# Applied Chemistry Project

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| **Project title** | Electrochemical Approach for Improved Analysis of Tea Leaves with Thin Layer Chromatography |


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# Electrochemical Approach for Improved Analysis of Tea Leaves with Thin Layer Chromatography

**by**
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**In Partial Fulfillment for the Degree of Bachelor of Science**
**Program in Applied Chemistry (International Program)**
**Department of Chemistry, Faculty of Science**
**Chulalongkorn University**
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Project Electrochemical Approach for Improved Analysis of Tea Leaves with Thin Layer Chromatography

By Ms. Benyapha Phoomtrakul and Ms. Pichaya Watthanawareekun

Accepted by Department of Chemistry, Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science Program in Applied Chemistry (International Program)

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## Abstract

Tea is one of the most consumed beverages worldwide with potential benefits derived mainly from antioxidant properties. During fermentation and preservation, tea leaves undergo oxidation and hydrolysis leading to formation and degradation of various tea constituents and affecting the antioxidant capacity. This study developed an analytical approach to compare chemical fingerprints of various types of tea and the oxidation/reduction products by using electrolysis combined with thin layer chromatography (TLC). Electrolysis approach was performed on TLC plates followed by the TLC separation using the selected mobile phase system of toluene, ethyl acetate and formic acid (2:9:1). Effects of electrolysis voltage and time were investigated in details with the suitable conditions of 1V and ≥1 min. The TLC fingerprints after the electrolysis revealed the increasing amount of less polar nonvolatile components which improved the tea fingerprints compared with the sample without electrolysis. The approach may also reveal greater antioxidant properties of Earl Grey tea than Oolong Tea as indicated by the longer oxidation time required to change the Earl Grey tea fingerprint. The additional TLC spots were also selectively cut and desorbed at 80°C and the volatile compounds in the headspace were sampled with solid phase microextraction (SPME) and analyzed with GC-MS. This could detect several peaks albeit with their small areas which could not be identifiable. Electrolysis of the tea solution was then performed by using stainless steel spring coils as the electrodes at 9V for 5 min. The SPME GC-MS analysis revealed degradation of several volatiles with the increasing amounts of benzaldehyde (in both teas), 1,2 dihydro-linalool (in Earl Grey Tea) and alkanes (in Oolong Tea) after the electrolysis. With further development, the developed on-TLC plate electrolysis technique will allow assessment of tea quality in terms of antioxidants on one hand and quality control for improved processing, manufacturing, storage, and preparation in order to maximize the nutritional value on the other.

Keywords: Tea, Camellia Sinensis, GC-MS, Electrolysis, Electrocoagulation

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# Chapter 1
# Introduction

## 1.1 Introduction to the research problem and significance

Tea, one of the most popular beverages, is consumed by two thirds of the world's population [1]. It is obtained from leaves from plants named "*Camellia Sinensis*" [1]. The reason for its popularity is attributed to its unique sensory properties but recently, the scientific community has discovered the potential therapeutic benefits of this beverage [1,2]. In the past several years, there has been a growing interest in identifying the pharmacological and physiological effects of various types of tea [2]. Studies have discovered its potential benefits against cardiovascular diseases and cancer as well as antihypertensive, antiateriosclerotic, and hipochloresteroladmic properties mostly from activities of antioxidant flavonoids present [3-5].

Teas are classified into three main categories; fully fermented, non-fermented, and partially fermented [2]. Black tea, produced by fermentation of slightly withered leaves before being either smoked fired, flame fired, or steamed, is oxidized [1,3]. In contrast, green tea is prepared by dehydration of the leaves, thus avoiding oxidation of the polyphenols [1,3]. The third type, oolong tea, is a partially oxidized product prepared by withering fresh leaves in the sun, brushing slightly, and partially fermenting [1,3]. The three types of tea undergo a different degree of fermentation, steaming or roasting during their manufacturing. Fermentation leads to chemical oxidation and hydrolysis of chemical constituents in the leaves [6]. This causes an increase in volatile compounds [7]. On the other hand, the antioxidant capacity is reduced due to oxidative or thermal degradation of antioxidants such as caffeine, saponin, ascorbic acid, and non-catechin polyphenols [6]. Since processing methods significantly impact the concentration of chemical components inside the leaves, investigation into antioxidant activity of tea is crucial for quality control as they are associated with health benefits and attributed to their free radical scavenging activity [7.8].

Unlike others, this study will be focusing on the chemical compounds and antioxidant content of the three different types of tea prior to and after oxidation. Tea infusions are prepared in a way that reflects how consumers would prepare their teas. Method developed in the last decade for determination of antioxidants of plant extracts was based on the ability of an antioxidant to quench free radicals by hydrogen donation [9]. However, it could not identify the bioactive compounds in a mixture of plant material [9]. Not to mention, the procedure involved distillation, solvent extraction, and isolation of compounds by mass spectrometry and nuclear magnetic resonance spectroscopy; which are time-consuming [9].

According to a research by Zeng et al. (2013), electric field treatment has been reported as a green and effective method to accelerate aging of wine. The result showed a slight increase in content of free amino acids after exposure to direct current (DC) electric field [10]. In order to apply this theory, electrolysis of tea samples was performed on a Thin-layer chromatography (TLC) plate. TLC is a technique extensively found to be a rapid and convenient approach to physically separate chemical components of a sample with the detection approach [11]. Despite the facts that the chemical composition of tea is highly complex, with 2,000 components, chromatography is essential for investigation of multi-components mixture in tea leaves, identification of aromatic compounds as well as quantitative analysis of tea bioactive compounds [12]. Chromatography is a separation technique, used in analysis and isolation of individual components based on how they interact with the stationary phase and mobile phase inside a column [12]. Gas chromatography-mass spectrometry (GC-MS) is a qualitative analytical method used to carry out the determination of volatile compounds [10,11]. Solid phase microextraction (SPME) was incorporated as a preparation method. It is a type of headspace sampling for investigating the volatile components in the space above a sample in a sealed container [13].

## 1.2 Research objectives

The objective of this research is to develop TLC based approach allowing online investigation of the chemical profile of various types of teas grown in Thailand before and after electrolysis on TLC plates and to identify volatile compounds by using head-space solid phase microextraction (HS-SPME) GC-MS in conjunction.

## 1.3 Literature search

### *1.3.1. Primary Compositions in Tea*

The identification of volatiles is crucial in order to characterize teas. Many studies have looked into these volatiles and identified the components involving GC-MS and SPME techniques. **Figure 1** below shows the primary components found in the majority of teas [13].

