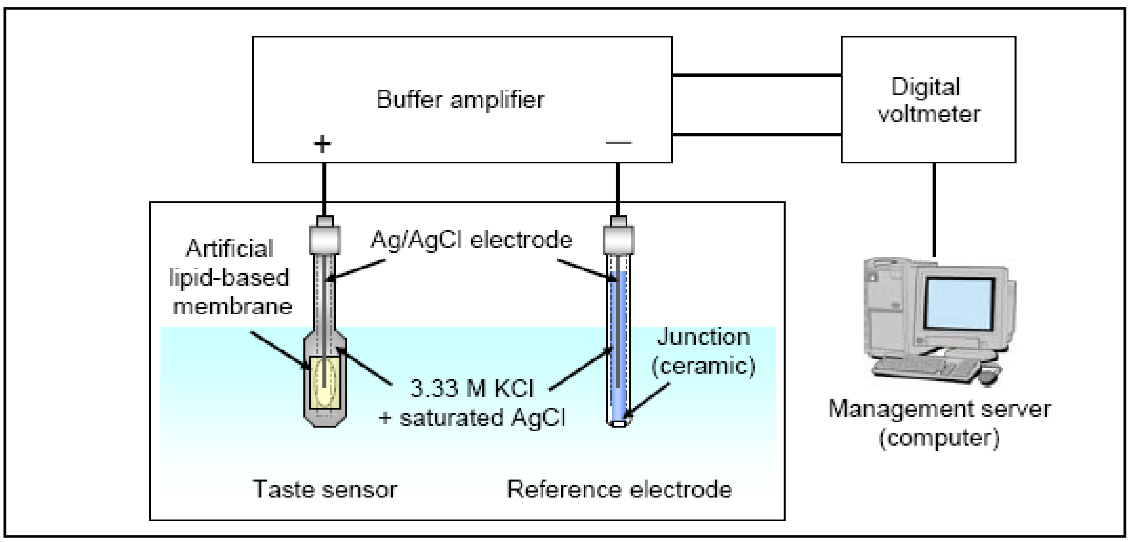

Fig. 2. Schematic diagram of taste sensing system (Kobayashi, Hamada, Yamaguchi, Ikezaki, & Toko, 2009).

There have been a number of extended studies involving applications of taste sensor including beverages manufacture of beer, wine, milk, coffee, green tea as well as in food stuff production of rice, pork and tomato. Other pharmaceutical and industrial uses were also investigated (Gastl, Hanke, &

Back, 2008; Harada, Sakurai, Hondo, Yasui, & Owaki, 2014; Hayashi, Chen, Ikezaki, & Ujihara, 2008; Kobayashi *et al*., 2010; Mizota *et al*., 2009).

2.1.1.2 Voltammetric ET

Voltammetric ET was firstly introduced to the world in 1997 by Fredrik Winquist and co-workers from Linkoping University / Sweden (Del Valle, 2010). It's a sensitive, powerful, versatile and simple device, which composed mainly of six working electrodes of gold (Au), iridium (Ir), platinum (Pt), palladium (Pd), rhenium (Re) and rhodium (Rh), with a reference electrode and auxiliary electrode made of stainless steel (Fig. 3). The number of working electrodes used depends mainly on the type of application employed in (Mohamed & Adel-Mageed, 2010). An electrochemical reaction at the working electrode from the redox active species on the solution (reduced or oxidized) produces a current, which depends on the type of the working electrode and the applied potential (Winquist, 2008). One obstacle can affect the sensitivity of voltammetric ET when all compounds in the measured sample are electrochemicaly active under the applied potential. The poor sensitivity problem can be overcome by using pulse voltammetry or covering the working electrode with a membrane that permit only gasses to pass through (Baldwin *et al*., 2011; Wang *et al*., 2015).

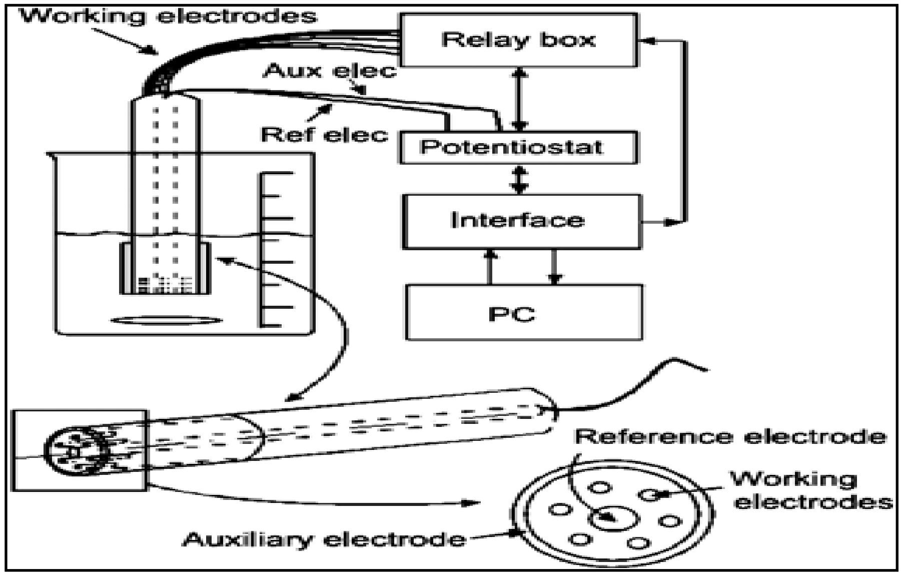

Fig. 3. Voltammetric electronic tongue (Riul Jr, Dantas, Miyazaki, & Oliveira Jr, 2010).

Different kinds of information could be obtained from the measured samples depending on the technique being used, including cyclic, stripping, and pulse voltammetry (Tonle & Ngameni, 2011). Various published studies demonstrated applications of voltammetric ET in discrimination of rice with different pretreatment (Ahmad Bhawani, Fong, & Mohamad Ibrahim, 2015), detection and monitoring of nitrate, nitrite and ammonium levels in water (Nunez *et al*., 2013), food analysis (Alghamdi *et al*., 2010) and pharmaceutical industries (Gupta, Jain, Radhapyari, Jadon, & Agarwal, 2011; Soderstrom, Boren, & Krantz-Rulcker, 2005).

2.1.1.3 Potentiometric ET

A novel potentiometric sensor was developed around 1996 in St. Petersburg University / Russia by Andrey Legin and co-workers (Jones, 2010). It's the most widely used sensor among others, because of its familiarity, cost and simplicity in preparations and usage since their selectivity can be simply modified by changing the transducer construction (Ciosek & Wroblewski, 2011; Mimendia *et al.*, 2010). The measurements based on the potential difference between two electrodes (Fig. 4).

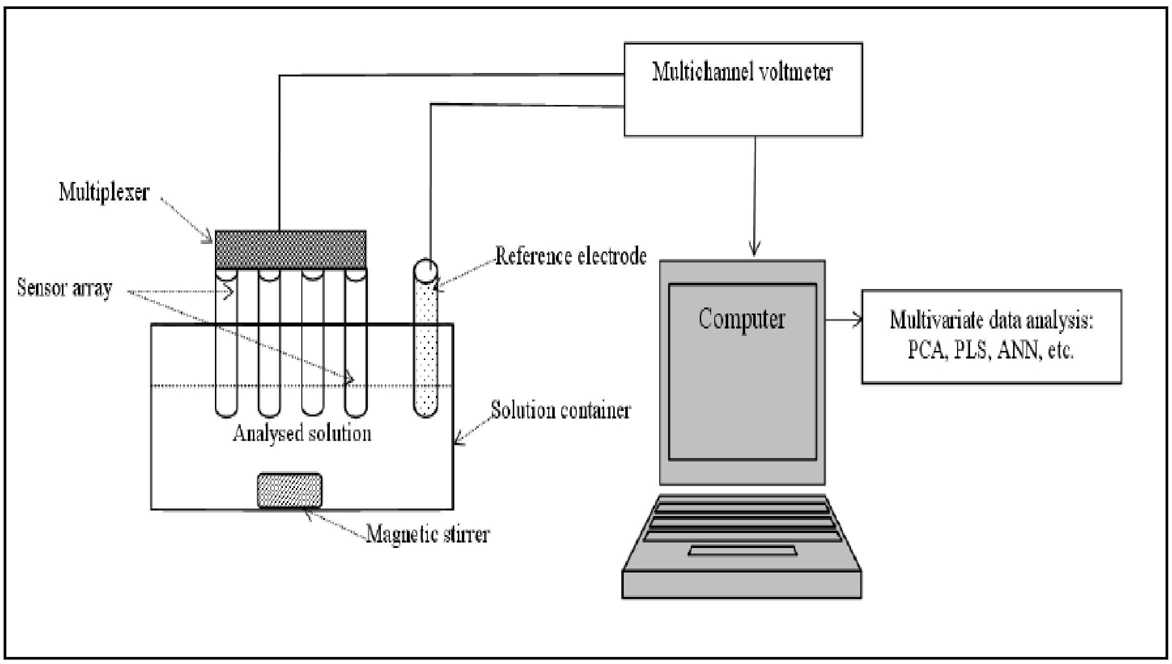

Fig. 4. Schematic diagram of potentiometric electronic tongue (Abu-Khalaf, 2006).

Ion-selective electrodes (ISEs) are the largest group among potentiometric sensors. The sensor-array must be characterized with low-selectivity and high cross-sensitivity sensors (Lvova, Kirsanov, Di Natale, & Legin, 2014). These two terms play a crucial role in the ET work and industry. Selectivity is defined as the ability of a sensor to measure a potential of a compound in presence of other interfering components. Whereas, the sensitivity toward several compounds in the solution being analyzed without any interactions refer to as cross-sensitivity. Measurements of cross-sensitivity depend mainly on the slope of the calibration curve in a plot that represents how concentration changes affect change of receiving signal (Riul Jr *et al.*, 2010).

Potentiometric ET became a key instrument in multiple applications including pharmaceutical and pediatrics applications, in which the bitterness is evaluated to indicate the taste masking efficiency and active ingredients detection in plant extracts (Abu-Khalaf *et al.*, 2018; Latha & Lakshmi, 2012; Lin *et al.*, 2016; Qneibi *et al.*, 2018;), the monitoring of environmental systems (Mimendia *et al.*, 2010) and quantification of immobilized protein in pharmaceutical production (Voitechovic, Korepanov, Kirsanov, & Legin, 2018). PVC ion-selective electrodes was used for the potentiometric flow injection analysis of distigmine in its pharmaceutical formulation and biological fluids (Issa & Khorshid, 2011) and in the determination of amprolium HCl in its single and combined dosage form and in chicken liver (Basha, El-Rahman, Bebawy, & Salem, 2017) and many other purposes in a wide range of aspects.

2.1.2 Applications of ET

A considerable amount of literature has been published on ET applications. These studies focused on various aspects including food industry for quality control, beverage flavor analysis, process of monitoring and works of adulterations and origin tracing. Also, pharmaceutical and pediatrics uses in taste masking were a major area of interest. ET received considerable critical attention in environmental monitoring processes and finally in commercial purposes and national safety services (Abu-Khalaf *et al*., 2018; Alonso, Istamboulie, Noguer, Marty, & Munoz, 2012; Apetrei & Apetrei, 2013; Dias, Alberto, Veloso, & Peres, 2016; Gutierrez-Capitan *et al*., 2013; Kutyla-Olesiuk, Zaborowski, Prokaryn, & Ciosek, 2012; Lichtenstein *et al*., 2014; Pein, Preis, Eckert, & Kiene, 2014; Podrazka, Baczynska, Kundys, Jelen, & Witkowska Nery, 2017; Qneibi *et al*., 2018; Sliwinska, Wisniewska, Dymerski, Wardencki, & Namiesnik, 2016; Vagin, Eriksson, & Winquist, 2016).

Kalit *et al.* (2014) reviewed different types of ET used in dairy products applications, including a hybrid ET that was used to analyze six samples due to their fermentation yield, and data was interpreted by a PCA plot. The obtained data were interpreted and used to construct prediction model to predict compounds concentrations. Furthermore, spoilage of milk preserved at room temperature was followed using ET and PARC techniques to characterize its microbial properties. Finally, the taste sensor has been also used to discriminate between milk products from healthy and infected glands and milk adulteration.

An electronic tongue with potentiometric solid-state electrodes for pork freshness evaluation was introduced by Gil *et al.* (2011) to detect organic materials during stages of spoilage. PCA was performed in the study for qualitative evaluation of pork freshness, showing the degree of pork freshness by analysis of the potential on electrodes.

Other studies focused on bitterness evaluation in pharmaceutical industry. They evaluate coated micro-particles of Ibuprofen and roxithromycin (Janczyk *et al*., 2010) and taste of crude drugs and Kampo Formula using also ET (Anjiki *et al*., 2011).

Campos *et al.* (2012) used a voltammetric electronic tongue to measure certain water parameters that controls water quality, 28 and 11 samples were used from wastewater treatment plant. After applying the sensors, PLS was employed to correlate the signal obtained by the ET and the concentration level of certain compounds in the samples. ET was successfully able to determine most of the pollution parameters, including chemical oxygen demand (CODs), soluble biological oxygen demand (BODs), ammonia (NH_4-N), orthophosphate (PO_4-P), sulphate and alkalinity.

Commercially, ET was widely used for benchmarking of the products and the stability regarding various storage conditions, time or packaging (shelf time) (Korposh, Selyanchyn, James, Tatam, & Lee, 2014). ET can be also used for microorganism's detection (Heras, Pallarola, & Battaglini, 2010) and molecular recognition (Alzeer *et al*., 2014; Baldwin *et al*., 2011; Del Valle, 2012; Gupta *et al*., 2010; Gutierrez-Capitan *et al*., 2014; Kalit *et al*., 2014; Ramamoorthy *et al*., 2014; Smyth & Cozzolino, 2012; Wadehra *et al*., 2016; Wang *et al*., 2015).

Several studies have been made on tea. The ability of multi-sensor array to discriminate between different samples of green and black tea using array of cross-sensitive sensors from twenty-two electrodes was examined by Papieva *et al*. (2011), the data was processed by means of PCA. The polyphenols and caffeine were quantitatively analyzed under conditions of micellar electro-kinetic chromatography and UV detection to give a complete profiling of the tea samples, the ability of the sensors to determine the concentration of caffeine was proved with a relative error of 5-15% with respect to the reference chromatography.

Wang *et al.* (2017) conducted a study in which black tea samples was analyzed using both sensory taste panel and ET to find a correlation between them. The results of both tests were compatible and the different types of tea show differences in the taste response. Also, good taste was found to have high intensity of the umami flavor while bitter taste has low intensity of the umami.

The effect of production date of certain green tea brand called Biluochen on tea quality was assessed using ET by Wu *et al*. (2011). Taste scores were obtained by chemical evaluation methods (*i.e.* calculating optical density values of amino acids and tea polyphenols) and used as a reference measure. One-way analysis of variance (one-way ANOVA) was used to analyze the effect of production date on tea quality and sensory scores, then a PLS model was established to check the relationship between ET signals and taste scores. Results showed significant effect of production date on ET signals and tea quality. Parameters of PLS plot shows correlation coefficient of 0.906 and root mean square error of prediction of 4.077, which represent an excellent evaluation ability by ET.

Other research about tea analysis were carried out by several researchers (Kumar, Ghosh, Tudu, & Bandyopadhyay, 2017; Kundu & Kundu, 2016;

Mondal, Roy, Tudu, Bandyopadhyay, & Bhattacharyya, 2017; Saha, Ghorai, Tudu, Bandyopadhyay, & Bhattacharyya, 2016; Zhi, Zhao, & Zhang, 2017).

The ginseng plant growing year might be identified by a taste sensor. A TS-5000Z model of ET with eight connected sensors was employed by Cui *et al.* (2013) to identify differences and similarities among samples of different ages, depending on increasing correlation between ginosenoside content and growing year. The relation between various tastes saltiness, sourness, umami and ginseng content were 0.974, 0.941 and 0.943, respectively.

Eckert *et al.* (2011) investigate lozenges, which is an herbal extract including sage as its major component. A taste sensory system SA402B with seven sensors was used to control the quality of products made from herbal extracts. This provides insight of ET in pharmaceutical applications. In that study an unknown sample was analyzed, in comparison to a reference sample, to check whether it meets the quality requirements. HPLC, GC and human taste panel used to confirm the taste sensor results.

2.1.3 Advantages of ET

Recent evidences suggest that ET has a variety of advantages over other analytical methods, including its global selectivity, relatively low cost, rapid detection time, wide potentials for applications, reliable, objective, simple, highly sensitive, versatile considering its possible sensors modifications, results reproducibility and allows different kinds of samples to be tested at the same time. It does provide an opportunity to examine toxic materials that tasters cannot taste and will not get exhausted like human tongue.

ET also avoids uncertainty, since the testing process is label-free with an automatic measure, it does not require skilled person to operate, can be modified into handhold instrument for in situ monitoring, allows multiple properties of sample to be examined and it's easy to prepare the samples (Alzeer *et al.* 2014; Baldwin *et al.* 2011; Del Valle 2012; Gupta *et al*. 2010; Gutierrez-Capitan *et al*. 2014; Kalit *et al.* 2014; Ramamoorthy *et al*. 2014; Smyth and Cozzolino 2012; Wadehra and Patil 2016; Wang *et al*. 2015).

2.1.4 Limitations of ET

Despite its long success, ET has a number of constrains, like any technical devices. It's mainly affected by some environmental factors, especially temperature, humidity or moisture causing sensor drift. Also finding the suitable sensor type for the applied research could require some efforts. One cannot deny that this emerging technology is still under a lot of research and studies to improve its work and limit its defects (Nagy, Kovacs, Szollosi, & Fekete, 2013).

In potentiometric sensors, which depends on ions for measurements, the results measured highly affected by solution changes and the adsorption of the chemicals in the solution, which will lead to direct impact on the transferred charge causing sensor drift (Bratov, Abramova, & Ipatov, 2010; Ciosek & Wroblewski, 2007). In order to minimize that effect of adsorption, electrodes can be washed with specific solvents (Muratova, Kartsova, & Mikhelson, 2015).