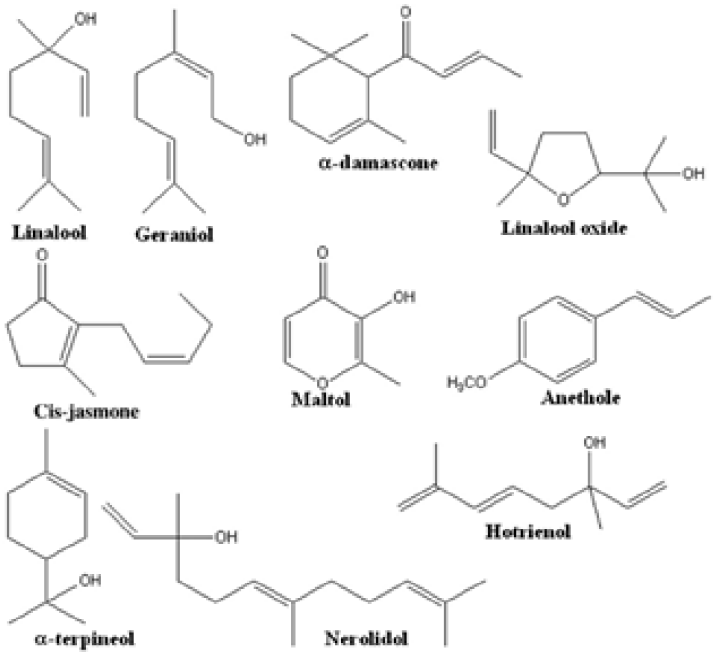


**Figure1**. Structure of main constituents found in most teas [13].

In 1975, a research conducted by Vitzthum et al. discovered several volatile components in black tea. Fifty six compounds were identified in black tea by using a combination of glass capillary gas chromatography and mass spectrometry. The main components were reported as pyridines, prazines, quinolines, thaizoles, aromatic amines, and carbonyls [14]. Few years later, lipids in black teas degraded to volatiles during the manufacturing process was reported. Also, the levels of linalool and salicylate increased after rolling and fermentation. However, most volatiles were lost during the firing process. Selvendran's experiment concluded that the flavor of the black tea was dependent on volatile carbonyl compounds which were initiated by enzymatic breakdown of membrane lipids [15].

The volatiles in non fermented green tea were investigated and reported in 1981. A total of 89 compounds, including 17 hydrocarbons, 17 alcohols, 16 aldehydes, 13 ketones, 8 esters, 2 ethers, 1 acid, and 5 others were detected by GC-MS. Other major components included 2,6,6-trimethyl-2-hydroxycyclohexanone, linalool, geraniol, cis-jasmone, para-ionone, cyclohexanone, 5,6-epoxy-para-ionone, indole and caffeine [16].

The composition of oolong tea was analyzed in 1995 by the use of GC and GC-Fourier transform infrared spectroscopy coupled to mass spectrometry (GC-FTIR/MS). Forty-nine compounds were identified from Chan Pin oolong samples. The brewed extract consisted of high levels of 2,6-dimethyl-3,7-octadiene-2,6-diol, 2-phenylethanol, benzyl alcohol, linalool oxides I, II, and III, and hexanoic acid. Other important components were geraniol and 3,7-dimethyl-l,5,7-octatrien-3-ol. The largest quantity was found to be 2,6-dimethyl-3,7-octadiene-2,6-diol [17].

*1.3.2 Antioxidant Activity in Tea*

Polyphenols are the tea components contributing to antioxidant activity in teas [18]. Because of their antioxidative property, tea has gained a great deal of attention [19]. Therefore, the tea component helps reduce risks of getting cancers from antioxidant activity and becomes an effective chemopreventive factor for toxic chemicals [20]. Studies have shown that extractions of polyphenols in tea were related to antimutagenic, antidiabetic, antibacterial, anti-inflammatory and hypocholesterolemic qualities [21-24]. Tea can be an important source of minerals with antioxidative properties [19].

*1.3.3 Electrolyzed Water in Food Industry*

Electrolysis of water and beverage is popular in the food industry for the purpose of sanitization to eliminate microorganisms in food [25,26]. However, this technique exhibits disadvantages for sanitizing, and the process is not effective in eliminating bacterial spores [27]. Electrolyzed water requires low production costs and gives wider favoring implementation in food and the food industry [28].

Water electrolysis is a technique to remove water and produce highly pure hydrogen and oxygen by using electricity for the electrochemical decomposition of water as shown in **Figure 2**, with no pollution being generated [29,30,31] since it is not efficient in a sustainable society to produce hydrogen from non-renewable sources. Water electrolysis processes exhibit many advantages: simple, high purity, zero-pollution, plenty of sources, and good energy carrier [32].

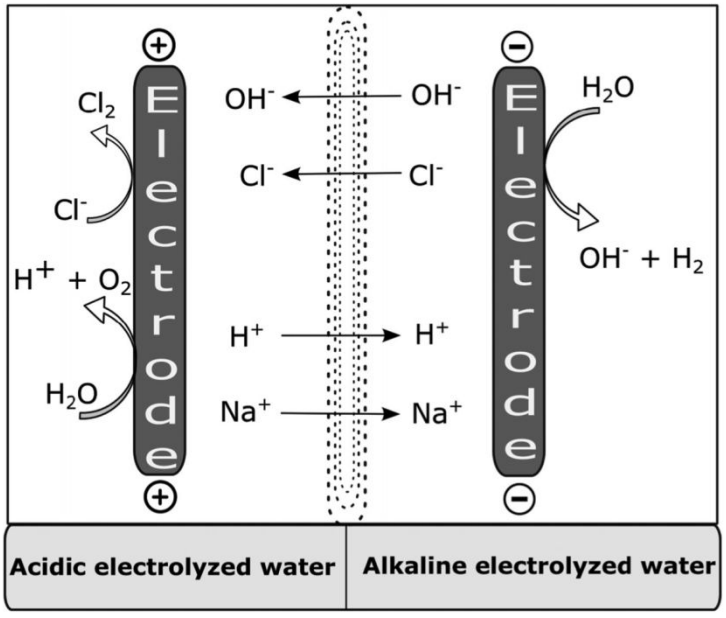


**Figure 2**. Application of electrolysis of water and an electrolytic cell, consisting of anode and cathode electrodes connected through an external power supply and separated by a septum or diaphragm [31].

### *1.3.4 Vanillin*

Vanillin is a crystalline solid in white and is known as one of the most popular and common flavors in food and beverage industries [33]. Vanillin can be naturally produced from *Vanilla planifolia*, which can be grown in countries like Mexico, Tonga, Madagascar, Tahiti, and Indonesia; or wet polymerization of lignin and synthetic biotransformation [34]. Visualization of components on TLC plates requires the use of vanillin which gives a range of pink colors. Phenolic compounds in tea, vanillin, and acid are reagents used to detect phenols by changing color on the spot [35].

### *1.3.5 Thin Layer Chromatography*

Thin Layer Chromatography (TLC) is a fast, easy, cost effective, and time minimization technique to analyze crude samples. Although this technique is limited in the use of quantitative analysis, TLC is still a common separation technique due to its notable advantages in qualitative analysis [36]. Polar substances migrate close to the eluent front. Compounds could be characterized by their polarity. Low polarity substances will have higher retention time being eluted due to the increasing adsorptivity of compounds along with its polarity [37].

### *1.3.6 Gas Chromatography-Mass Spectrometry*

GC-MS is the method for qualitative analysis of volatile compounds. However, the technique is time consuming and requires additional techniques such as SPME or dynamic headspace (DHS) [38,39]. Headspace-SPME focuses on the study of flavor impact in a mixture of volatile organic chemicals. HS-SPME/GC-MS method contributed to distinguish aromatic compounds. Therefore, GC-MS technique is more commonly used for analyzing volatile and semi-volatiles substances due to its usefulness of the built-in library. GC provides highly efficient separation of volatile mixtures according to different boiling points of the components and their interactions with stationary phase. The separated compounds are then detected with MS offering compound identification and quantification based on different characteristic *m/z* of the compounds. Compound identification can be further confirmed by comparison between the experimental retention indices with the library data. This technique has been used in the pharmaceutical field identifying impurities but the analysis is not as wide as applications in food and beverage industries [40,41].

# Chapter 2
# Experimental

## 2.1 List of equipment and instrument

- Beaker 50 mL (Schott, Germany)
- Beaker 250 mL (Duran, Germany)
- Beaker 500 mL (Duran, Germany)
- Beaker 1000 mL (Duran, Germany)
- Measuring Cylinder 10 mL (Simax, Czech Republic)
- Measuring Cylinder 50 mL (Simax, Czech Republic)
- Electronic Scale (Mettler Toledo, Ohio, USA)
- Watch glass (Earth Chemelab, Thailand)
- Forceps (Mira, Japan)
- Stirring Rods (Earth Chemelab, Thailand)
- Parafilm (American National Can Co., Chicago, USA)
- Delicate Task Wipers (Kimtech, Spain)
- Micropipette 1000uL (eppendorf Research plus, Germany)
- Micropipette 20uL (eppendorf Research plus, Germany)
- Micropipette Tips (eppendorf Research plus, Germany)
- TLC Silica Gel 60 F 20x20 cm (Merck, Germany)
- Hot Plate (Schott, Germany)
- DC Power Supply (Korad, Korea)
- Electrical wire (Korad, Korea)
- Glass Vial 20mL (Agilent, USA)
- Vial septa 20mL glass vial (Agilent, USA)
- Crimp (Agilent, USA)
- Aluminium Crimp Seals 20mm (National Scientific, USA)
- 1.5 mL Centrifuge Tube (Eppendorf, Germany)
- Hot water bath (edelstahl rostfrei, Germany)
- Thermometer (Earth Chemelab, Thailand)
- SPME Fiber (Supelco, USA)
- SPME holder (Supelco, USA)
- GC-MS System 7890A (Agilent, USA)

## 2.2 Chemicals and reagents

Ethyl acetate and Toluene were supplied by J.T. Baker (New Jersey, USA) and formic acid was obtained from Fisher Scientific UK (Loughborough, England). All the reagents were at an analytical grade and utilized without any further purification.

Three Samples of tea leaves were purchased from a local grocery store. Earl Grey and oolong were used as fully fermented and partially fermented samples respectively. Both are harvested and processed by Boon Rawd Brewery Farm

(Chiang Rai, Thailand). The non fermented type, green tea, was produced by Phufah (Nan, Thailand).

## 2.3 Experimental procedure

### *2.3.1 Preparation of Tea Infusions*

The infusions were prepared by brewing 3.5g of tea leaves (Earl Grey, oolong, and green tea) with 7.5-mL water. They were placed on a hotplate at 100°C for 5 minutes with constant stirring. After cooling down to room temperature, the tea leaves were removed with a spatula and brewed tea mixtures were obtained.

### *2.3.2 Vanillin Stock Preparation*

5 g of vanillin was dissolved with 500-mL ethanol. After vanillin solution was homogenized with the solvent, ethanol, the solution was transferred into a beaker. The beaker containing vanillin solution was placed on an ice bath at temperature 0-10 °C and 1.5% sulfuric acid was slowly dropped and stirred into the solution in a total of 5 mL sulfuric acid.

### *2.3.3 Selection of Mobile Phase*

Brewed Earl Grey, oolong, and green tea mixtures were loaded on separate 4.5 cm × 6.0 cm TLC plates coated with 175-225 μm layers of silica gel. Tea samples from different brands were loaded 2 μL for one spot each, on the same plate by using Eppendorf pipettes. Spots were applied 1.5 cm from the bottom and and 1.5 cm apart from each other as shown in **Figure 3**.

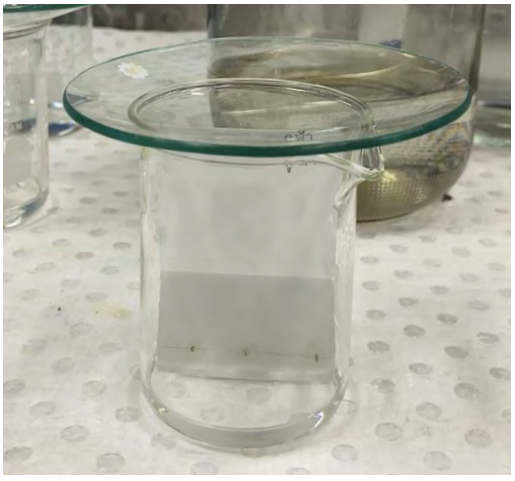

**Figure 3.** Setup of TLC chamber during selection of mobile phase.

The mobile phase was prepared by a mixture of toluene, ethyl acetate and formic acid. The analysis was performed and adjusted the mobile phase ratio for three times including (5:6:1), (1:10:1), and (2:9:1). Prior to performing TLC, the

mobile phase chamber was put in equilibrium for 10 minutes within a homogeneous system. The ideal condition was found to be 2:9:1.

*2.3.4 Electrolysis on TLC Plates*

There were six spots loaded at different times (starting from 5min, 2min, 1min, 20s, 10s after the electrolysis and control) on a TLC plate for each type of tea: earl grey, oolong, and green tea. Each sample (2 μL) was loaded onto the plate as a spot. Voltage was then applied for the electrolysis period of interest followed by alternative reload of the sample onto the adjacent spot. The overall process was repeated three times. After the first spot was loaded, voltage was applied for 5min by connecting the TLC plate with a Korad KA3005D digital control DC power supply via an electrical wire either cathode (- electrode) or anode acted (+ electrode). The electrolysis at the cathodic TLC was performed with the anode connected to an Al foil; whilst, the electrolysis at the anodic TLC was performed with the cathode connected to the foil. A paper was then inserted between any set of cathode and anode. This resulted in a capacitor-like electrolysis cell consisting of the TLC plate/paper/Al foil sheets in close contact as shown in **Figure 4**. This allows electrolysis of tea on a TLC plate without any electrolyte solution.