In case of using voltammetric sensors, the applications being used in are restricted to the ones that involve redox-active species due to its mode of action (Martinez-Bisbal *et al*., 2017). Also, when using different noble elements on the working electrode, more explanation and research must be done to illustrate differences between these electrochemical reactions (Bueno, De Araujo, Salles, Kussuda, & Paixao, 2014; Martinez-Bisbal *et al*., 2017; Novakowski, Bertotti, & Paixao, 2011).

Many parameters must be fulfilled in the Japanese version of the taste sensor, including equal threshold on the membrane with the human taste threshold; similar respond mechanism with that of the human tongue and must be able to detect interactions with taste substances (Ikezaki, 2014). Until recently, there has been no reliable evidence that taste sensor can totally replace human taste sense, no artificial membrane works exactly like taste buds. Furthermore, signal transduction and processing mechanisms is distant from the one done by neurons, and the mode of action of human brain is much more complex than electronic sensing technique (Toko, 2013). The debate about this issue has gained fresh prominence with many arguing that human-like sensors can be done even with some biological differences bearing in mind that it requires deep investigation and efforts (Kobayashi *et al.*, 2010; Nakamoto, 2016). However, the design and sensing mechanisms of different releases of commercial ET might be helpful in minimizing such defects. Also combining data of different sensors may be a good suggestion (Del Valle, 2012). Haddi *et al.* (2011) fused data from potentiometric and voltammetric sensors to discriminate three types of beer, in which a sensor array of three voltammetric sensors coupled with six potentiometric sensors were employed. The obtained data was used to construct PCA model to detect the different groups of beer. About 96% of the samples were classified successfully.

Both electronic nose (which mimic human nose) and electronic tongue was used to improve their outcome by fusion of data for eight different Chinese liquor samples, leading to a significant improvement in accuracy and further classification of the samples (Men *et al.*, 2013).

2.2 Multivariate data analysis (MVDA)

Recent developments in all analytical equipment have heightened the need for appropriate method to interpret the obtained data and display them in a useable form. Through the present research the complex set of data extracted from the sensor array need to be interpreted. MVDA is used for that purpose. MVDA is also called chemometrics. The term chemometrics was firstly introduced to the science society in 1971 by Svante Wold, a Swedish professor at Umea University. Wold proposed a novel definition for chemometrics as: “it is the art of extracting chemically relevant information from data produced in chemical experiments” (Kayondo, 2012). Recently, Bruce Kowalaski defined chemometrics as: “the chemical discipline that uses mathematical and statistical methods to design or select optimal procedures and experiments, and to provide maximum chemical information by analyzing chemical data” (Lavine, Brown, & Booksh, 2015). Both definitions are almost very similar to each other.

MVDA can be used in two main purposes, either for exploring the data set, *i.e.* identification and classification, or finding a correlation between two sets of variables, *i.e.* quantification and correlation (Kumar, Bansal, Sarma, & Rawal, 2014).

In ET, a couple of parameters control which method to use: depending on the research purpose and the type of data obtained. Considering the first parameter, ET can be used either for qualitative or quantitative analysis. A qualitative analysis can be done using PCA, partial least squares-discrimination analysis (PLS-DA) and ANN (Gutierrez-Capitan *et al*., 2014). For quantitative purposes, PLS or back propagation neural network (BPNN) can be employed (Wesoléy, Ceto, Del Valle, Ciosek, & Wroblewski, 2016). The linear or nonlinear relation between the sensor signal and species concentration is also an important parameter in determining the technique being used. As for the linear relation, one can use PCA, LDA, DFA, HCA, SIMCA and PLS. However, for non-linear response, ANN is appropriate candidate to use (Baldwin *et al*., 2011; Men *et al*., 2013; Roussel, Preys, Hauchard, & Allemand, 2014).

2.2.1 Principal component analysis (PCA)

Principle component analysis (PCA) technique was firstly introduced in 1901 by Karl Pearson (Chen, Chen, & Jin, 2011). It is used to reduce a set of data and explore possible relations between groups of variables in order to construct a predictive model. The reduction process yields a group of uncorrelated variables called principle components (PCs) or factors. PC1 is responsible for most variation in the data, the following PCs is orthogonal to the previous ones and have less data variability. In describing samples and variables spread, two dimensions are employed: scores and loadings plot. The former represents the relationship between samples. The latter describe the relationship between variables on the PCs axis. Fig. 5 shows an example of how scores plot looks like (Jolliffe & Cadima, 2016; Kayondo, 2012; Moffat, 2015;).

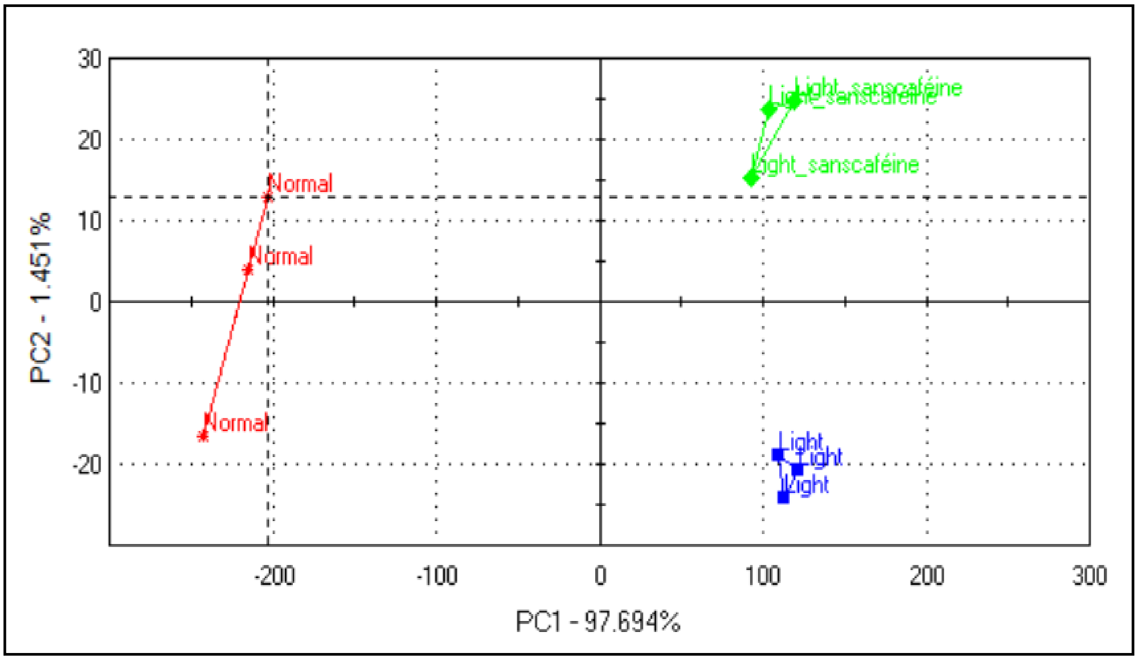

Fig. 5. Example of PCA scores plot that represent samples grouped into three distinct groups, with values of PC1 on X-axis and PC2 on Y-axis.

2.2.2 Discriminant function analysis (DFA)

The idea of DFA grew in 1930s by Fisher from UK, Hoteling from US and Mahalanob from India (Dudzik & Kolatorowicz, 2016). It’s a tool used mainly for determining variables that can classify data into distinct groups. When the whole sample set is grouped based on the distance (*i.e.* Euclidian distance) between samples of the same group, the results then can be saved as a model to classify unknown samples based on the proximity to the center of the groups (Dudzik & Kolatorowicz, 2016; Gagne, 2014).

2.2.3 Partial least squares (PLS)

It was firstly aroused by Wright in the 1920s in economics, and then used widely in chemistry and physics (Pirouz, 2006). It has characteristics of both PCA and multiple regression (Sawatsky, Clyde, & Meek, 2015). The purpose of using PLS model is to extract a set of dependent variables from a huge set of independent variables, by establishing a linear relation between the dependent matrix (X) (*i.e*. ET signals) and the independent one (Y) (*i.e.* samples concentrations and taste panel scores). The latent variables are determined and then PCA is performed on both matrixes (X and Y) to determine the inner relationship (Garcia & Filzmoser, 2017; Stone, 2012).

2.3 Taste panel

In a human taste panel, a group of healthy people is recruited to taste samples of the study, then judge it based on defined parameters through previously planned procedures. The human taste buds convert the taste into electrical signal that will be transferred to the brain through the nerves where it is analyzed. The outcome of this test is displayed in well-designed statistical form to ensure less bias probability (Eckert *et al*., 2013; Eshleman, 2016).

Human volunteers are chosen based on the purpose of the study especially on medical uses, but in all cases, they must be trained on the sample tasting procedures, taste intensity and how to choose the appropriate scale. Volunteers should keep on mind not to take any food, beverage or gum one hour before the test (Hutchings, 2011; Kilcast, 2010). Taste sensing panel usually used to detect the five different tastes (*i.e.* bitter, sweet, salty, sour and umami), texture, flavor and aroma. Therefore, different types of tests can be employed including (Amerine, Pangborn, & Roessler, 2013; Anand, Kharb, Kataria, Kukkar, & Choudhury, 2008; Fellendorf, O'Sullivan, & Kerry, 2017; Kerry, 2009; Lesschaeve & Noble, 2010; Lin *et al.*, 2017; Martinsdottir, 2010; Sandgruber & Buettner, 2014; Stefanowicz, 2013)

1- Discrimination tests: used to detect differences between samples using two types of tests, ranking or difference tests. Both are used in quality control, flavor and bitterness applications.

2- Scaling tests or scoring: use a score sheet to collect data on specific sample, and usually employed on dairy industry for quality and physical appearance.

3- Expert tasters: a group of qualified and trained tasters with long experience in tasting products especially wine, coffee, beer and tea. Four descriptive methods (*i.e.* appearance, texture, taste and odor) are used to describe the total picture of the product in industrial research for development and improvement of food and pharmaceuticals.

4- Affective tests: tasters just decide whether they like or dislike a product based on its aroma or taste in determining sweetness or bitterness. Comparing between two samples is done by multiple paired comparison test, but when using acceptance test, tasters choose between a scale of nine points ranging from ‘like extremely’ to ‘dislike extremely’ named hedonic scale (*i.e.* 1-9), which was used in this study.

2.4 Medical plants and antimicrobial effect

In the history, development of medical plants has been thought as a key factor in treating several diseases including the ordinary, infectious or even epidemics. The art and knowledge of dealing with plants that have healing properties were transmitted from one generation to the other over the years. Nowadays, the scientific community is facing an increased resistance of the pathogens against antibiotics, thus several studies aim to provide a clear insight of chemical composition of medical plants and mode of interaction between plants’ metabolites and microbes.

Recently, a wide research started for new plant species that could be helpful (Silva & Fernandes Junior, 2010; Van Wyk & Wink, 2015).

Researchers have emphasis on the active ingredients of plants, produced as secondary metabolites for their treatment properties. Silva *et al*. (2009) tested the antimicrobial resistance against *E. coli* and *S. aureus* strains using essential oils extracted from rosemary (*R. officinalis*), clove (*Caryophyllus aromaticus* L.), ginger (*Z. officinalis*), lemongrass (*C. citratus*), peppermint (*M. piperita*) and cinnamon (*Cinnamomum zeilanicum Blume*). The results showed a noticeable antimicrobial effect of ginger oil against *S. aureus*, while *E. coli* was greatly affected by both clove and cinnamon oils. Studying the inhibition effect of *Punica granatum* fruit (*i.e.* pomegranate) against 38 *S. aureus* strains was done by Silva *et al.* (2008). Results showed potential antibiotic effect of the pomegranate extract against 22 of the 38 strains being tested with inhibition zone of 10 to 36 mm in diameter.

The phenolic compounds presented in chamomile were found to have antimicrobial activity against *Staphylococcus aureus* in a research done by Asolini *et al*. (2006). Furthermore, Zampini *et al*. (2009) studied the antimicrobial effect of 11 plant species originated in Argentine and inhibition effect was observed toward one or more of the tested bacteria including: *S. aureus, E. faecalis, E. coli, K. pneumoniae, Proteus mirabilis, Enterobacter cloacae, Morganella morganii* and *P. aeruginosa.*

Other studies focused on the antioxidant activity for plant extracts or oils. For instance, Proestos *et al.* (2013) examined a group of aromatic plants in Greece for their oxidation capacity, total phenolic contents, reducing power and stability through oxidation. The popular Palestinian plant *Salvia palaestina* (Lamiaceae) leaves were used to detect both antimicrobial and antioxidant activity. Sabbobeh *et al.* (2016) noticed an increase of the antioxidant activity with time and with increasing concentration of the essential oils. The extracted oils found to contain eucalyptol (47.09%) and camphor (8.73%) after GC-MS analysis. In this study a disc diffusion method was used to estimate the antimicrobial activity of essential oils against two bacterial strains *Staphylococcus aureus* and *E. coli* compared to gentamicin. The effect of essential oils on *S. aureus* was greater than that of gentamicin, while the essential oils and gentamicin had nearly the same activity on *E. coli*.

3. Materials and Methods

3.1 Electronic tongue (ET)

Astree II ET (Alpha MOS, Toulouse, France) was used, which was patented and developed by Alpha MOS. It has seven solid potential sensors that are chemically modified field effect transistors (ChemFET). These sensors consist of two parts: the sensitive layer and the transducer. These sensors are coated with specific membrane (chemical compounds) to induce both cross sensitivity and cross selectivity. A covalent bond will form between specific molecules and the coated membrane leading to variation in potential between each sensor and Ag/AgCl reference electrode (Alpha MOS, 2009; Haraguchi, Yoshida, Kojima, & Uchida, 2016). This edition of Astree II ET can be used for food industry studies and other purposes, *i.e.* pharmaceutical applications (Podrazka *et al*., 2017).

The device consists of four parts including: the liquid auto-sampler, a sensory array, a data acquisition electronic unit and advanced software, called Alpha Soft (Ver. 12.4), for data analysis (Fig. 6). When performing an experiment using ET, 150 ml beaker is filled to two-thirds of their volume with the prepared sample (*i.e.* 100 ml), other beakers (about 4 of the 16) is filled with distilled water for sensors washing. Each sample requires about 120 seconds to be analyzed, followed by washing process to minimize the carryover effect from the previous sample. Both measuring and washing takes about 3 minutes for each sample. Usually 5 or more replicates are performed to ensure reproducibility of the results (Alpha MOS, 2009).

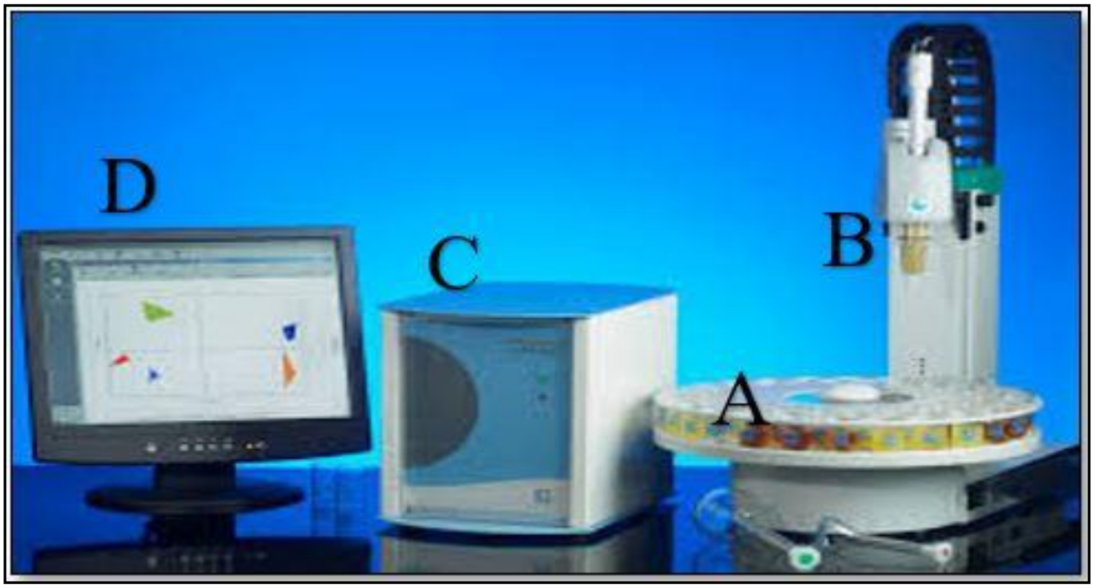

Fig. 6. Alpha MOS Astree II ET parts: (A) liquid autosampler, (B) electrochemical sensor array, (C) acquisition unit, (D) Alpha Soft software (Alpha MOS, 2009).

3.2 Herbal tea samples

Four different herbal tea brands were provided by the local Bajjora Factory in Tulkarm; Lipo, Morning, Nanus and Gasobal. The black and green tea samples were purchased from local supermarket. The tea samples were cooled to room temperature (about 25°C) before being tested. Table 1 provides a brief description of each of the samples.

Table 1. Brief information about herbal tea brands provided by Bajjora Factory, Tulkarm.

<table>
<tr><th>Tea brand</th><th>Components</th><th>Uses</th></tr>
<tr><td>Morning tea

</td><td>Chamomile, Zingiber officinalis.</td><td>Helps pregnant women to get rid of morning sickness symptoms.</td></tr>
<tr><td>Lipo tea

</td><td>Green tea, Cassia italca, Fennel, Cumin, Ginger, Cinnamon, Flax seeds, Portulace oleracea.</td><td>Helps in decreasing weight, destroying lipids and cholesterol.</td></tr>
<tr><td>Gasobal tea

</td><td>Anise, Fennel, Fenugreek, Thyme, Marshmallow, Oak bark, Licorice, Pomegranate, Nettle.</td><td>Helps in treatment of the digestive system and colon problems irritation and pain.</td></tr>
<tr><td>Nanus tea

</td><td>Oak bark & leaves, Black seeds, Pumpkin, Sumac, Gum Arabic, Anise.</td><td>Helps those suffering from enuresis.</td></tr>
</table>

3.2.1 Sample preparation

Six grams of each tea sample were weighed and added to a 250 ml of boiled deionized water, the mix was infused for about 10 minutes, then filtered by several layers of white gauze pads as shown in Fig. 7. Finally, the filtrate temperature cooled to 25℃ (room temperature) before being submitted to ET analysis (Adnan *et al.*, 2013; Xu, Yan, Ye, Fu, & Yu, 2013).

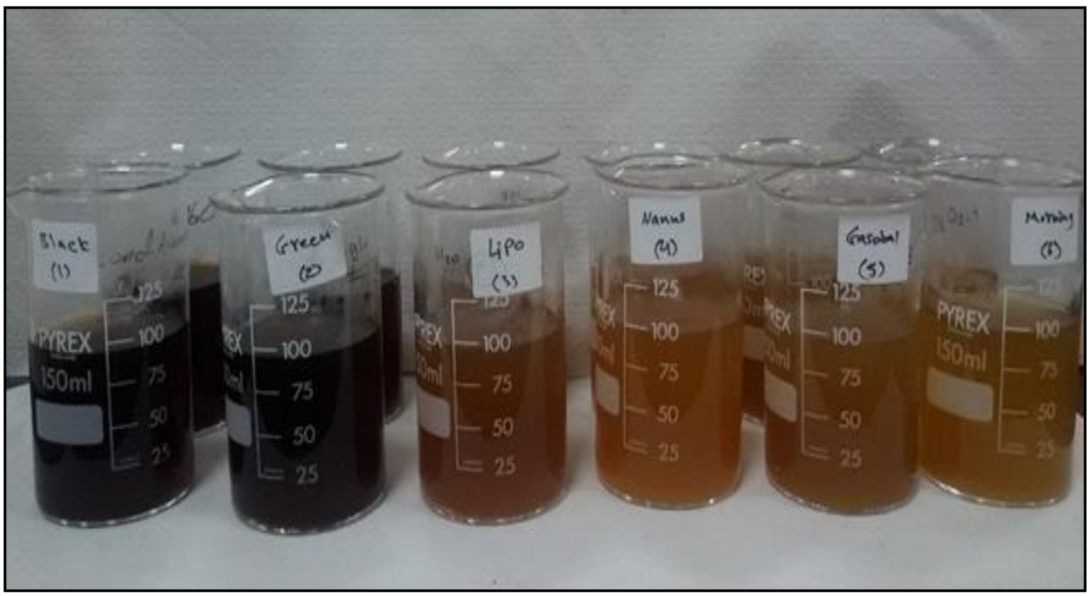

Fig. 7. Tea solutions after filtration prior to ET analysis.

3.2.2 Tea measurements.

Each of the six samples was labeled and placed in an autosampler for analysis (Fig. 8). Four beakers contain distilled water were used for sensors cleaning. Then an array of seven sensors (ZZ, JE, BB, CA, GA, HA and JB) and Ag/AgCl reference electrode detection system completed the potential difference measurement. Two samples (*i.e.* duplication) of each tea were measured by ET in one run. Each sample was measured three times (*i.e.* triplications), in which three points per tea sample can be seen in scores plots in data analysis section.

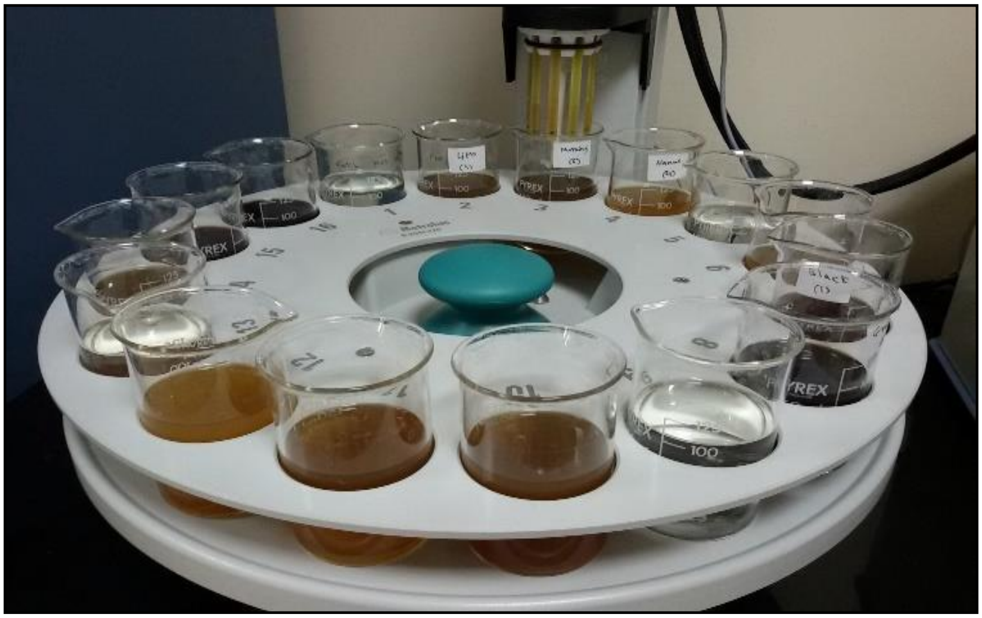

Fig. 8. Herbal tea samples on the ET autosampler

3.2.3 Taste panel

To establish a correlation between ET signals and taste panel scores, a sensory evaluation of several characters of the six tea samples were performed by six students from the same collage at PTUK (*i.e.* consumer test). Fifty percent of the tasters were females. The participants were trained for about 20 minutes before taste any of the samples. Tasters were chosen to be non-smoking, healthy and their ages ranged from 21-22 years. The experiment was conducted in controlled environment with suitable temperature, humidity, light and no other food or chemicals around (Fig. 9).

Fig. 9. Tasters training session before sample tasting and evaluation.

Six grams of each tea brand was mixed with 250 ml boiled deionized water, soaked, filtered then presented to the taste panel once at a time every 15 minute using porcelain cups. The samples were tasted twice before filling the evaluation form (Fig. 10), which was previously designed to record their observation during the test of four parameters: color, taste, flavor and overall acceptability. They were asked to choose between a scale of nine points called hedonic scale, ranging from ‘like extremely’ to ‘dislike extremely’ (Adnan *et al*., 2013; Dong *et al*., 2017).

Data from different experiments in this research were analyzed using Microsoft Excel (Microsoft Corporation, USA), Alpha Soft software (Ver. 12.4, Toulouse, France) and Unscrambler software (Ver. 10.4 Camo Software, Olso, Norway).

Sample number: .... Taste panel of tea samples Date: ....

Characteristics & Attributes	Grading Categories			
	Color	Flavor	Taste	Overall acceptability
9= Like extremely				
8= Like very much				
7= Like				
6= Like slightly				
5= Neither like nor dislike				
4= Dislike slightly				
3= Dislike moderately				
2= Dislike				
1= Dislike extremely				

Fig. 10. Taste panel questionnaire used to evaluate tea samples using hedonic scale.

3.2.4 Mixing two different tea brands

To investigate the ability of ET to discriminate between two herbal tea brands (Nanus and Morning tea) that were the closest to the reference tea, *i.e*. green tea, according to the distance values obtained by ET analysis (will be shown in results and discussion section). Solutions were mixed at different concentrations in a serial dilution as shown in Table 2. Then samples were measured by ET. Several runs on ET were carried out at different times and showed similar results. However, in the results section, the result of one average run is shown (*i.e.* due to the limitation of the capacity of 16 beakers autosampler).

Table 2 Volumes of Nanus and Morning tea used in the mixing.

Sample name	Volume of Nanus tea (N) (ml)	Volume of Morning tea (M) (ml)
N100M0	100	0
N90M10	90	10
N80M20	80	20
N70M30	70	30
N60M40	60	40
N50M50	50	50
N40M60	40	60
N30M70	30	70
N20M80	20	80
N10M90	10	90
N0M100	0	100

3.2.5 Spectrophotometric analysis

Caffeine is a chemical compound used in many drinks, foods and medicines for mental alertness and as pain relievers. It was also found in more than 60 plants worldwide, especially tea leaves. However, high levels of caffeine (more than 400 mg per day) are harmful (Dobrinas et al. 2013). People tend to control their daily consumption and to choose healthy products with minimum caffeine content. In this study, the levels of caffeine in each of the six tea samples were detected using DR 6000™ UV-Vis spectrophotometer (Hach, Germany) at wavelength of 273 nm. Where 6 grams of tea was dissolved in 250 ml boiled deionized water, diluted 40 times then placed in quartz cuvettes and measured in duplication (Fig. 11).

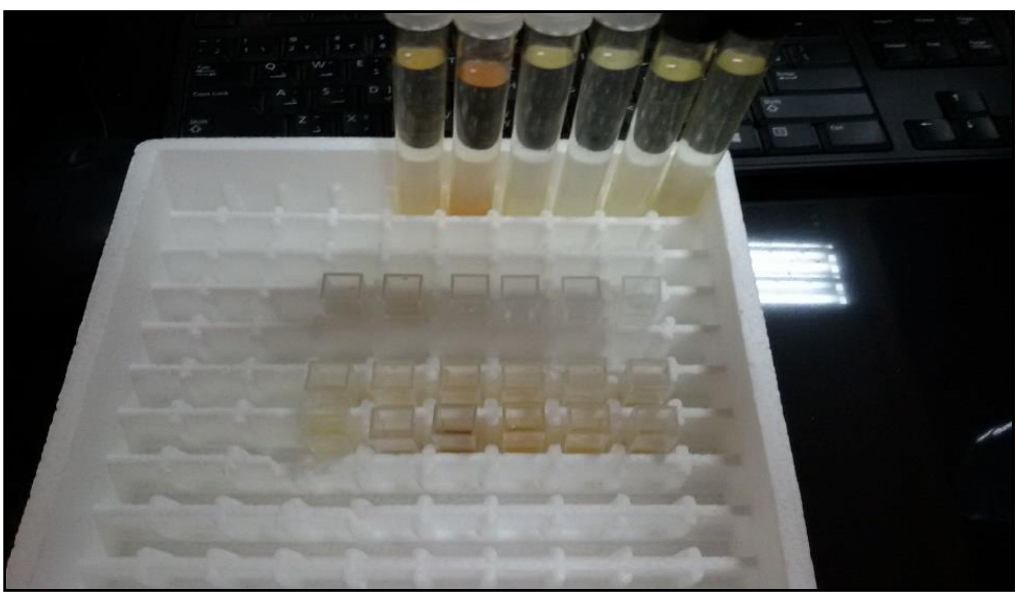

Fig. 11 Quartz cuvettes with tea samples prior to spectrophotometric analysis.

Stock standard solution of 1000 ppm caffeine was prepared by dissolving 197.9 mg caffeine in 200 ml deionized water, then analyzed by DR 5000TM UV-Vis spectrophotometry (to determine λ_{max}). Different volumes of the standard were diluted with water to create multiple concentrations (Table 3). Each of the prepared solutions was analyzed using spectrophotometer, then the data obtained used to perform a linear regression of absorbance versus concentration and constructing a calibration curve by which the concentration of caffeine in each tea sample can be calculated (Ahmad Bhawani *et al*., 2015; Rehman & Ashraf, 2017; Wanyika *et al*., 2010).

Table 3. Standard solutions absorbance values.

Concentration of standard caffeine solution (ppm)	Absorbance (AU)
100	1.799
50	0.901
40	0.736
30	0.542
20	0.367
10	0.187

PLS model was built using Unscrambler 10.4 software (Camo Software, Olso, Norway) between the average of ET signals and caffeine concentration of each tea sample. Through this model the ability of ET to predict caffeine concentration was tested.

3.3 Relax granules production batches

Relax granules is now being used as food complement to improve gastro intestinal tract functions, lower cholesterol and bad lipids levels. Also, it could be used in weight loss programs. Table 4 describes Relax granules ingredients and their medicinal values.

3.3.1 Samples collection

Eleven different samples were provided by Bajjora Company in Tulkarm, each sample was from different production batches (Fig. 12). These samples were analyzed to detect shelf life or the effect of time on the stored granules over one year using ET. Table 5 shows the production date of each sample.

Fig. 12. Some of the Relax samples produced at different production batches provided by Bajjora Company (Tulkarm, Palestine).

Table 4. Relax granules ingredients and their functions.

Ingredient	Medicinal function
Oats	Contain high percentage of powerful soluble fibers called beta glucan that helps in cases of constipation. Used widely in diet programs because it provides feeling of fullness. Encourage useful bacterial growth in intestines (Meydani, 2009).
Wheat Germs	It contains 18% of dietary fibers so it beneficial to relieve constipation, digestion of germs and excess lipids (Tai *et al.*, 2013).
Flax seeds	It contains soluble fibers that easy bowel movement and insoluble ones that let water bind to the stool making it softer (Goyal, Sharma, Upadhyay, Gill, & Sihag, 2014).
Fennel	It reduces the pain results from swelling colon movement and reduces constipation (Kian, Bekhradi, Rahimi, Golzareh, & Mehran, 2017).
Mint	It increases bile secretion and flow which facilitate digestion (Aggarwal & Kunnumakkara, 2009).
Caraway	It helps to get rid of gases associated with GIT disturbance (Khajeali, Kheiri, Rahimian, Faghani, & Namjo, 2012).
Pimpinella Anisum	It is used in mood relaxation, so helps to relieve colicky pain (Rocha & Fernandes, 2016).
Coriander	It helps in digestion problems including bowel spasms, diarrhea and gases. Also motivate pancreas to release insulin, so it helps in diet (Kansal, Sharma, & Lodi, 2012).
Prunus mahaleb	It works as sedative for digestion trace pains especially colon and helps to get rid of gases (Li *et al.*, 2016).

Table 5. Eleven Relax samples produced at different production batches.

Sample No.	Production date
1	February 2017 - (2/2017)
2	January 2017 - (1/2017)
3	November 2016 - (11/2016)
4	October 2016 - (10/2016)
5	September 2016 - (9/2016)
6	August 2016 - (8/2016)
7	July 2016 - (7/2016)
8	June 2016 - (6/2016)
9	April 2016 - (4/2016)
10	March 2016 - (3/2016)
11	February 2016 - (2/2016)

3.3.2 Sample preparation

Nine grams of each sample (one tea spoonful) were weight and mixed with 200 ml of boiled deionized water, infused for about 10 minutes (Fig. 13) and filtered by white gauze pads. The resulted solution was cooled to 25°C (room temperature) before being submitted to ET analysis. Similar results were got from several trials, which were carried out at different times. However, in results section, the result of one run is shown (*i.e.* due to the limitation of the capacity of 16 beakers autosampler), in which each sample was measured three times by ET.

Fig. 13. Relax samples before being filtered.

3.3.3 Relax granules competence

Three products: Konsyle powder, Jungborn and Normalax have the same usage and function as laxative, *i.e.* to improve intestinal tract movement and compete relax in the pharmaceutical local market. ET analysis is performed on these three products to find out which one is more closely related to Relax, since it's made of natural herbs while the others from chemical constituents. Table 7 contains brief description about these competence products ingredients.

Nine grams of each product (Relax produced in 02/2017, Konsyl, Normalax and Jungborn) was mixed with 200 ml boiled deionized water, infused for a while, filtered and cooled to room temperature prior to ET analysis as shown in Fig. 14. All the previous products have gone through these steps except Konsyl, which was too thick to be filtered or analyzed, thus it was excluded from the analysis. Two measurements (*i.e.* duplication) for each baker were performed in one run. Two beakers of each product were measured, with three times per baker.

Table 6. Konsyl, Jungborn and Normalax ingredients.

Product name	Ingredients	Manufacturing company
Konsyl powder 	Psyllium Hydrophilic Mucilloid 3.5g	Manufactured by Konsyl Pharmaceuticals Inc., USA.
Jungborn granules 	Senna powder 6.52%	Manufactured by Taro Pharmaceutical industry Ltd., Haifa Bay.
Normalax 	100% Polyethylene glycol 3350	Manufactured by Taro Pharmaceutical industry Ltd., Haifa Bay.

Fig. 14. Relax, Konsyl, Normalax and Jungborn solutions, respectively (from right to left).

4. Results and Discussion

4.1 Herbal tea samples

Three sensors (ZZ, JE and HA) were used for data analysis. They were the most contributing sensors for the data. Values of standard deviation (SD) (*i.e.* ranged between 10.7-30.0 mv) show reproducibility. Low values of SD (less than 30 mv) and coefficient of variation (CV) (*i.e.* ranged between 0.4%-3.3%) represent good reliability of the sensors in data detection and samples measurement (Table 7).

Table 7. Sensors' mean, SD and RSD values in tea samples analysis.

Brand Name	**ZZ**			**JE**			**HA**		
	Mean	SD	RSD%	Mean	SD	RSD%	Mean	SD	RSD%
Black	2252.3	13.0	0.6	1656.1	19.8	1.2	861.1	24.4	2.8
Gasobal	2491.1	12.7	0.5	1672.9	22.0	1.3	895.9	27.3	3.0
Green	2389.4	13.9	0.6	1654.4	20.2	1.2	861.0	24.0	2.8
Lipo	2501.3	18.3	0.7	1679.0	23.9	1.4	909.3	30.0	3.3
Morning	2486.4	10.7	0.4	1664.7	21.6	1.3	904.4	29.8	3.3
Nanus	2469.5	12.9	0.5	1698.4	22.2	1.3	905.5	25.3	2.8

4.1.1 Six tea brands analysis

PCA was used to analyze ET signals of six tea brands, to investigate the ability of ET to distinguish the finger print of each brand. There was a reasonable discrimination between six tea brands. PCA scores plot provided grouping of the brands, with some overlapping for the Gasobal, Morning and Lipo tea. The first principle component (PC1) explained about 78 % of the total variance, and the second component (PC2) explained about 21 % of total variance. Both components explained 98.3% of the total variance (Fig. 15).