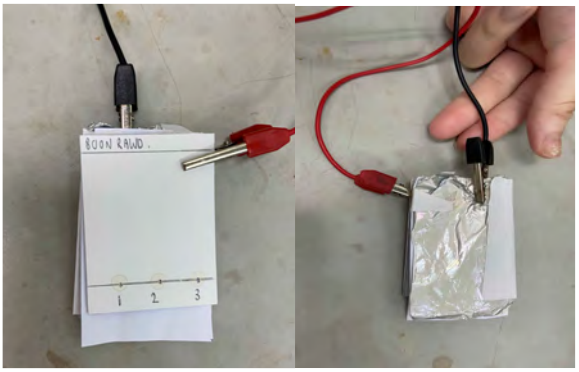

**Figure 4**. Setup of electrolysis on TLC plate with aluminium foil.

For the power supply control, press the ‘M1’ button to select voltage and current. Once the voltage was adjusted into a different target value, the electrolysis of tea on a TLC plate was performed with different time. The voltages were applied to cathodic TLC and anodic TLC at 1, 2, 5, 10 and 20V. Once the target voltage was set, the current was run by pressing the ‘Off/On’ button.

Moreover, the electrolysis was performed within different times: 10s and 20s, and 1, 2, 3, 4, 5, 10, 15 and 20 min in order to select a suitable condition for further reaction.

*2.3.5 Electrolysis of Tea Solution by Aluminium Spring Coil*

In order to monitor changes in chemical composition that will occur, the second approach, which was electrolysis of tea solution by aluminium spring coil was

conducted. This will allow us to compare the difference between chemical fingerprint as a result of two distinct types of electric current application. The electrolytic cell applied in this part is composed of small aluminium spring electrodes inserted into the larger one, which were connected to anode (+) and cathode (-), respectively, and separated by a parafilm layer. Two electrical wires were used as the connecters to complete a DC circuit, with the tea solution (Earl Grey and Oolong) as electrolyte.

Earl Grey solution (1 mL) was applied as a control (before electrolysis reaction) and another 1-mL of Earl Grey (after 5min electrolysis) was transferred into a 1.5-mL centrifuge tube container. The small aluminium coil electrode was covered with parafilm on top and bottom without touching the larger aluminium coil electrode. The electrical wire was then connected to the aluminium coil electrodes. Voltage of 9V was applied to the aluminium spring coils for 5 min as shown in **Figure 5**. This resulted in the experimental current of 0.098-0.1A. The same method was performed for oolong tea solution. The sample solution after the electrolysis was collected into a vial for further analysis by HS-SPME/GC-MS and the result was compared with the control.

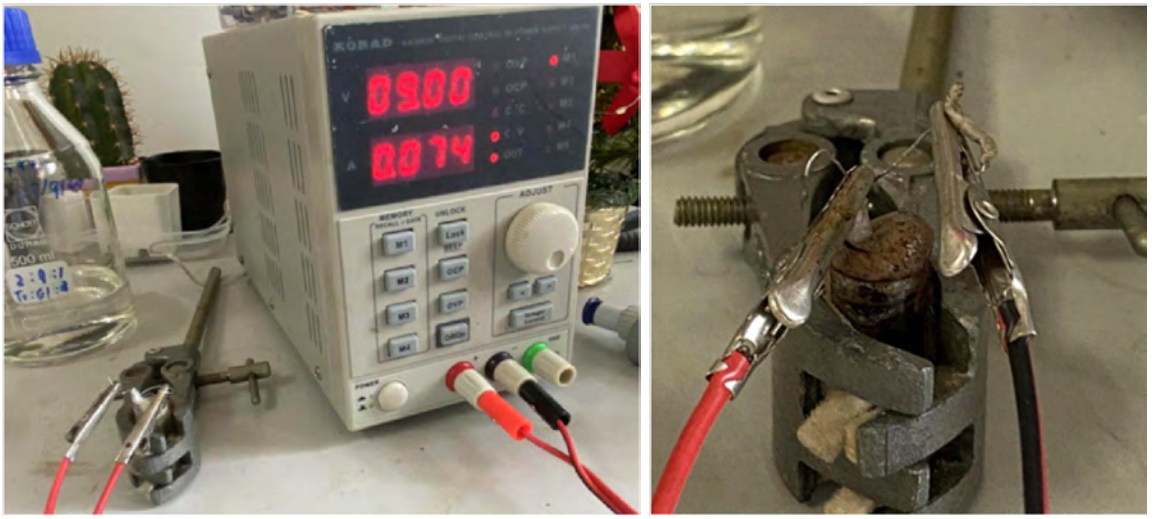

**Figure 5**. Set up of electrolysis with aluminium spring coil electrode.

*2.3.5 Thin Layer Chromatography After Electrolysis*

The TLC plate was removed from the electrical wire to perform subsequent separation. The mobile phase was prepared by a mixture of toluene, ethyl acetate and formic acid with a ratio of (2:9:1). After electrolysis, the chromatographic plate was transferred to 100mL beaker to perform TLC analysis.

The separation spots on TLC plates of Earl Grey, Oolong, and green tea were observed under Spectroline UV-viewing cabinet model CM-10 (Spectronic corporation, Westbury, New York) at a short wavelength of 254 nm.

Each TLC plate was dipped with a vanillin reagent and heated at 80°C within a fume hood to obtain the detection. The colored spots on the TLC plate were

observable with naked eyes within 30 seconds as a pinkish-red spot against a yellow background for both before and after electrolysis.

*2.3.6 Standard Solution*

Nine standard solutions, including vanillin acid, protocatebuic acid, succinic acid, sinapic acid, syringic, m-hydroxybenzoic acid, quercetin, resveratrol, and benzoic acid were diluted with methanol to achieve 1000 ppm concentration for each. A drop of each standard solution was spotted on the TLC plate from left to right, performed TLC with a solution of toluene, ethyl acetate and formic acid as a mobile phase at a ratio (2:9:1), to compare identify standards with tea compounds from the distance travelled over the chromatographic plate.

*2.3.7 Identification of Volatiles by GC-MS analysis*

*2.3.7.1 Electrolysis of Tea on TLC plate for GC-MS*

200-μL of Earl Grey tea samples were loaded on an 8 cm × 10 cm chromatographic plate on the same spot for five times resulting in a total volume of 1mL. The electrolysis on the plate was performed with the optimized voltage and time of 2V and 2min. This also performed for the Oolong sample.

*2.3.7.2 Thin Layer Chromatography*

After the chromatographic plate was electrolyzed, thin layer chromatography analysis with toluene, ethyl acetate, and formic acid as a mobile phase with ratio of (2:9:1) was performed

*2.3.7.3 HS-SPME*

The first separation spot (fraction 1), second separation spot (fraction2), and third separation spot (fraction3) were cut from the electrolyzed TLC plate. The headspace solid phase microextraction (HS-SPME) was performed with a 50/30 μm DVB/CAR/PDMS fiber and a holder was purchased from Supelco (Supelco, Germany). For HS-SPME procedure, 20-mL glass vials were closed with polytetrafluoroethylene (PTFE) coated silicone rubber septum. A 1cm x 3.5cm TLC plate containing the targeted sample (fraction1 or first separation spot, fraction 2, and fraction 3 respectively) were transferred into the glass vial separately. The vial was heated at 80°C for 60 minutes in a hot water bath as shown in **Figure 6**. The DVB/CAR/PDMS fiber was then collected after extraction was achieved.