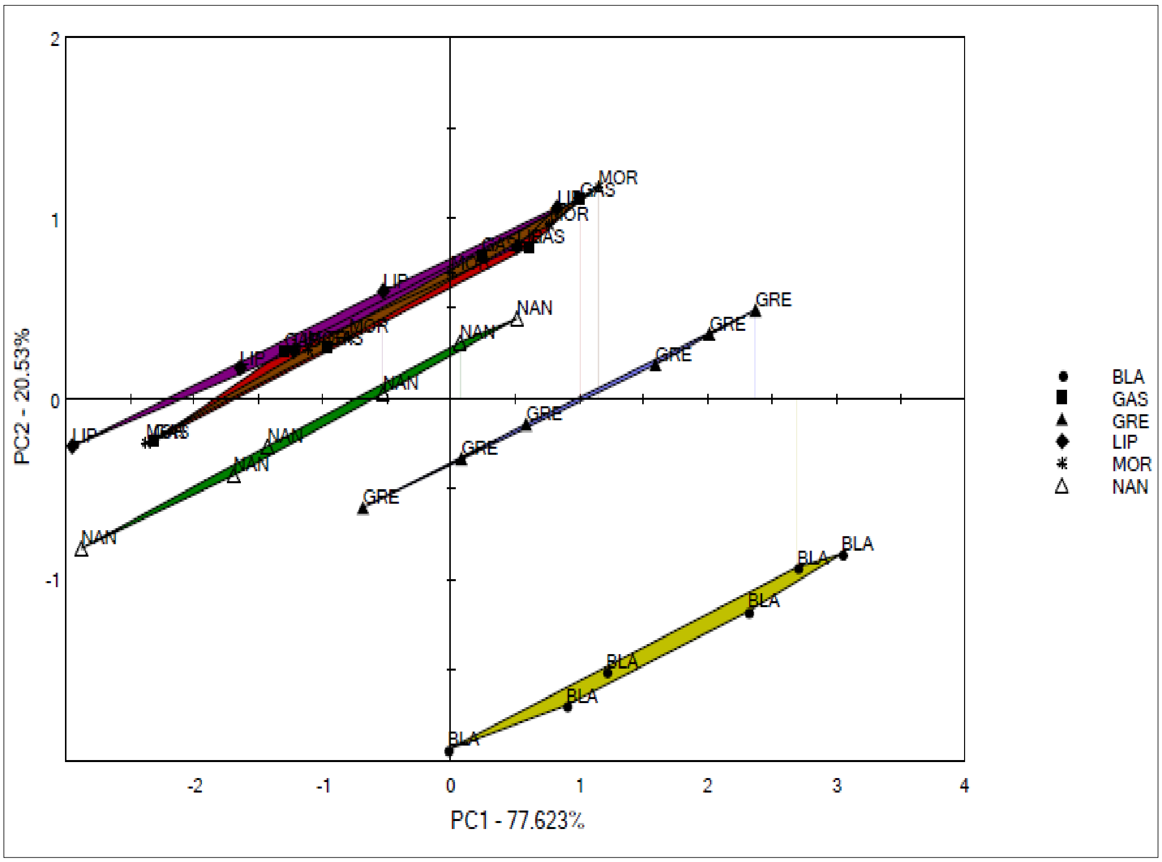

Fig. 15. PCA scores plot of the six herbal tea brands.

The three-dimension representation of PCA model in Fig. 16 shows distinct groups of the Black, Green and Nanus samples (marked with a circle). While samples of Lipo, Gasobal and Morning show some overlapping.

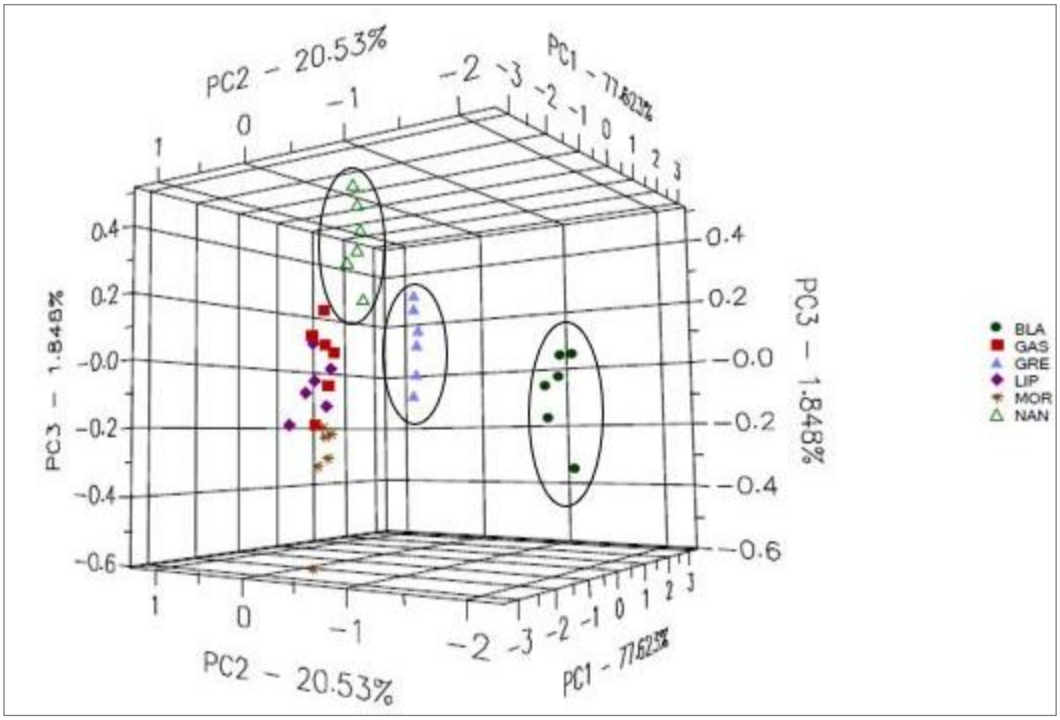

Fig. 16. Three-dimension PCA score plot for the six herbal tea brands.

In order to get a clearer picture of sample grouping DFA was used. The first discriminant function (DF1) and the second function (DF2) explained 99.2% and 0.76%, respectively. Thus, the total accumulated contribution rate of both two functions was 99.96%, indicating that they could explain majority of the original data information without any significant loss in the information (Fig. 17).

The figure indicates that different varieties of tea samples are distributed away from each other. This model shows large distance between groups and less spaces within the same group meaning that there was a good discrimination and high reproducibility values. Comparing the plots of PCA and DFA, more clear discrimination of the data was observed on the DFA model especially between the four herbal tea brands. This model can be used later to analyze unknown samples and detect any adulteration or mixing in the manufacturing process.

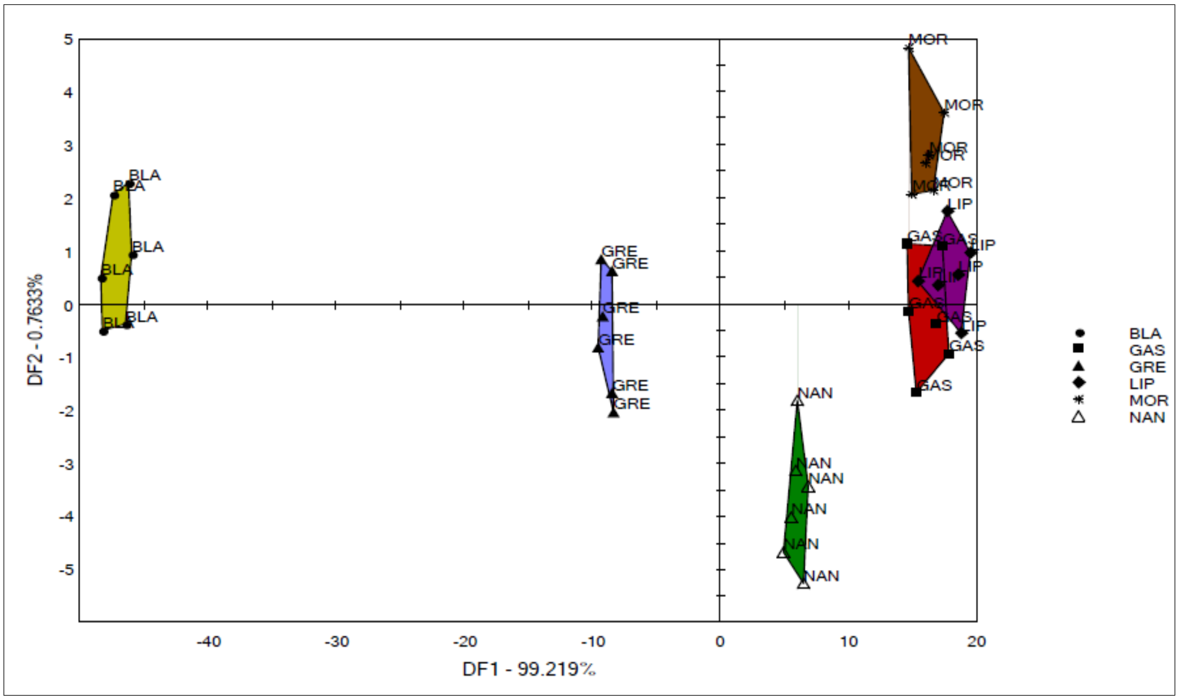

Fig. 17. DFA plot of six tea brands (Black, Green , Morning, Gasobal, Lipo, Nanus).

Using Green tea as an arbitrary reference (Abu-Khalaf *et al.*, 2018), due to its well-known taste, the distance between the five tea brands and the reference indicates that black tea (*i.e.* with a distance of 137.1) is the most distinguished group (*i.e.* the highest distance) followed by Lipo, Gasobal, Morning and Nanus with a distance of 124.3, 109.1, 109.1 and 101.6, respectively (Fig. 18). As the distances between each of the groups increase, the pattern discrimination indexes increase (This index takes account of the difference between groups). The closer the index to 100%, the greater the distance between the groups and the smaller the dispersion within groups, meaning that the most different product from Green tea is the black, with pattern discrimination index of 70.2%, and the most similar product is Nanus, with pattern discrimination index of 55.3%. Table 8 represents distance values and their pattern discrimination indexes at probability of 0.05.

Table 8. The distance and pattern discrimination index between groups of tea brands and the reference green tea at P=0.05

Product name	Reference sample	Distance	Pattern discrimination index (%)
Nanus	Green	101.6	55.3
Morning	Green	106.7	56.4
Gasobal	Green	109.1	58.1
Lipo	Green	124.3	61.4
Black	Green	137.1	70.2

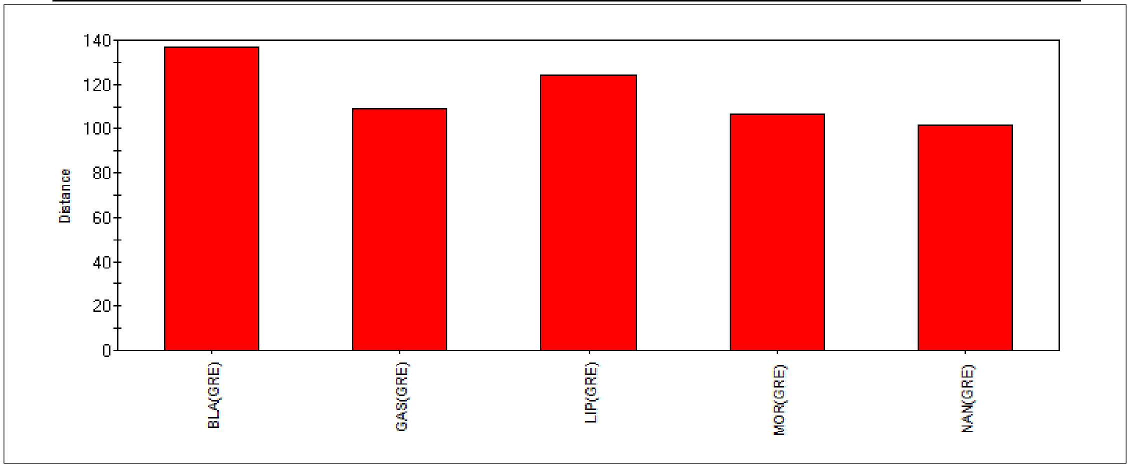

Fig. 18. Distance between each of the tea samples and Green tea as a reference.

4.1.2 Taste panel

The scores obtained from the taste panel of six tasters using hedonic scale were correlated to ET signals to reveal the ability of ET to predict taste of the tea brands. Four parameters; taste, color, flavor and overall acceptability; were studied.

4.1.2.1 Color

The color scores of the tea samples assigned by the tasters are displayed in Fig. 19. The highest average score was 6.2 for Black tea, while the lowest score was 3.0 for the Green tea. The total scores ranged from 3.0- 6.2, but these scores were very close to the other four herbal tea samples and in between the black and green color.

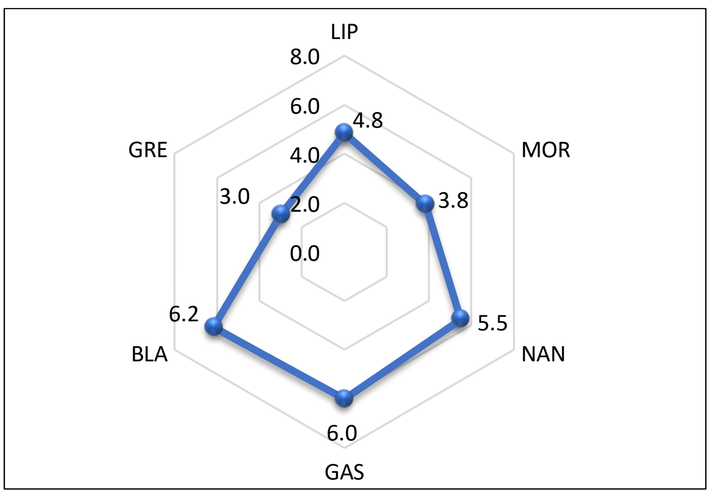

Fig. 19. Radar graph representing the average color scores evaluated by taste panel.

The SD values (*i.e.* ranged between 1.0-2.9) and the CV% values which were high for Green (74.5%), Nanus (53.3%), Morning (53.1%) and Lipo (43.8%), but low for Black (19.7%) and Gasobal (16.7%) tea in (Table 9) shows low precision and low reproducibility in color evaluation.

The color difference between the Black and Green tea can be explained by the present of colored chemical compounds resulted from oxidation of polyphenols. The low percent of these chemicals in Green tea gave the green color in different shades depending on the ratio between chlorophyll A (dark green) and chlorophyll B (yellowish-green in color). The Black tea fermentation and oxidation process during preparation produce theaflvins (yellow color) and therubigins (red color), which contribute to tea color depending on the ratio between them (Chaturvedula & Prakash, 2011; Kerio, Wachira, Wanyoko, & Rotich, 2013).

Table 9. Color scores of the six tea samples evaluated by taste panel.

Tea sample	T1	T2	T3	T4	T5	T6	Average	SD	CV%
Lipo	3	7	5	4	2	8	4.8	2.1	43.8
Morning	3	1	6	7	3	3	3.8	2.0	53.1
Nanus	6	1	8	8	8	2	5.5	2.9	53.3
Gasobal	6	6	4	7	6	7	6.0	1.0	16.7
Black	4	6	6	8	7	6	6.2	1.2	19.7
Green	1	1	2	7	5	2	3.0	2.2	74.5

Results of one-way ANOVA test (Table 10) are in line with Xiao & Wang (2009) work, show no significant difference (P> 0.05) in the tea brands colors, since the F value was less than $F_{critical}$. The null hypothesis (H◦: there is no significant difference between the color of the brands) is accepted, meaning that tasters cannot significantly distinguish between color of tea samples.

Table 10. One-way ANOVA test for color of different tea brands according to taste panel.

Source of Variation	SS	Df	MS	F	P-value	$F_{critical}$
Between Groups	47.5	5	9.5	1.9	0.12	2.5
Within Groups	148	30	4.9			
Total	195.5	35				

To evaluate the ability of ET to model the color of six tea brands, the average of ET signals for each sample (X-matrix) from three sensors (*i.e.* ZZ, HE and HA) and taste panel color scores (Y-matrix) were used to build PLS model (Fig. 20). Full cross validation was applied to the data set, due to the small number of samples. Several factors (*i.e.* low number of PCs, high R^2, high slope, RPD is greater than 2.5, low values of RMSE and the lowest difference between the RMSE of calibration and validation) should be considered when evaluating a PLS plot (Abu-Khalaf & Iversen, 2007b). Table 11 shows parameters of PLS calibration and validation models of tea samples. Two PCs explained about 96% and 43% of X and Y matrices, respectively. Values of R^2 (*i.e.* 0.3 for calibration and 0.54 for validation) indicates that there is no correlation between the color scores and the ET signals. Also, ratio of standard error of performance to standard deviation (RPD) values of 1.29 and 1.82 for calibration and validation sets, respectively, indicating that PLS was not able to model color scores using ET signals. This is due to the value of RPD that should be greater than 2.5 to accept the PLS model (Mireei *et a.l* 2010).

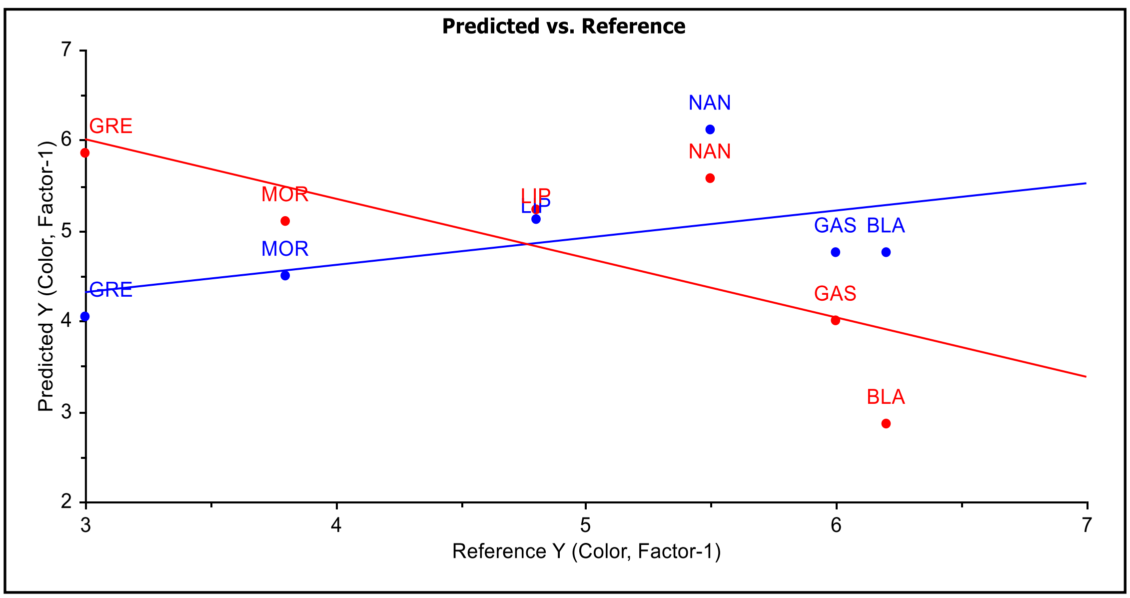

Fig. 20. The predicted average color scores (by PLS) versus reference color scores evaluated by taste panel (blue line for calibration set and red line for validation set).

However, ET is a device that generally characterizes taste not color, or even the chemical compounds that cause color of the herbal tea cannot be identified by ET, thus the model was invalid.

Table 11. PLS parameters of calibration and validation model for color determination.

Data set	Slope	Offset	RMSE	R^2	RPD
Calibration	0.30	3.41	0.97	0.30	1.29
Validation	-0.66	7.98	0.69	0.54	1.82

RMSE: root-mean-square error; R^2: squared correlation coefficient, RPD=SD/RMSE

4.1.2.2 Taste

Tasters' judgment on the taste of the herbal tea samples is presented in Fig. 21, with an average ranged from 2.2 to 6.7 The highest score was for the Morning tea and the lowest was for the Black tea. The four herbal tea samples had higher taste scores than Green and Black tea. Table 12 shows tasters' scores, SD and CV% values, with ranges of 0.7-1.5 and 13.2%-31.7%, respectively. Which can clearly show low dispersion and thus high reliability and precision.

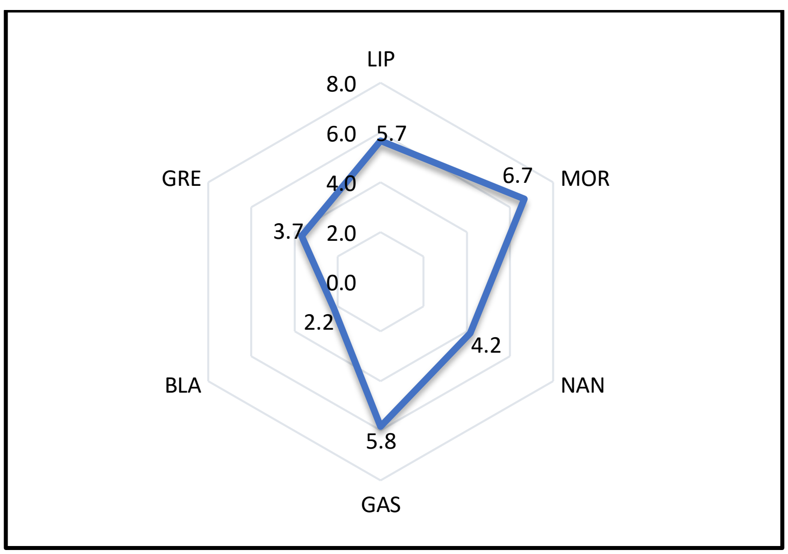

Fig. 21. Radar graph representation of average taste scores.

The better taste is balanced combination between the five basic tastes (sweetness, saltness, sourness, bitterness and umami). The chemical composition of these tea brands had direct impact on the taste in general. Different percentages of the biochemicals polyphenol, amino acids, caffeine, theaflavins and thearubigin shape the overall taste (Chaturvedula & Prakash, 2011; Hayashi, Ujihara, Chen, Irie, & Ikezaki, 2013).

Table 12. Taste scores of the six tea samples evaluated by taste panel.

Tea sample	T1	T2	T3	T4	T5	T6	Average	SD	CV%
Lipo	5	5	5	7	6	6	5.7	0.7	13.2
Morning	5	7	6	8	5	9	6.7	1.5	22.4
Nanus	4	6	3	5	4	3	4.2	1.1	25.6
Gasobal	7	5	5	7	6	5	5.8	0.9	15.4
Black	3	2	2	1	3	2	2.2	0.7	31.7
Green	3	4	5	4	2	4	3.7	0.9	25.7

Tasters was able to differentiate between the six samples significantly ($P<0.05$) as Table 13 shows. The F value was greater than the $F_{critical}$ so the null hypothesis ($H_\circ$: there is no significant difference between the taste of the tea brands) is rejected.

Table 13. One-way ANOVA test for taste of different tea brands according to taste panel.

Source of Variation	SS	Df	MS	F	P-value	$F_{critical}$
Between Groups	83.1	5	16.6	13.7	5.5 E-07	2.5
Within Groups	36.5	30	1.2			
Total	119.6	35				

The PLS plot was built in Fig. 22 to show whether ET can predict taste. Two PCs were used. PC1 and PC2 explained about 97% and 95% of X and Y matrices, respectively. Table 14 include parameters of PLS calibration and validation sets of the taste factor. The R^2 values of 0.97 and 0.94 represent a high correlation for both the calibration and validation sets, respectively. The RPD values (7.3 for calibration and 3.8 for validation) were greater than 2.5, and values of RMSE were low.

Table 14. PLS parameters of calibration and validation model for taste determination.

Data set	Slope	Offset	RMSE	R^2	RPD
Calibration	0.97	0.11	0.23	0.97	7.33
Validation	0.84	0.71	0.43	0.94	3.87

Therefore, after taking into consideration the previous indicators (*i.e.* high R^2, high slope, RPD is greater than 2.5 and low values of RMSE) the model can be considered successful in explaining the relation between ET signals and taste panel. This supports the claim that ET can predict taste of different tea samples efficiently (Saha, Ghorai, Tudu, Bandyopadhyay, & Bhattacharyya, 2014; Saha, Ghorai, Tudu, Bandyopadhyay, & Bhattacharyya, 2017).

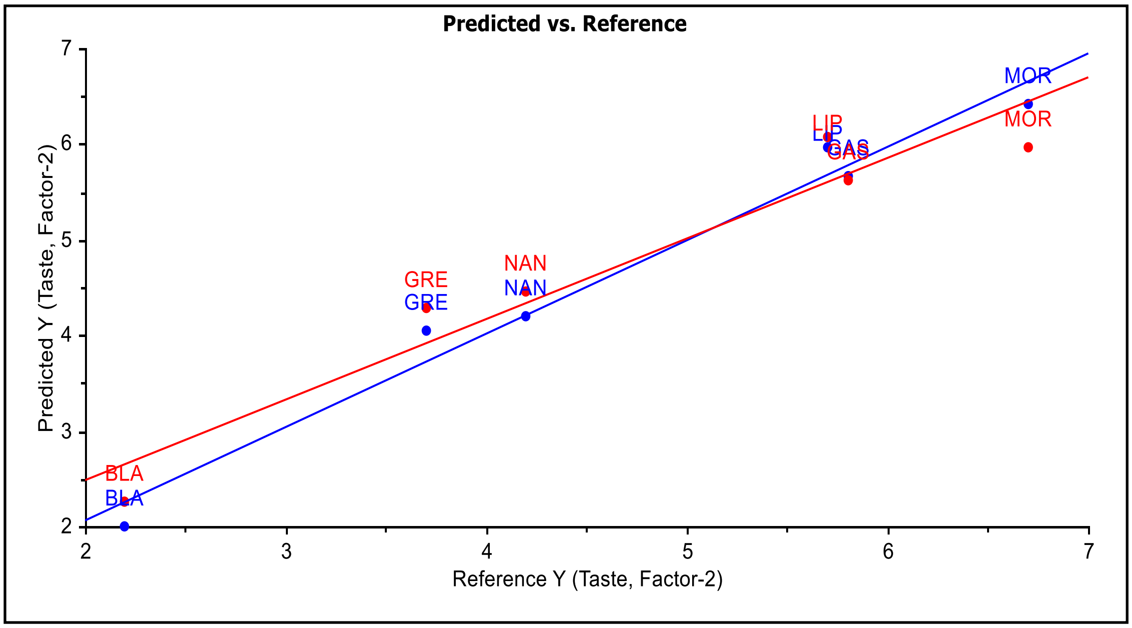

Fig. 22. The predicted average taste scores (by PLS) versus reference scores evaluated by taste panel (blue line for calibration set and red line for validation set).

Three of the seven sensors mentioned earlier (*i.e.* ZZ, JE and HA) had the ability to detect chemicals responsible for the most effective taste, in a manner similar to that of the human tongue. This also indicates that ET can be used in applications that humans can't tolerate, including tasting toxic materials or wide commercial and manufacturing products beside many applications discussed earlier (Lvova et al., 2016).

4.1.2.3 Flavor

Flavor can be described as the sensory impression of taste and smell (Auvray & Spence, 2008). The highest and the lowest flavor scores were for the Morning and Black tea, with values of 7.2 and 2.8, respectively. Three of the herbal tea brands (*i.e.* Lipo, Morning and Gasobal) had the highest scores (Fig. 23). Moreover, Green tea had higher score than Black tea, with values of 4.0 and 2.8, respectively. Data of flavor scores (Table 15) could be characterized with reliability and low dispersion, due to low values of SD and CV% with ranges of 0.7-1.5 and 17.2%-24.3%, respectively.

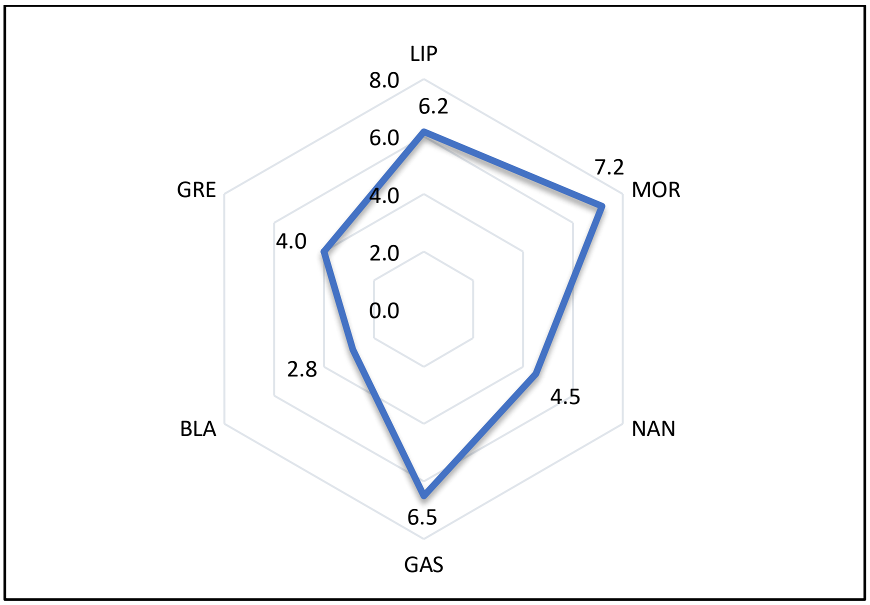

Fig. 23. Radar graph representation of average flavor scores.

Volatile compounds play major rule in the flavor of different types of tea depending on both the volatile's concentration and the compounds present.

These two factors are controlled by plants cultivar, growing conditions and preparation methods. Multiple pathways can result in aromatic volatile compounds in tea, favored by the factors above (Reineccius, 2016; Zheng, Li, Xiang, & Liang, 2016). For instance, Green tea contains a group of volatiles that provide a flower-like aroma including linalool, linalool oxides, geraniol, cis-jasmone, indole, coumarin, dihydroactinidiolide, methyl jasmonate, 6-chloroindole, 5,6-epoxy-β-ionone, trans geranylacetone, phytol and phenylethyl alcohol (Alasalvar *et al.*, 2012). Whereas, Black tea, with fruit like aroma contains 2-amylfuran, (E/Z)-2,6-nonadienal, 1-pentanol, epoxylinalool, (Z)-jasmone, 2-acetylpyrrole, farnesyl acetone, geranyl acetone, cadinol, cubenol and dihydroactinidiolide (Lee, Chambers, Chambers, Adhikari, & Yoon, 2013). The other herbal tea samples had similar constituents depending on the presented plant mixture, but from the taster's scores it was clear that it had many active ornamental volatiles in high concentration.

Table 15. Flavor scores of the six tea samples evaluated by taste panel.

Tea sample	T1	T2	T3	T4	T5	T6	Average	SD	CV%
Lipo	4	6	6	7	7	7	6.2	1.1	17.3
Morning	6	5	7	7	9	9	7.2	1.5	20.4
Nanus	4	3	4	6	5	5	4.5	1.0	21.3
Gasobal	7	7	5	8	7	5	6.5	1.1	17.2
Black	4	3	2	3	2	3	2.8	0.7	24.3
Green	4	3	5	4	5	3	4.0	0.8	20.4

The flavor of Green tea had superiority over the black tea (Green tea had higher flavor scores than Black tea) due to thearubigins, caffeine and catechin compounds variation due to controlled fermentation processes (Pou, 2016). Finally, Morning tea had the highest score, and this is in agreement with its role in relieving sickness of pregnant women through its smell and taste.

According to ANOVA test and the statistical parameters, there was a significant difference ($P<0.05$) between the tea brands flavor according to the panelist judgment (Table 16). The F value was greater than the $F_{critical}$, so the null hypothesis ($H_\circ$: there is no significant difference between the taste of the tea brands) is rejected.

Table 16. One-way ANOVA test for flavor of different tea brands according to taste panel.

Source of Variation	SS	Df	MS	F	P-value	$F_{critical}$
Between Groups	84.1	5	16.8	12.8	1.06 E-06	2.5
Within Groups	39.5	30	1.3			
Total	123.6	35				

To investigate the possibility of ET to predict taste of herbal tea, PLS with cross validation model was carried out (Fig. 24), using Unscrambler 10.4 (Como, Oslo, Norway). ET signals was obtained from three sensors including: ZZ, HA and JE. Two PCs was used to explain the total variation. PC1 and PC2 explained 97% and 93% of the X and Y matrices, respectively. PLS parameters in Table 17 indicate good ability of ET to predict flavor. R^2 value was 0.95 for calibration set and 0.88 for validation set, the RPD values for both the calibration set (*i.e.* 5.3) and validation set (*i.e.* 2.7) were greater than 2.5 and relatively small RMSE values. ET could successfully predict tea flavor meaning that ET was sensitive to taste-causing and smell-forming compounds in order to profile the overall flavor. The results support the use of ET in quality control for various commercial products and in testing consumers' acceptability.

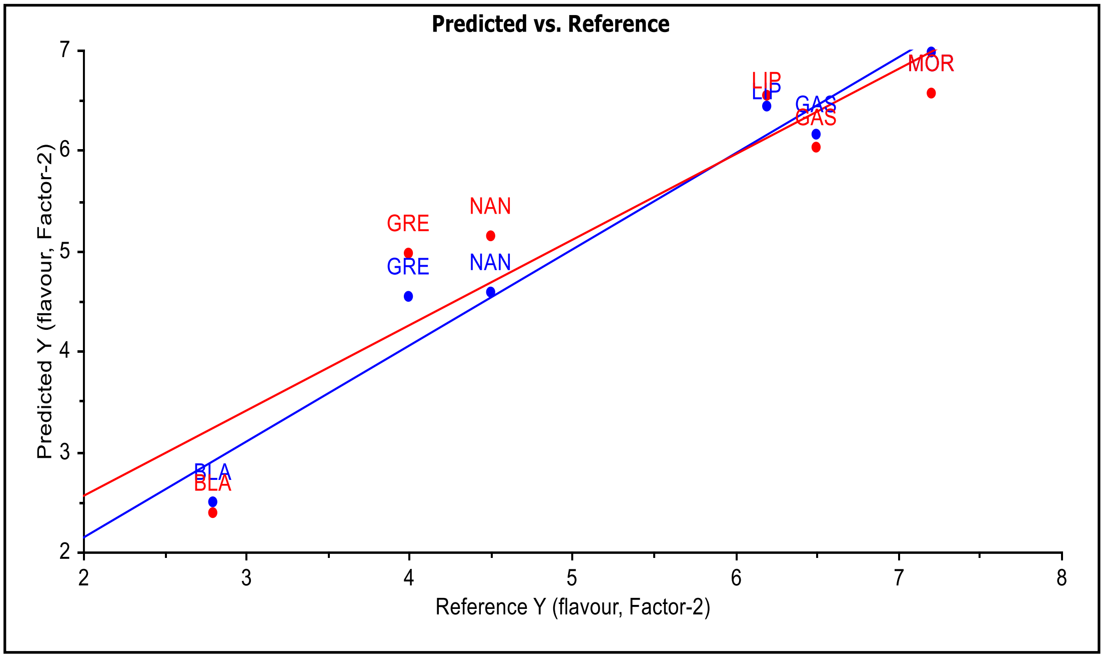

Fig. 24. The predicted average flavor scores (by PLS) versus reference scores evaluated by taste panel (blue line for calibration set and red line for validation set).

Table 17. PLS parameters of calibration model for flavor determination.

Data set	Slope	Offset	RMSE	R^2	RPD
Calibration	0.95	0.22	0.32	0.95	5.3
Validation	0.85	0.85	0.62	0.88	2.7

4.1.2.4 Overall acceptability

The highest score of overall acceptability was for the Morning tea (*i.e.* 6.5), and the lowest one was for Black tea (*i.e.* 2.0) (Fig. 25). The SD values, which ranged between 0.7-1.3, indicate high precision and low dispersion of the data (Table 18). Also scores of CV% (*i.e.* 13.6-50.0%) were good and represent a good reliability, except for the Black tea, which indicates varying opinions between the judges.

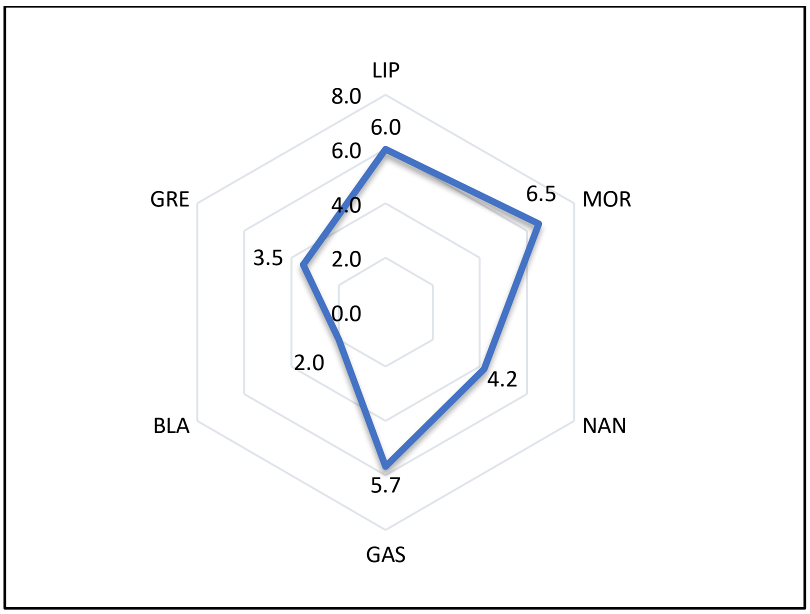

Fig. 25. Radar graph representation of overall acceptability scores.