**Figure 6**. Hot water bath set up where the vial is heated for HS-SPME.

*2.3.7.5 Desorption of tea VOCs compounds by GC-MS*

An Agilent 7890A gas chromatography system coupled with mass spectrometry detector was used to perform the analysis. HP-5 column capillary column (30 m x 0.25 mm i.d., 0.25 um film thickness) was equipped with purified helium (99.999%) as the carrier gas at a constant flow rate of 1 ml/min. The injection was performed under splitless mode at 220°C and was desorbed for 5 minutes. The oven temperature was held at 40°C for 5 min and then increased to 220°C at a rate of 4°C/min, and held at 220°C for 5 min and then increased to 240°C with the same rate, and finally held at 240°C for 5 min. The temperature of the ion source in MS was set at 230°C. Electron ionization voltage was -70eV. The mass spectra were acquired over the mass range of 30-300 Da with a scan time of 100 ms.

# Chapter 3
# Results and Discussion

## 3.1) Optimization of mobile phase

Mobile phase is an essential factor that determines efficiency of the separation of components of tea. Mobile phase systems consisting of toluene: ethyl acetate: formic acid with three different ratios: 5:6:1, 1:10:1, and 2:9:1, were investigated. After separation, pinkish-colored spots appeared after dipping the chromatographic plate in a vanillin solution and heated. The difference between polarities of these three mixtures influenced the distances at which the chemical compounds migrated along the TLC plate. The mobile phase with highest polarity was with the ratio of 1:10:1 followed by 2:9:1 and 5:6:1. As shown in **Figure 4C**, the spots interacted more strongly with the stationary phase and were not well separated (with 2 clearly separated spots) using the mobile phase ratio of 5:6:1, suggesting a higher polarity is required. Although increasing polarity of the solvent enhanced the movement of analytes, the polarity of the mobile phase with the ratio of 1:10:1 was found to be too high (**Figure 7A**), since the compounds traveled too rapidly upwards the plate and were not well separated with 2 separated spots. The suitable mobile phase ratio resulting in 3 clearly separated spots was at 2:9:1 as shown in **Figure 7B**.

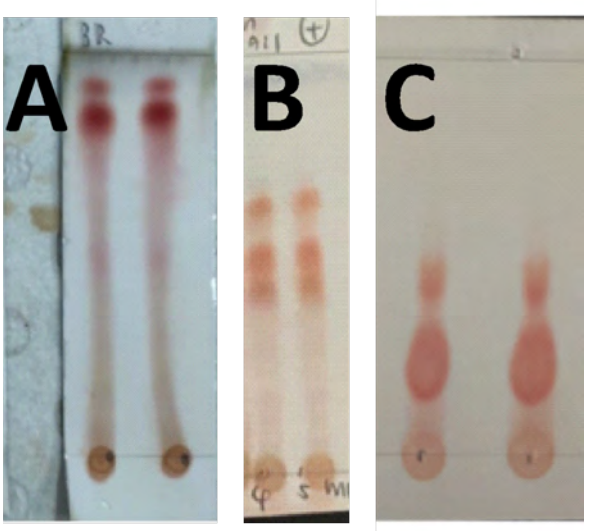


**Figure 7**. TLC results obtained using different mobile phases with the Toluene: Ethyl Acetate: Formic Acid ratios of (A) 5:6:1, (B) 2:9:1 (C) 1:10:1.

## 3.2) Optimization of Voltage and time of electrolysis

Upon selection of the optimal voltage and time for electrolysis, various conditions were evaluated for the Earl Grey and Oolong tea sample. Voltages were tested at five levels: 1, 2, 5, 10, and 20V, for both anodic and cathodic TLC plates as shown in **Figure 8**. For each voltage, electrolysis time was varied as control (before electrolysis), 10s, 20s, 1min, 2min, and 5min.

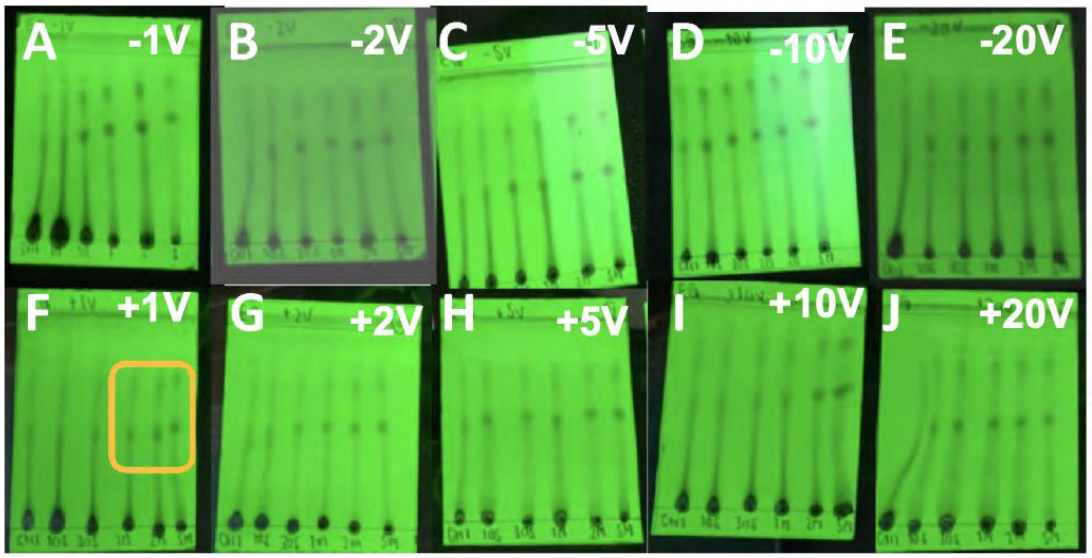


**Figure 8.** Effects of voltages on the electrolysis on-TLC plate results for the Earl Grey tea sample; at -1V, -2V, -5V, -10V and -20V (cathodic) from A-E, respectively, and +1V, +2V, +5V, +10V and +20V (anodic) from F-J, respectively. The electrolysis time was varied from Control (0s), 10s, 20s, 1min, 2min and 5min from left to right tracks, respectively, on each TLC plate.

Earl Grey sample, the electrolysis at +1V or -1V only showed minimal contrast for the separated components (same as the control before the electrolysis). The electrolysis on cathode (-) of Earl Grey sample at 1V in **Figure 8** (8A) and the electrolysis on anode (+) of Earl Grey sample at 1V in **Figure 8** (8F), using the same set of timeframe, appeared similar, and so did the oolong sample in **Figure 9** (9A) and (9F). This 1-dimensional TLC did not show separating spots as clear as in 2-dimensional technique.