The acceptability of food and beverage depends on two factors: -

1- The personal desire and knowledge of sample therapeutic benefits, fragrance and antioxidant properties. The previous idea consumers had about herbal tea as natural, safe and healthy plays major rule in its acceptance (Higa, Koppel, & Chambers, 2017).

2- The effectiveness of active ingredients (Chiang, Ying, Ping, & Cheng, 2011).

These two factors help in the overall profiling of each tea sample. In a study where green tea is decaffeinated (*i.e.* the concentration of caffeine is reduced), which changed the original tea flavor and reduce its acceptability by the tasters (Lee, Lee, Kim, & Kim, 2009), meaning that the presence or absence of certain ingredients in certain percentage will affect the overall acceptability of tea. The sweetness of tea is another point to consider since people usually drink Black tea with sugar which might affect the overall acceptability since no sugar was used in our study.

Table 18. Overall acceptability scores of the six tea samples evaluated by taste panel.

Tea sample	T1	T2	T3	T4	T5	T6	Average	SD	CV%
Lipo	5	7	6	7	5	6	6.0	0.8	13.6
Morning	7	4	6	7	7	8	6.5	1.3	19.4
Nanus	4	3	4	5	5	4	4.2	0.7	16.5
Gasobal	4	7	5	6	7	5	5.7	1.1	19.5
Black	2	1	2	4	2	1	2.0	1.0	50.0
Green	5	2	3	4	3	4	3.5	1.0	27.4

The ANOVA test parameters presented in Table 19 indicates significant difference ($P<0.05$) between the tea samples, since the value of F is greater than the $F_{critical}$, so the null hypothesis that assume no difference between the tea brands is rejected. This illustrates that tasters can efficiently discriminate between these tea samples with regards to the overall acceptability.

Table 19. One-way ANOVA test for overall acceptability of different tea brands according to taste panel.

Source of Variation	SS	Df	MS	F	P-value	$F_{critcal}$
Between Groups	89.1	5	17.8	15.2	1.8 E-07	2.5
Within Groups	35.2	30	1.2			
Total	124.3	35				

To investigate the ability of ET to predict the overall acceptability of herbal tea brands, PLS model was constructed with full cross validation. PC1 and PC2 explained 98% and 95% of the X and Y matrices, respectively. (Fig. 26). Parameter of calibration and validation sets in PLS regression (Table 20) denote the excellent correlation between the acceptability scores from the taste panel and the predicted acceptability by ET signals. The R^2 for both calibration (*i.e.* 0.98) and validation (*i.e.* 0.93) indicates an excellent correlation. The RPD values were greater than 2.5 (*i.e.* 9.84 and 3.94 for calibration and validation, respectively), which infers of the model acceptance with high confidence.

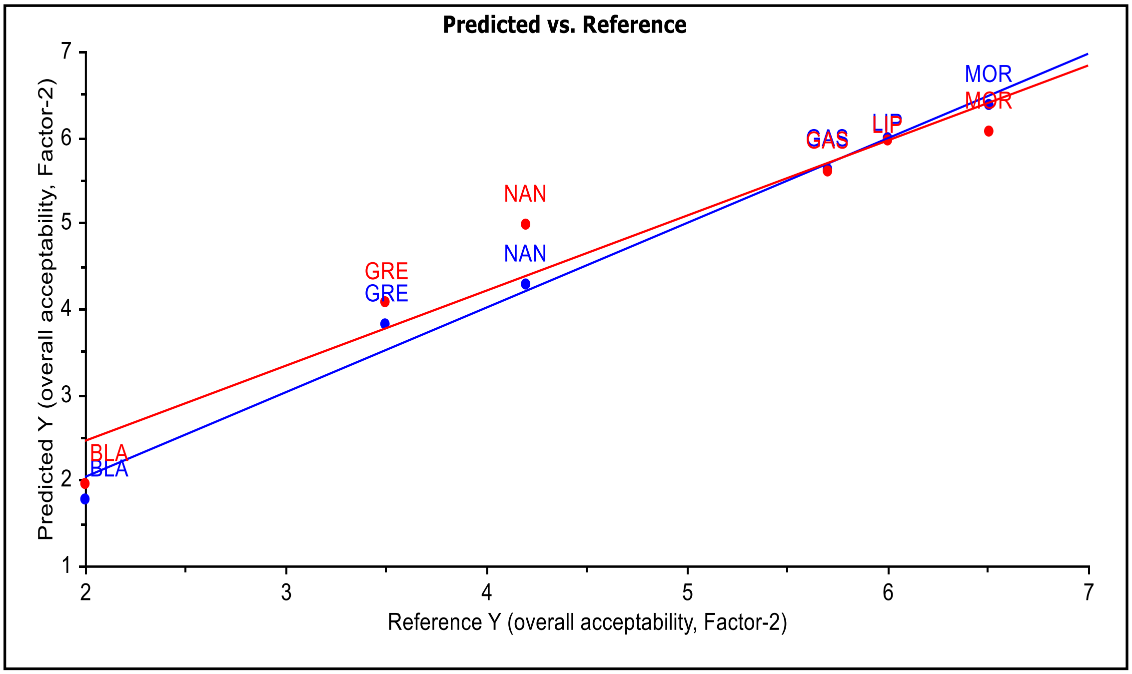

Fig. 26. The predicted average overall acceptability scores (by PLS) versus reference scores evaluated by taste panel (blue line for calibration set and red line for validation set).

The ability of ET to predict the overall acceptability of tea can be generalized to be employed in process of quality control of commercial products and in pharmaceutical applications including taste masking and pediatrics (Immohr, Hedfeld, Lang, & Pein-Hackelbusch, 2017)

Table 20. PLS parameters of calibration and validation model for overall acceptability.

Data set	Slope	Offset	RMSE	R^2	RPD
Calibration	0.98	0.06	0.18	0.98	9.8
Validation	0.87	0.70	0.45	0.94	3.9

4.1.3 Morning and Nanus tea mixing.

Further investigation of ET discrimination ability was revealed, when the closest two herbal tea brands (*i.e.* Morning and Nanus) to the reference Green tea (as shown in Table 8) were mixed, in certain concentrations, and measured by ET. The most sensitive sensors to the taste of the mixture were: ZZ, JE and BB, with SD values of 2.8-29.4 mv, which are less than 30 mv, and RSD of 0.11-3.1, which reflects reliability and reproducibility of the sensors (Table 21).

Table 21. Sensors response and reliability for Morning and Nanus tea mixture analysis.

Sample name	ZZ			JE			BB		
	Mean	**SD**	**RSD%**	**Mean**	**SD**	**RSD%**	**Mean**	**SD**	**RSD%**
N0M100	2537.7	2.8	0.11	1765.9	11.3	1.1	2694.1	21.1	1.3
N10M90	2621.1	3.3	0.12	1852.5	12.8	3.1	2798.0	22.9	2.2
N20M80	2541.5	4.7	0.18	1773.6	13.9	1.4	2705.1	23.0	1.9
N30M70	2551.4	5.3	0.21	1782.8	14.5	1.9	2704.4	24.3	2.2
N40M60	2555.5	6.4	0.25	1786.9	15.5	2.0	2726.6	24.6	2.2
N50M50	2568.7	7.0	0.27	1798.5	16.9	2.3	2744.6	25.1	2.5
N60M40	2583.0	7.4	0.29	1808.4	17.3	2.6	2755.0	26.6	2.6
N70M30	2588.0	8.7	0.34	1814.1	18.1	2.8	2771.0	27.2	2.5
N80M20	2599.7	9.5	0.37	1822.9	18.2	2.9	2774.7	27.9	2.3
N90M10	2606.8	10.0	0.38	1832.8	19.0	2.9	2783.1	29.7	2.2
N100M0	2618.6	11.1	0.42	1842.1	20.2	3.1	2802.4	29.4	2.4

The PCA scores plot of the mixing process shows a good discrimination ability, and a clear pattern was observed (Fig. 27). PC1 explained 98.6% of the total variance, where PC2 explained 1.3% of total variance. Thus, ET can be used to detect differences of closely related products, which can open a door for a lot of applications in food and industry. This is in agreement with Lvova *et al.* (2018), who stated that defects and adulteration during manufacturing process are expected to be easily detected by ET.

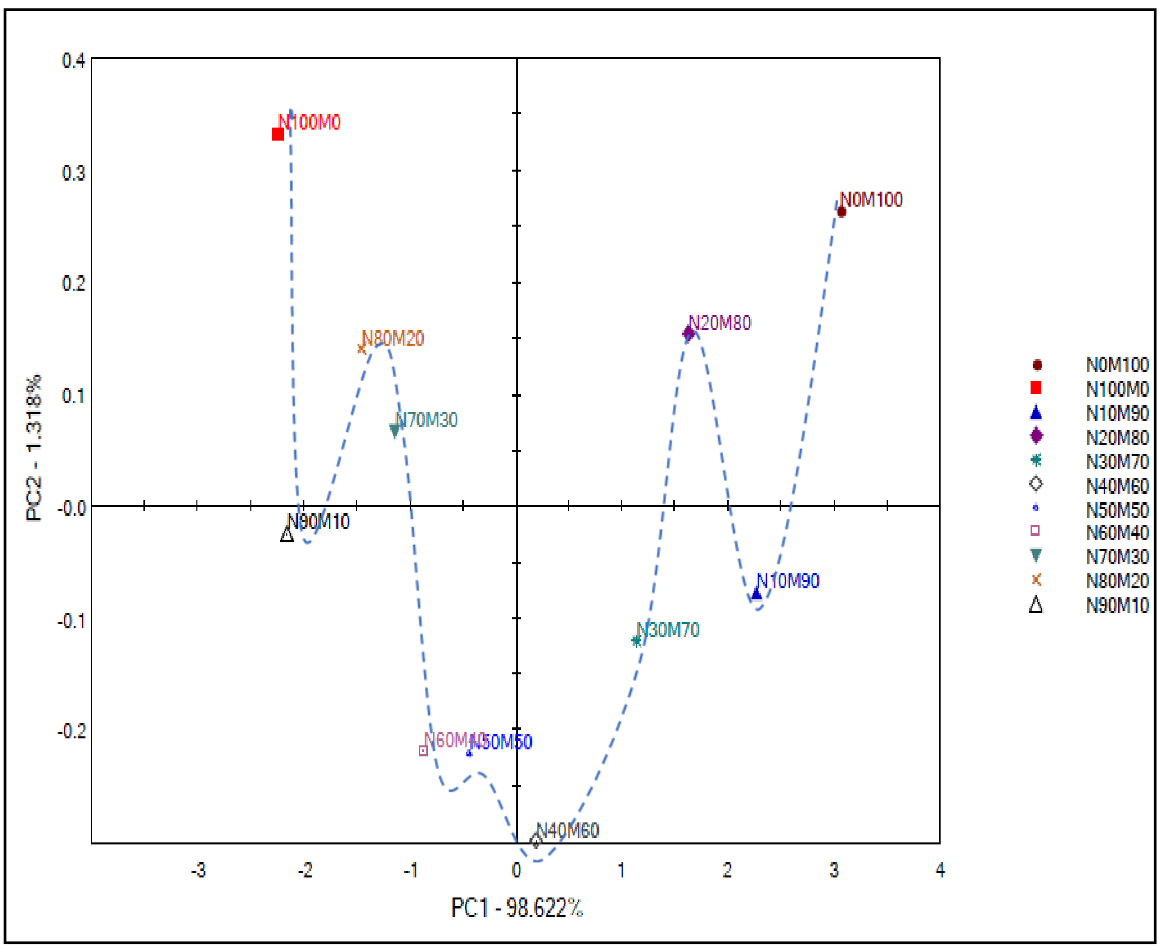

Fig. 27. PCA scores plot for the solutions resulted from mixing Morning and Nanus brands at different concentrations.

4.1.4 Spectrophotometric analysis of caffeine content

Measuring the absorbance of caffeine in herbal tea samples solutions, using DR 6000^{TM} UV-Vis spectrophotometer (Hach, Germany), helped in determining the concentration of caffeine in each tea brand, through the calibration curve shown in Fig. 28. Values of caffeine absorbance in the standard solution are shown in Table 22.

Table 22. Concentration of caffeine standard solution and its absorbance.

Conc. of standard caffeine solution (ppm)	Absorbance (Au)
100	1.799
50	0.901
40	0.736
30	0.542
20	0.367
10	0.187

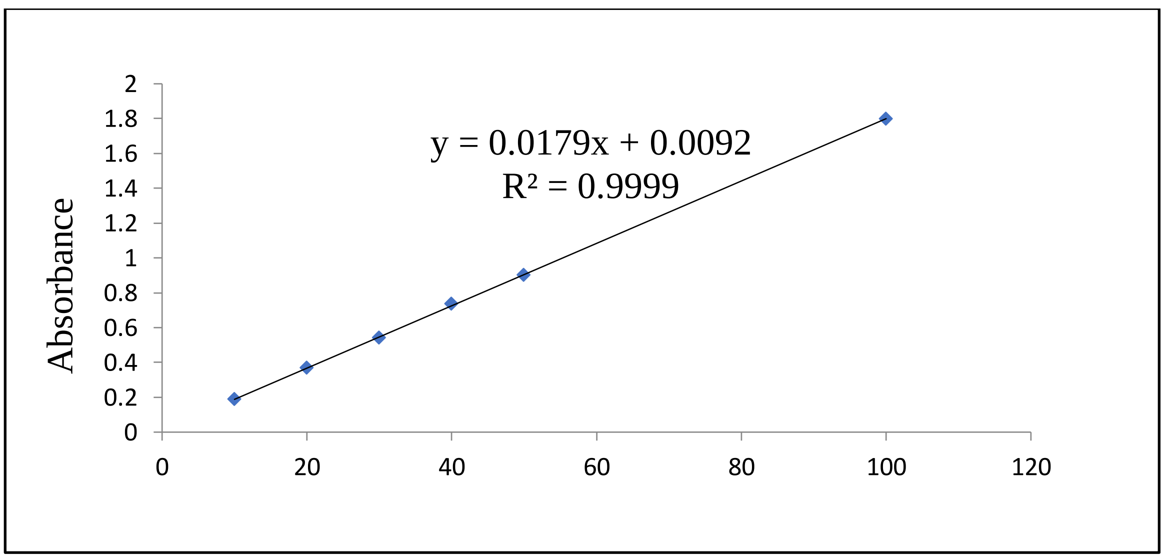

Fig. 28. Calibration curve of absorbance vs. caffeine concentration of standard solution.

The calibration equation was used to calculate caffeine concentration in each of the six herbal tea samples, the obtained values are listed in Table 23. These results were employed as a reference (Y-matrix) to test ET ability to identify certain components in the tea samples mentioned earlier, especially caffeine as Papieva *et al*. (2011) carried out.

Table 23. Values of caffeine concentration calculated from the calibration equation.

Sample name	Absorbance (Au)	Caffeine concentration of herbal tea (ppm)
Lipo	0.103	210.4
Nanus	0.094	190.3
Morning	0.081	160.3
Gasobal	0.138	286.9
Black	0.225	482.9
Green	0.214	458.7

PLS model (Fig. 29) was constructed between the ET signals and concentration of caffeine. Both PC1 and PC2 explained 88% and 96% of the X and Y matrices, respectively. Table 24 represents parameters of caffeine calibration, which reflects good discrimination, high reliability and relatively small error of ET quantitative analysis compared to the data obtained by spectrophotometer.

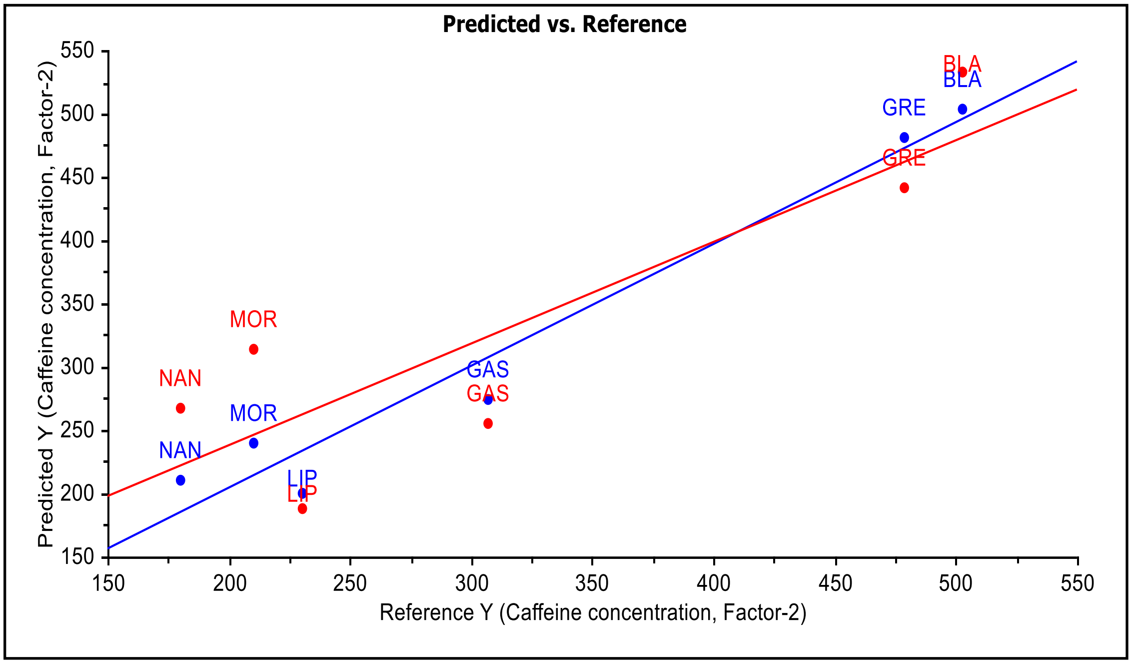

Fig. 29. The predicted average caffeine concentration (by PLS) versus reference scores evaluated by spectrophotometer (blue line for calibration set and red line for validation set).