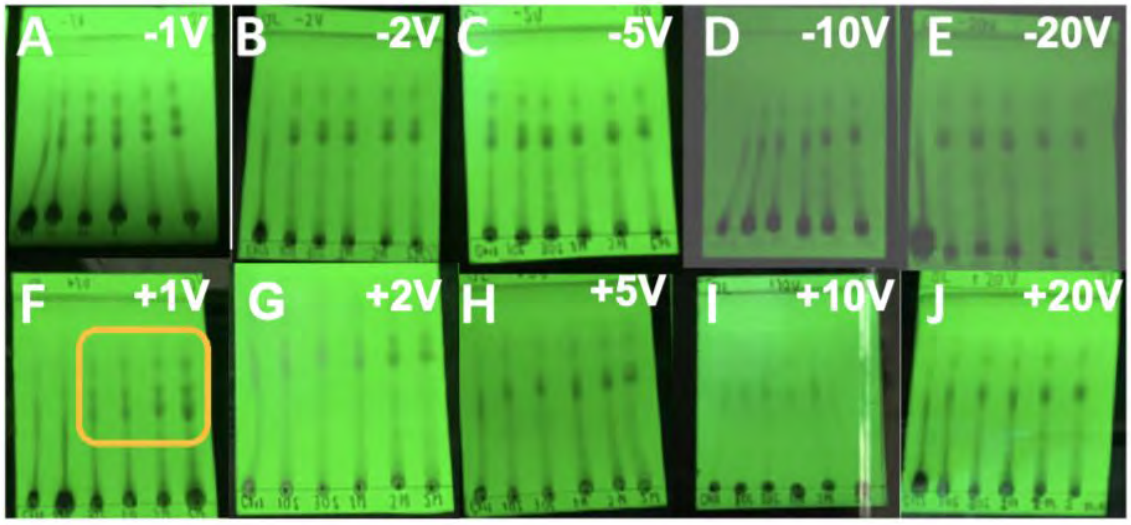


**Figure 9.** Effects of voltages on the electrolysis on-TLC plate results for the Oolong tea sample; at -1V, -2V, -5V, -10V and -20V (cathodic) from A-E, respectively, and +1V, +2V, +5V, +10V and +20V (anodic) from F-J, respectively. The electrolysis time was varied from Control (0s), 10s, 20s, 1min, 2min and 5min from left to right tracks, respectively, on each TLC plate.

According to **Figure 9**, the electrolysis performed at 2V started to show the changes as the time increases, starting from control (before electrolysis) in both Earl Grey and oolong tea samples.The separating process disappeared on some plates in **Figure 9I** due to the low volume of formic acid in mobile phase mixture. The evaporation occurred and acidity decreased after re-using the same mobile phase solution more than 5 times.

The electrolysis between cathode and anode started to showed differences at 5V, where cathode electrolysis of Earl Grey sample at 5V shown in **Figure 8C** and anode electrolysis of Earl Grey sample at 5V shown in **Figure 8H**, using the same set of timeframe, appeared different in migration distance, and so did the oolong sample. The electrolysis on cathode(-) showed additional spots above the first separation points as compared to electrolysis on anode(+), and so did the electrolysis at 10V in **Figure 8C-8I**.

The electrolysis between cathode and anode of Earl Grey sample at 20V appeared similar in **Figure 8E**, **8J**. Electrolysis of oolong tea at -20V in **Figure 9E**, **9J** showed slightly more spots separation progress as the reaction time increases and clearer component migration over the chromatographic plate. However, the optimized voltage was 2V at 2min. The spot separations were found to be the most effective with the condition of 1V for ≥1min, where the spots of the oxidized/reduced products travelling at appropriate distances and clearly observed corresponding to the increasing
amount of less polar components. Furthermore, the TLC fingerprints after the electrolysis were improved compared with that of the same sample without electrolysis, *e.g.* compare the rightmost track (electrolyzed sample) with the leftmost (control sample) on any TLC plate in **Figures 8** and **9**. The benefit of this can be demonstrated by comparing the conventional TLC fingerprints of Earl Grey and Oolong teas are very similar (see the leftmost tracts in **Figure 8A** and **9A**). On the other hand, their fingerprints could be clearly differentiated using our developed electrolysis on TLC approach (*e.g.* see the rightmost tracts in **Figure 8A** and **9A**). In addition, the approach may also be correlated with some antioxidant properties of the tea samples. To this end, TLC fingerprint of Earl Grey tea changed more slowly with the electrolysis time (see the circled region in **Figure 8F**) than Oolong Tea with the shorter oxidation time required to change its fingerprint (see the circled region in **Figure 9F**). This may be related to the greater antioxidant property of Earl Grey tea.

### 3.3) TLC of Standard Solution

TLC under the same separation condition as that applied for the tea samples was also performed for 9 different phenolic compound standards with the results shown in **Figure 10**. Since all the standards are considerably hydrophobic, they were eluted at the top of the TLC plate above the regions that all the tea and the

oxidized/reduced tea components occupied in both Earl grey and oolong tea samples. This indicates no phenolic compounds were produced as the products of the on-TLC plate electrolysis.

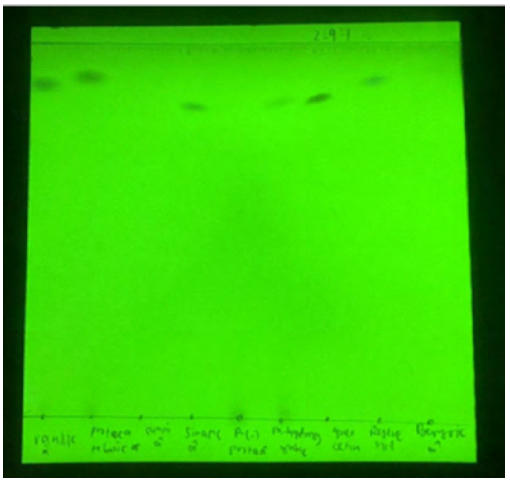

**Figure 10**. 1000 ppm Standard solutions after performing thin layer chromatography in a total of 9 standards.

## 3.4) GC-MS Analysis

The two approaches on GC-MS analysis: electrolysis on TLC plates and electrolysis/electrocoagulation of spring coil, were performed for both Earl Grey and Oolong tea samples.

### *3.4.1) Electrolysis on TLC Plates*

In order to determine volatiles by GC-MS, a total 1 mL of each sample was gradually loaded onto the TLC plates, 200μL each for 5 times. The components of Earl Grey separated into a total of 3 fractions as shown in **Figure 11** and 2 fractions for oolong tea, after electrolysis on TLC plate at optimized condition, 2V for 2min, which can be observed from **Figure 8B**, **8G**, **9B**, and **9G.**

The component of control (sample before electrolysis) could not be obtained, since the analytes did not migrate up the TLC plate as shown in **Figure 11**(A). Consequently, GC-MS analysis was conducted using fractions where targeted separation spots were most obvious and clearly defined in **Figure 11**(B).

The volatile compounds in tea samples (Earl Grey and oolong) were extracted by HS-SPME followed by desorption analysis with GC-MS. The fraction compounds of Earl Grey and oolong samples extracted were listed in **Table 1-5** and followed by chromatograms obtained were shown in **Figure 12, 13, 14, 15** and **16** respectively.

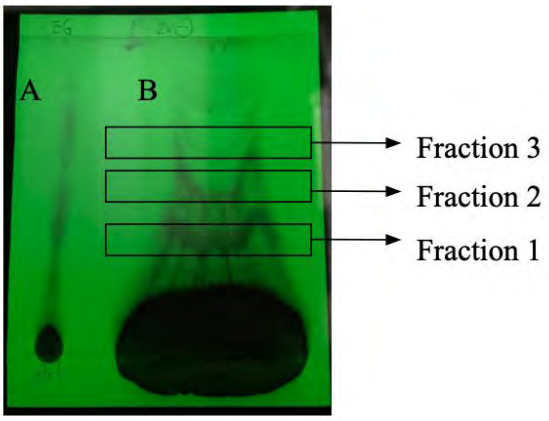


**Figure 11**. TLC plate of an Earl Grey sample with (A) Control Sample and (B) Three fractions obtained after electrolysis on TLC plates of Earl Grey sample at an optimized condition, 2V, 1A for 2 minutes.

- Earl Grey tea

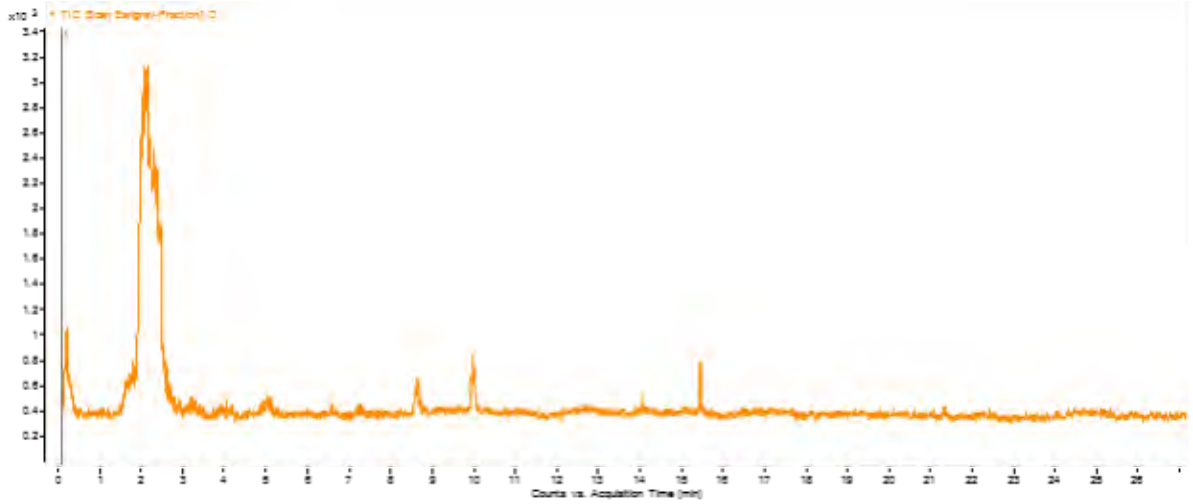

**Figure 12.** Chromatogram of compounds detected in fraction 1 of Earl grey sample.

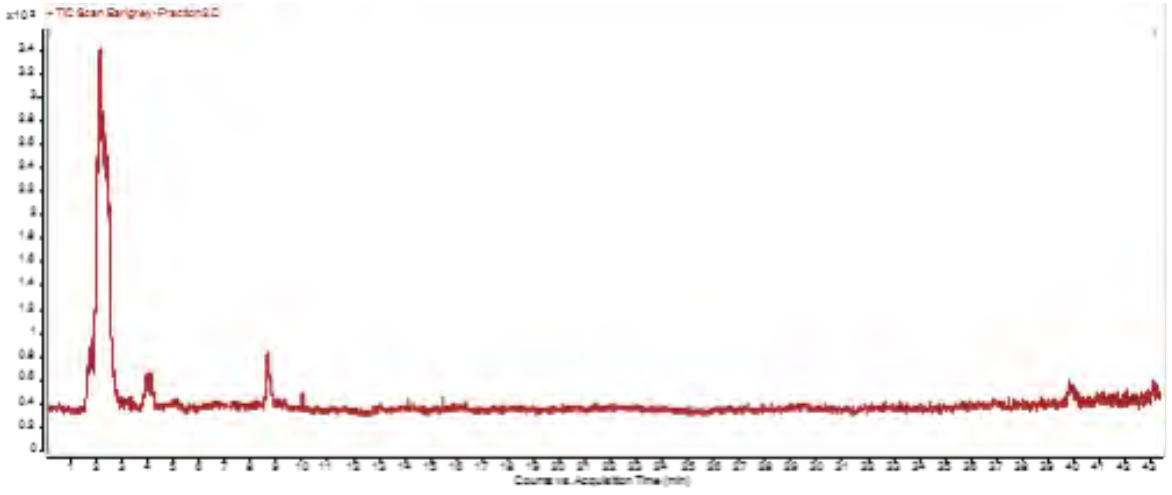

**Figure 13**. Chromatogram of compounds detected in fraction 2 of Earl grey sample.

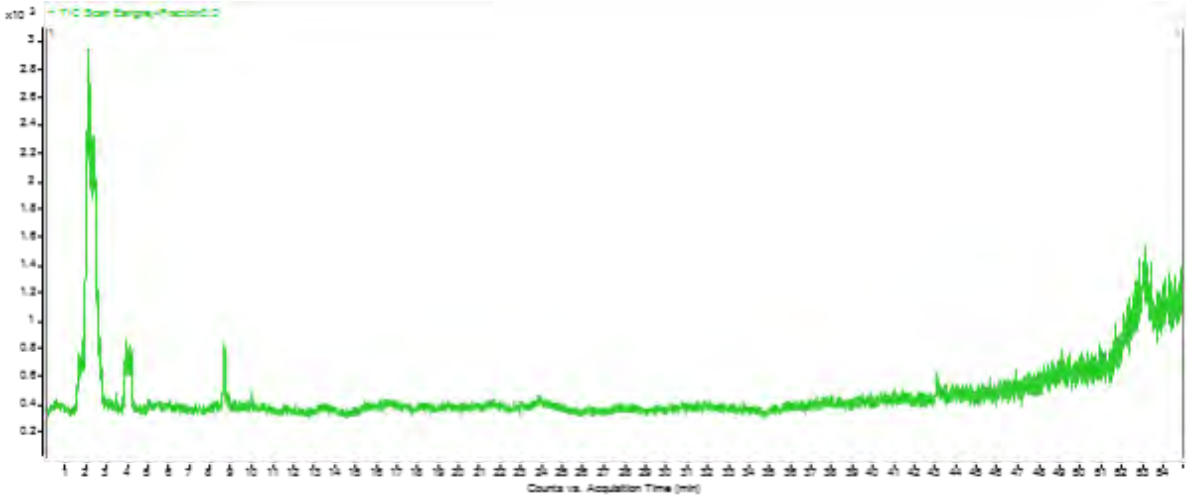

**Figure 14.** Chromatogram of compounds detected in fraction 3 of Earl grey sample.

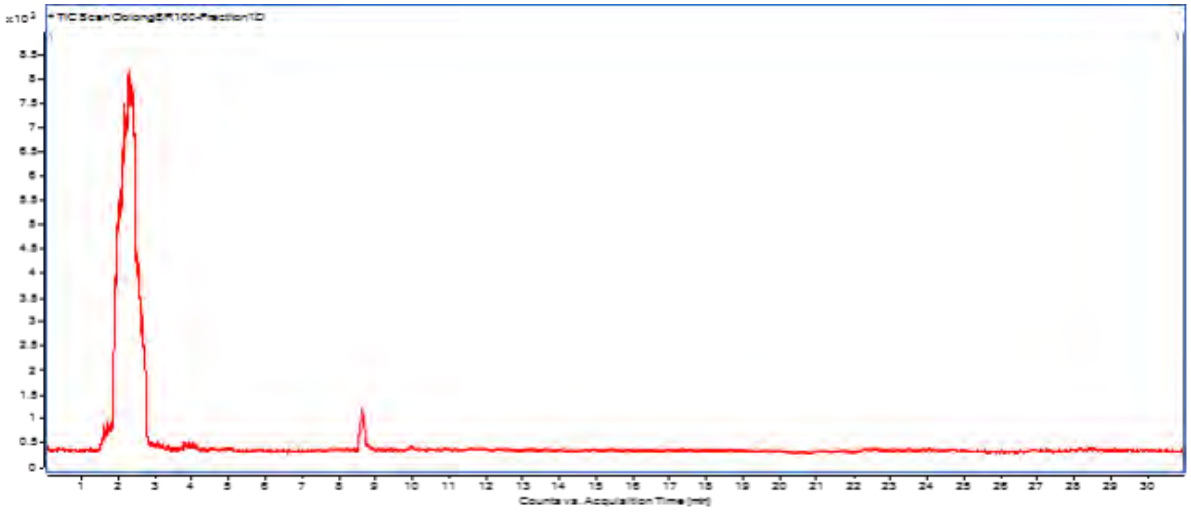

**Figure 15.** Chromatogram of compounds detected in fraction1 of Oolong tea sample.

**Figure 16**. Chromatogram of compounds detected in fraction 2 of Oolong tea sample.

Although a large volume, 1mL, of each tea sample was used in the experiment, only few compounds were detected by GC-MS from the analyzed fractions. In

addition, the detected compounds were only present in a minimal amount and therefore identified compounds are estimated. According to a study conducted by Suchatanugal et al. in 2020, a similar method was applied to analyze chemical composition in perfume samples using TLC techniques developed [42]. In the study, samples of perfumes were loaded onto TLC plates and further analyzed with HS-SPME/GC-MS [42]. However, over 60 compounds were detected [42]. This suggests that chemical compounds in tea mixtures are more prone to vaporization and oxidation from application of electrochemical reactions in comparison to perfumes.

### *3.4.2) Analysis of Volatile Products from Electrolysis with Spring Coil Electrodes*

As seen from **Figure 17** and **18,** various compounds undergo changes after treatment with electric current in **Table 3** with **Table 6**. GC-MS analysis revealed decrease in peak area of several compounds such as Nerol acetate, eventually many compounds were entirely degraded and could not be detected at all after electrolysis, for instance, β-Myrcene, γ-Terpinene, Nonanoic acid, 9-oxo-, methyl ester, and 2-Methylbutyl octanoate in Earl grey sample. However, some compounds showed a rise in peak area including Benzaldehyde in Earl grey tea and Dodecane, Tridecane, Tetradecane, Pentadecane, and Heptadecane in Oolong tea samples. Others completely changed into new chemical components for example, 2-Cyclohexane-1-ol, 1-methyl-4-(1-methylethyl)-,cis- in Earl grey tea transformed into linalool and Buprofezin into Benzaldehyde in Oolong tea sample.