Full cross validation was performed on the calibrated data, due to the small number of samples tested. The model is acceptable according to Mireei *et al.* (2010), since the RPD values is greater than 2.5 (*i.e.* 4.3 for calibration and 3.0 for validation), with R^2 value of more than 0.9 for both calibration (*i.e.* 0.93) and validation (*i.e.* 0.90), indicating that there is a good correlation between data.

ET had the possibility to predict values of caffeine concentration. Thus, it can be stated that ET can be considered as a powerful tool for quantitative analysis for some chemical compounds presented in tea.

Table 24. PLS parameters of calibration and validation model for caffeine determination.

Data set	Slope	Offset	RMSE	R^2	RPD
Calibration	0.93	20.9	32.8	0.93	4.3
Validation	0.80	61.3	47.3	0.90	3.0

Caffeine is an alkaloid belongs to methylxanthine family, present as bitter white crystals. It is responsible for some taste characteristics of tea (Wanyika *et al*., 2010). The basic properties of caffeine in tea resulted from the presence of couple unpaired electrons on the nitrogen atoms as caffeine structure (Fig. 30). The black tea sample had the highest value of caffeine (503.01 ppm *i.e*. 0.05%) among the other five brands (Table 23), but still within the acceptable range (less than 4%) according to Parvathy, Luiz, & Varkey (2014). Different factors account for the different caffeine content in each sample, and therefore in ET signals, including growing environmental conditions, such as leaf position on the tree, soil composition and climatic changes. Other reasons could be varying plucking season, brewing conditions, variety and cultivar of the plant (Buratti, Casiraghi, Minghetti, & Giovanelli, 2013;Gramza-Michaleowska, 2014).

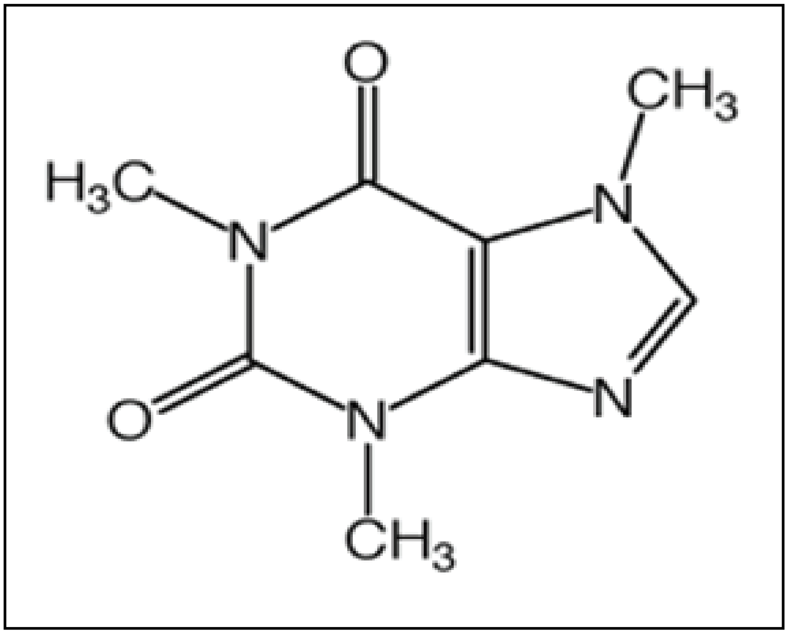

Fig. 30. Structure of caffeine (Pohanka & Dobes, 2013).

4.2 Relax granules

4.2.1 Relax batches analysis

Eleven samples of the herbal mixture Relax (Table 5), each from different production batches, were analyzed to investigate the ability of ET to follow production time or characteristics of the product. Two sensors were the most sensitive to Relax batches, *i.e.* ZZ and JE. Values of standard deviation (SD) ranged between 4.9-10.2 mv, which shows reproducibility. Low values of SD (*i.e.* less than 30 mv) and low RSD% values ranged between 0.22%-0.57% (Table 25), reflect low dispersion and good reliability in the measuring process.

Table 25. Sensors response and reliability for Relax samples analysis.

Sample Name	**ZZ**			**JE**		
	Mean	SD	RSD%	Mean	SD	RSD%
01_17	2268.1	10.2	0.45	1663.7	9.5	0.57
02_16	2241.6	5.6	0.25	1676.9	6.6	0.40
02_17	2285.7	6.6	0.29	1680.4	8.6	0.51
03_16	2285.7	6.9	0.30	1690.4	6.2	0.36
04_16	2272.0	6.3	0.28	1674.0	6.7	0.40
06_16	2274.5	6.5	0.29	1672.9	6.4	0.38
07_16	2268.9	4.9	0.22	1669.6	6.0	0.36
08_16	2271.6	5.5	0.24	1681.4	6.1	0.36
09_16	2263.4	8.2	0.36	1670.4	8.7	0.52
10_16	2267.1	8.4	0.37	1670.1	8.0	0.48
11_16	2265.5	9.4	0.42	1669.9	9.1	0.55

PCA scores plot in Fig. 31 was used to group different samples and figure out the relationship between them. PC1 and PC2 explained 81.55% and 18.45% of the total variance, respectively. Both PCs described 100% of the total variance. Some groups could be easily distinguished, especially sample named 2_16, while the others are close to each other with some overlapping.

To study the relationship and similarity between samples, the fresh sample from production batch 2_17 (*i.e.* produced February 2017) was used as a reference. The distance from the reference sample ranged between 0.97-3.3. The most distinguished group (Table 26) was 2_16 with the highest distance and highest pattern discrimination index of 3.3 and 78.1%, respectively. However, all the other groups were close to the reference sample (can be seen from the distance values) and no pattern was observed that relate production batches time to their distance from the reference.

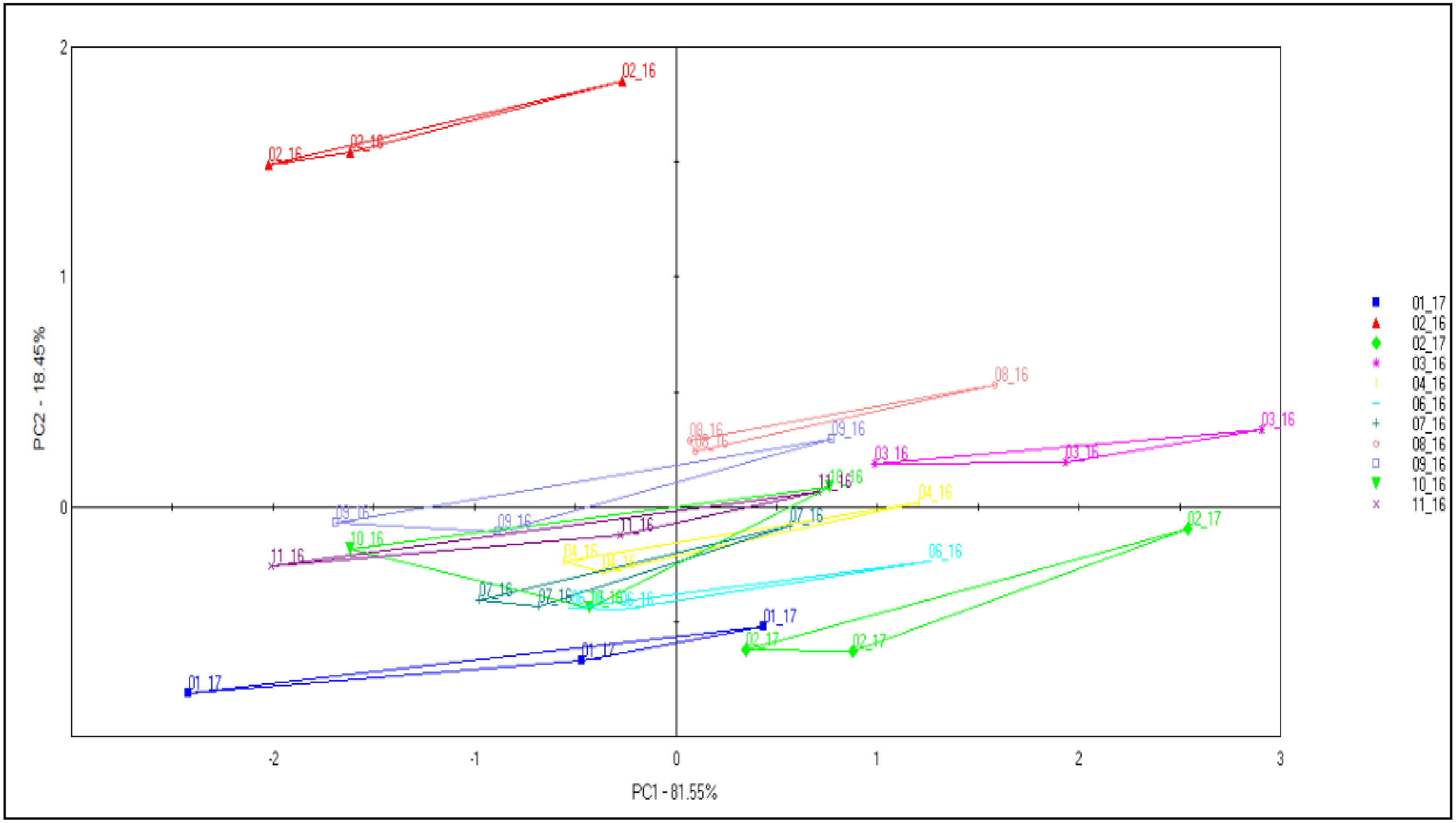

Fig. 31. PCA scores plot for analysis of Relax production batches.

Table 26. Distance values between eleven Relax batches at $P<0.05$

Product names	Reference samples	Distances	Pattern discrimination index (%)
02_16	02_17	3.3	78.11
01_17	02_17	2.1	47.8
09_16	02_17	1.92	47.72
11_16	02_17	1.81	42.6
10_16	02_17	1.7	43.03
07_16	02_17	1.62	48.4
04_16	02_17	1.19	31.17
06_16	02_17	1.1	28.12
08_16	02_17	1.05	27.54
03_16	02_17	0.97	23.38

Furthermore, DFA analysis carried for more clarification (Fig. 32). The first and second discriminant functions explained 98.6% and 1.39% of the total variance, respectively. The total accumulated contribution rate of the first two discriminant functions is 99.99%, indicating that the model can explain the whole variation of the original data, without any significant loss in the information.

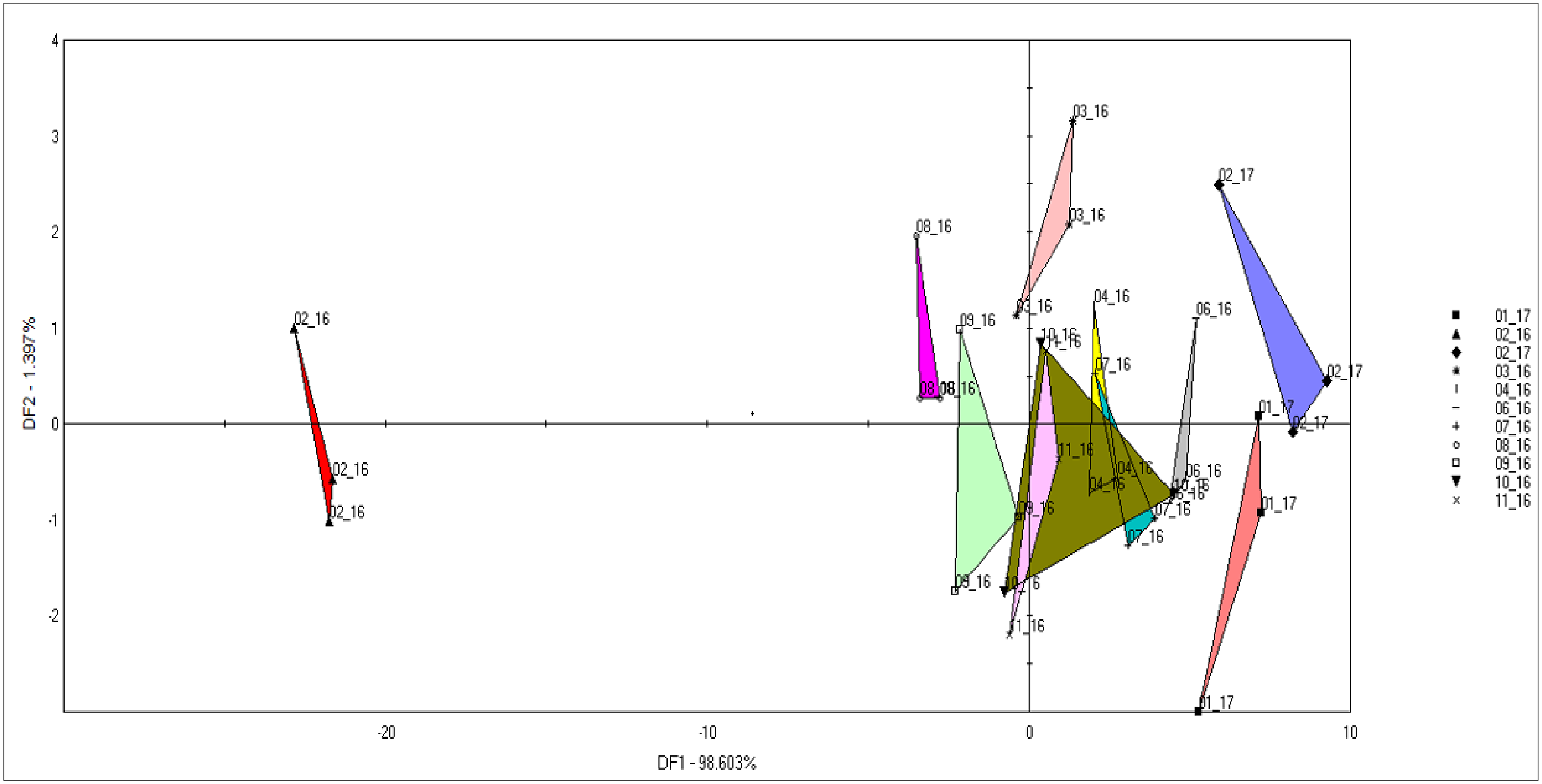

Fig. 32. DFA model for different Relax production batches.

The DFA model clearly shows that sample 2_16 lies on the far left of the figure, away from all the other samples groups, while 2_17 (the reference sample) is located to most right of the model. All samples (except 2_16) are relatively close to each other, indicating that the storage time (shelf-life) have little or no effect on the analyzed batches ensuring the good quality of this local product. For sample 2_16, it was found that the manufacture company had stopped using one of the herbs (*i.e.* Prunus) in the mixture. Despite its presence in low quantity, ET can detect that small difference reflecting the high discrimination and sensitivity of this taste sensor in predicting the composition of samples.

4.2.2 Relax competence

Relax is a mixture of herbs that work together in order to improve gastro-intestinal tract functions or laxative. Many other products are being sold in pharmacies for the same purpose (Table 6). Two foreign alternatives to Relax having the same price was analyzed using ET. Fig. 33 displays PCA model for the competing products (*i.e.* Normalax and Jungborn) and Relax. A clear grouping of the samples was noticed. PC1 and PC2 accounted for 99.9% of the total variance (PC1 explained about 98.5% ot the total variance and PC2 about 1.4%, most of data was explained using only two PCs).

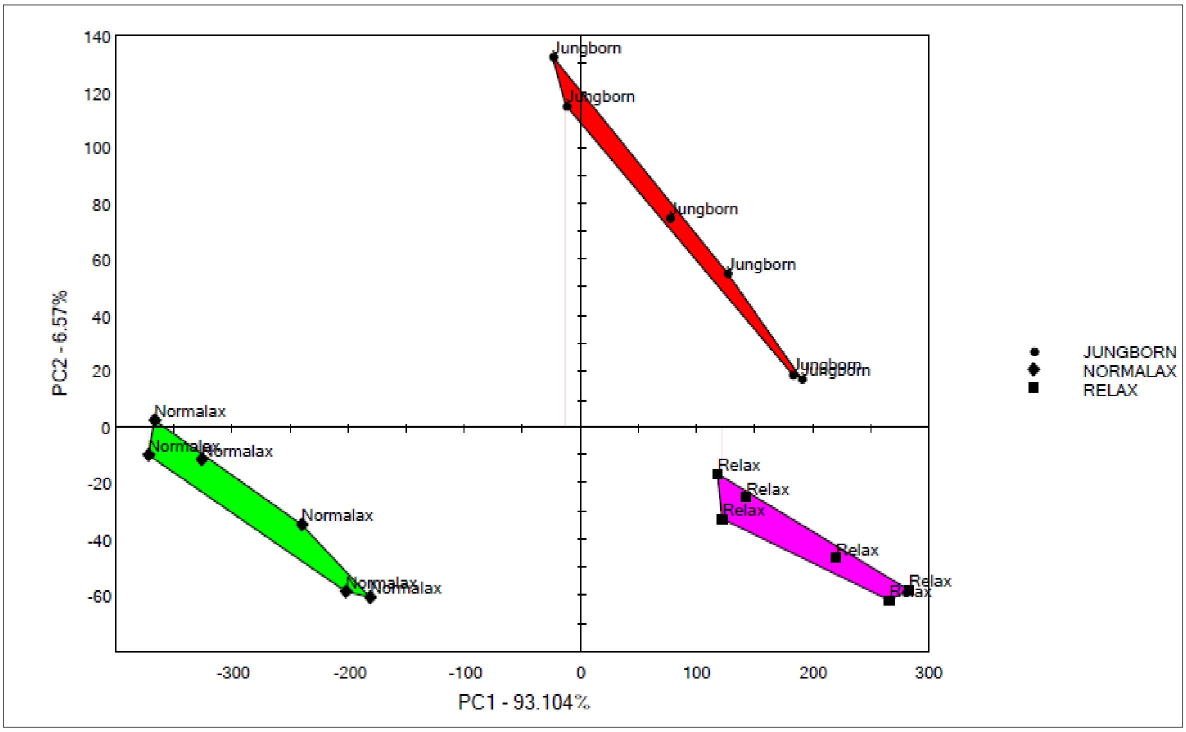

Fig. 33. PCA scores plot for Relax and its competence.

To investigate the relationship between samples, Fig. 36 used to show the similarity between them, using Relax as reference.

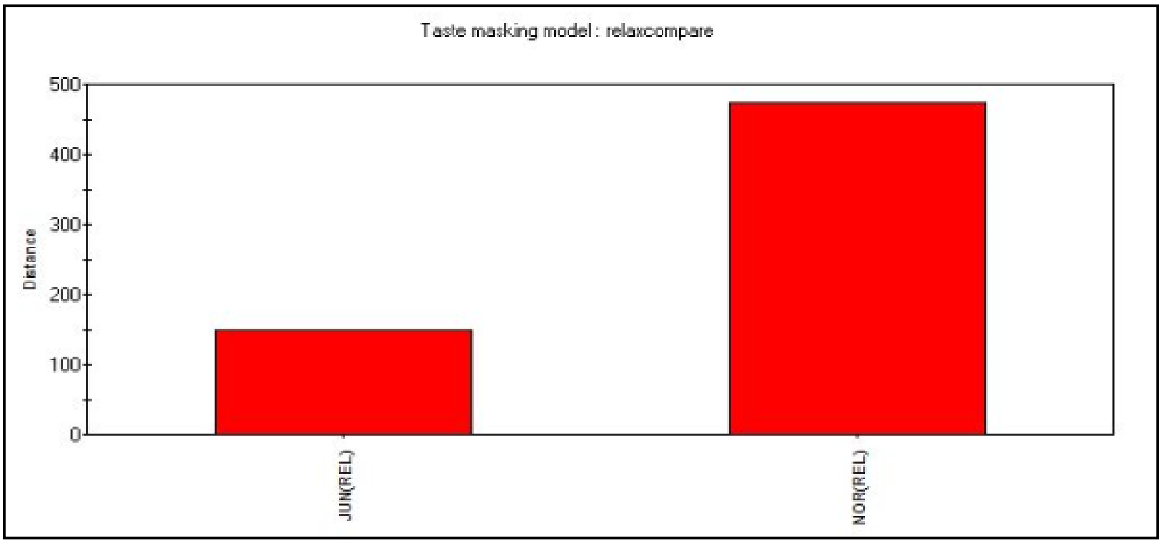

Fig. 34. Distance representation between Relax and its competence.

Values of distance (Table 27) illustrate that Jungborn granules was the most closed product to Relax with a distance of 1.46. This could indicate that the active constituents of Jungborn are much more similar to Relax herbal mix than Normalax.

Table 27. Distance values between Relax and its competence.

Product names	Reference samples	Distances	Pattern discrimination index (%)
Normalax	Relax	3.56	88.4
Jungborn	Relax	1.46	48.1

All the previously mentioned products have almost same price. The obtained results confirm that the local Relax proved its ability to compete with foreign products, with no side effects, since it made totally of natural herbs. Whereas, Jungborn is made of senna powder that could cause the bowels to stop functioning normally and might cause dependence on laxatives if used for more than two weeks. Also, long-term use of Jungborn can also change the amount or balance of some chemicals in the blood (electrolytes) that can cause heart function disorders, muscle weakness, liver damage and other harmful effects. On the other side, Normalax which had polyethylene glycol as major constituent might cause nausea, abdominal cramping, or gas. Excessive number of bowel movements, persistent diarrhea, severe or persistent stomach/abdominal pain, bloody stools, or rectal bleeding, while using this medication is possible according to the medicine's brochure.

5. Conclusion and Recommendations

The results reported the possibility using ET and MVDA as a very efficient analytical tools for quality control of local herbal medicines. These tools are promising research field. This will contribute in helping and supporting the improvement of local herbal medicines market and open a door for further applications.

Acceptable accuracy and relatively low cost could be achieved when using ET technology, due to its unique properties including: fast sample preparation, reliability, identifying and quantifying liquid compounds.

The ET was successfully able to distinguish between different herbal tea brands and predict several of their taste parameters; *i.e.* taste, flavor and overall acceptability. It could also identify a specific pattern, when mixing two herbal tea brands. Furthermore, it could predict caffeine content in each of the tea brands. Moreover, ET was extremely helpful in quality control of different Relax (*i.e.* herbal product) production batches and identifies the closest alternative being sold in the local market.

The examined herbal mixtures proved to be promising treating agents against one of the tested bacterial strains (*i.e. S. aureus*). A wider research can be done to test other therapeutic properties of such products.

Further research is needed to provide a better knowledge regarding the quality of herbal plants available in Palestine, and possible future applications that can utilize ET in quality control and mimicking taste to save time and money.

فحص عينات مزيج من الأعشاب الطبية باستخدام مجس التذوق ’اللسان الالكتروني‘ والتحليل المتعدد العوامل

حنين ماجد طه

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Abstract (in Arabic)

تلعب النباتات الطبية العشبية دوراً مهما في مختلف التطبيقات الصحية، ويركز العلماء في الوقت الحاضر على الأساليب التحليلية وتطبيقات القياس الكمي لتمييز هذه الأعشاب من أصناف وأنواع متقاربة، الا أن هذه الأساليب والأدوات لها عيوب جعلت العلماء يبحثون عن تقنيات جديدة في ظل الطلب الدائم على هذه النباتات.

أجريت هذه الدراسة لتقديم مجال بحثي ناشئ من تكنولوجيا المستشعرات، وهو اللسان الالكتروني. حيث تم استخدام جهاز اللسان الالكتروني وتحليل البيانات متعدد المتغيرات في مراقبة جودة الادوية العشبية المحلية المنتجة من شركة بجورة في طولكرم - فلسطين، واختبار قدرة اللسان الالكتروني على تذوق وتمييز بعض عينات الشاي محلية الصنع والمستخرجة من الأعشاب. حيث تم اختبار أربع عينات من الشاي العشبي، بالإضافة لعينات من الشاي الأخضر والأسود، باستخدام اللسان الالكتروني، كما تم استخدام الشاي الأخضر كمرجع للمقارنة بين العينات لدراسة علاقات التماثل فيما بينها، ثم تحليل الاشارات الناتجة من اللسان الالكتروني باستخدام التحليل المتعدد المتغيرات، بالإضافة الى استخدام أساليب مختلفة للتحقق من البيانات الناتجة من اللسان الالكتروني، مثل اختبار التذوق البشري (اختبار المستهلك) وقياسات الطيف الضوئي لمحتوى الكافيين من عينات الشاي. كذلك تم خلط نوعين من الشاي المتقاربين بالطعم وبتراكيز مختلفة واختبارهما بواسطة اللسان الالكتروني. وأخيراً تم دراسة منتج محلي يدعى "ريلاكس" (وهو مزيج عشبي يستخدم كملينات) لقياس مدى ثبات تركيبته مع مرور الزمن، بالإضافة الى مقارنته مع منتجاته البديلة في الأسواق.

أوضحت النتائج قدرة اللسان الالكتروني على أن يميز بنجاح بين عينات الشاي الستة، كما انه استطاع التنبؤ ببعض خصائص اختبار التذوق البشري ومحتوى الكافيين في عينات الشاي. كما تمكن اللسان الالكتروني من تمييز خليط من نوعين من الشاي المتقاربين في الطعم بكفاءة عالية.

أما بالنسبة لعينات الريلاكس، فقد اشارت النتائج الى تأثير ضئيل على العينات بمرور الزمن، كما وتم اثبات قدرة هذا المنتج على منافسة المنتجات الأجنبية.

توفر النتائج الاجمالية معلومات أساسية حول إمكانية استخدام جهاز اللسان الالكتروني، بمساعدة تحليل البيانات متعدد المتغيرات، في اختبار مزيج النباتات الطبية ولأغراض مراقبة الجودة، وإمكانية استخدامه في تطبيقات زراعية وبيولوجية متعددة مستقبلاً.

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Appendix A

Antimicrobial activity test

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Antimicrobial activity test

To evaluate the medicinal value of each herbal tea, the antibacterial activity against *Escherichia coli (*gram-negative) and *Staphylococcus aureus (*gram-positive) bacteria was studied. Disc diffusion method was used, in which Mueller Hinton Agar (MH Agar) (the best medium for these purposes) was prepared by suspending 38 g of medium in one liter of deionized water, heated with agitation, boiled for one minute to dissolve all the medium and autoclaved at 121°C for 15 minutes. Finally, the cooled MH agar was poured on 90 cm sterile petri dishes on a level and horizontal surface to give uniformity. The inoculums were spread on the autoclaved petri dishes using a sterile cotton swab and incubated overnight at 37°C. Whatman No. 1 filter paper discs (12 mm in diameter) were soaked in the prepared samples solutions then placed carefully on the inoculated dishes and stored at 37°C in an incubator overnight. Finally, the zone of inhibition was measured and recorded (Bakht *et al.*, 2011; Chan, Soh, Tie, & Law, 2011; Tuysuz, Dosler, Tan, & Otuk, 2016).

The test was performed in triplicates. The obtained results were analyzed using ANOVA test to check if the antimicrobial effect of tea varies significantly between both of the bacterial strains and between the tea brands.

The antimicrobial activities of all tea brands against two bacteria strains *E. coli* and *Staphylococcus aureus* were assessed by the presence or absence of inhibition zones presented (Table A. 1) using disk-diffusion method. The test was performed in triplicates and variable levels of antibacterial activity against tested bacterial strains had been noticed.

Table A. 1. Average inhibition zones (mm) of antibacterial test of the six tea brands against E. coli and Staphylococcus aureus using disk-diffusion method.

Bacterial strain / Tea brand	*E. coli*	*S. aureus*
Control	0.0	0.0
LIP	1.8	3.3
MOR	1.2	1.2
NAN	2.2	1.0
GAS	2.2	3.0
BLA	0.7	7.3
GRE	1.2	4.0

Results of ANOVA test (Table A. 2) indicate that bacterial strains had different effect on each of the tea brands. There was a significant difference ($P<0.05$) in the effect of both the bacterial strains on tea, in the inhibition ability between the tea brands and the antibacterial activity. The F values were greater than the $F_{critical}$, so the null hypothesis (H_0: there is no relation between different tea brands and the bacterial strains) is rejected.

Table A. 2. ANOVA test parameters for the relation between inhibition zones of different tea brands against two bacterial strains.

Source of Variation	SS	Df	MS	F	P-value	$F_{critical}$
Sample	29.2	5	5.8	21.7	3.5 E-08	2.6
Columns	28.1	1	28.1	104.4	3.2 E-10	4.3
Interaction	56.5	5	11.3	42	4.2 E-11	2.6
Within	6.5	24	0.3			
Total	120.2	35				

Fig. A. 1 shows that generally, the inhibitory effects on *S. aureus* (*i.e.* gram-positive) are greater than on *E. coli* (*i.e.* gram-negative) bacteria. The obtained data was in agreement with literature, which stated that gram-positive bacteria are more sensitive to different examined herbal plants than gram negative bacteria (Gharachorloo & Amouheidari, 2016; Li *et al.*, 2015). This could be due to the fact that gram negative bacteria contain an additional unique outer membrane that prevent some materials from entering the cell thus protect it from any antibacterial drug, while gram positive bacteria lack suck membrane and have only inner one (Sabbobeh *et al.*, 2016).

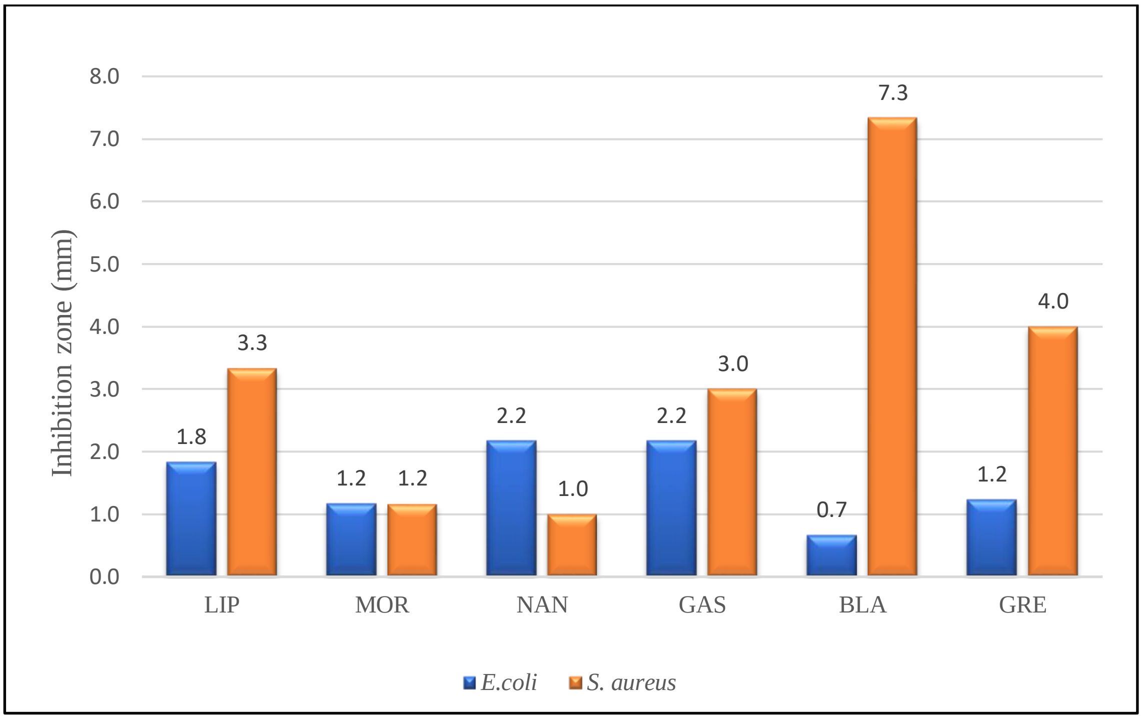

Fig. A. 1. Antimicribial average effect of six tea brands against *E. coli* and *S. aureus*.

The six tea brands reacted differently against the two bacterial strains (Fig. A. 2). Black tea had the higher inhibitory activity on *S. aureus* followed by Green tea. However, Morning tea had equal effect on the tested strains. Low effect was noticed for all the brands against *E. coli.*

Medial plants contain huge variety of secondary metabolites including flavonoids, terpenoids, tannins and alkaloids which are well known of their antimicrobial properties (Chan *et al*., 2011). The presence of these chemical constituents beside the nature and number of active components involved in plants is affected by plant genetic variation, age, growing environmental conditions, and the storage conditions including temperature, humidity and light exposure period (Elkhair *et al*., 2010).

These factors can be of great interest in explaining the different effect of each of the tea brands on the tested bacterial strains.

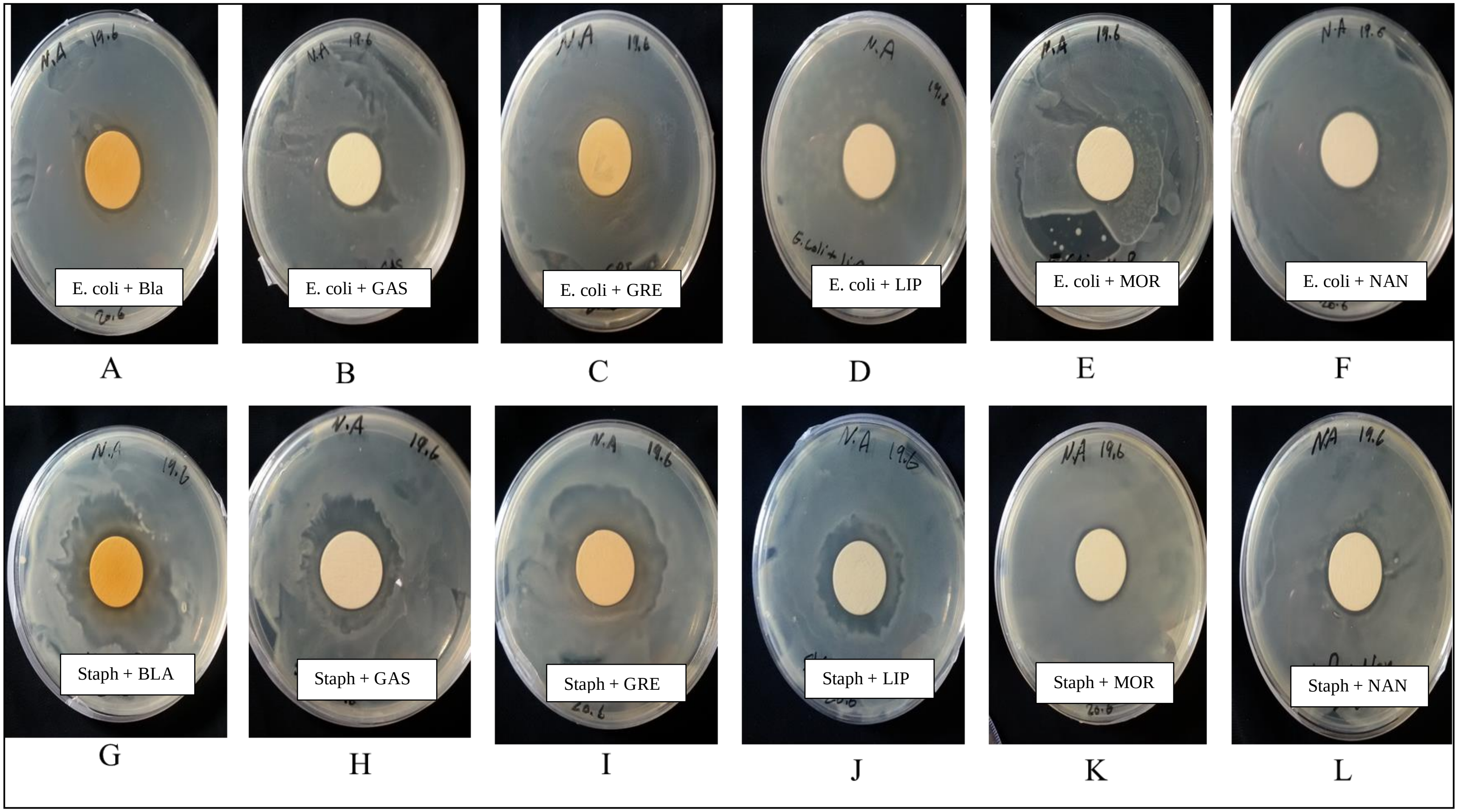

Fig. A. 2. The first row shows the inhibition zones for Black (A), Gasobal (B), Green (C), Lipo (D), Morning (E), and Nanus (F) samples against *E. coli*. The second row shows the inhibition zones for Black (G), Gasobal (H), Green (I), Lipo (J), Morning (K), and Nanus (L) samples against *S. aureus*.

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