This indicates that the chemical composition of teas undergo degradation and formation, resulting in decrease or increase in existing compounds and even origination of new ones. The mechanism behind this incident is electrochemical reactions, involving electrolysis and electrocoagulation. Electrolysis causes production of hydrogen gas at the cathode and removal of water from the system [43]. While electrocoagulation, hydrogen produced at the cathode and metal ions from corroded spring coil at the anode bind with the hydroxide groups, contributing to chemical change in components of tea as atoms and ions are interchanged by the removal or addition of electrons from the external circuit [43,44]. In short, electrochemical reactions take place at the interface of electrodes, in this case, a spring coil, causes interference with electrolytes [45]. Electricity causes movements of electrons from one element to another as a part of redox reaction, also known as oxidation-reduction [45]. Most importantly, oxidation is known to have an impact on formation and degradation of compounds thereby, causing change in chemical profile of the samples[6].

**Table 1.** Compounds detected in untreated Earl Grey tea sample (control).

| Earlgrey-Ctrl-ColiEC | | | | | | |
|---|---|---|---|---|---|---|
| Name | Time | Retention index | | | Match | R Match |
| | | Literature | Area | Area% | | |
| Benzaldehyde | 8.68 | 962 | 38683 | 0.031 | 666 | 758 |
| β-Myrcene | 9.644 | 991 | 116640 | 0.094 | 778 | 858 |
| 2-Carene | 10.418 | 1001 | 29219 | 0.024 | 622 | 724 |
| trans-β-Ocimene | 11.2 | 1049 | 50087 | 0.040 | 709 | 799 |
| 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- | 11.559 | 1038 | 91255 | 0.074 | 776 | 811 |
| γ-Terpinene | 11.888 | 1066 | 40731 | 0.033 | 672 | 679 |
| cis-Linalool oxide | 12.366 | 1074 | 228107 | 0.184 | 805 | 831 |
| Linalool oxide | 12.913 | 1074 | 417290 | 0.336 | 728 | 799 |
| Linalool | 13.743 | 1099 | 106000280 | 85.413 | 917 | 917 |
| 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylet | 14.167 | 1122 | 37759 | 0.030 | 633 | 702 |
| cis-Verbenol | 14.474 | 1142 | 91731 | 0.074 | 655 | 705 |
| 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylet | 14.736 | 1122 | 22147 | 0.018 | 619 | 665 |
| Camphor | 14.87 | 1145 | 27940 | 0.023 | 689 | 756 |
| β-Pinene oxide | 14.995 | 1156 | 5578 | 0.004 | 508 | 575 |
| Nerol oxide | 15.251 | 1153 | 30239 | 0.024 | 736 | 768 |
| Tricyclo[2.2.1.0(2,6)]heptan-3-ol, 4,5,5-trim | 15.624 | 1149 | 17307 | 0.014 | 545 | 644 |
| Epoxylinalol | 15.749 | 1178 | 7886 | 0.006 | 673 | 760 |
| Terpinen-4-ol | 16.027 | 1177 | 405134 | 0.326 | 886 | 913 |
| α-Terpineol | 16.561 | 1189 | 4256793 | 3.430 | 883 | 903 |
| 2-Cyclohexen-1-ol, 3-methyl-6-(1-methylet | 17.098 | 1208 | 27852 | 0.022 | 587 | 693 |
| cis-Carveol | 17.489 | 1229 | 17548 | 0.014 | 585 | 663 |
| Citronellol | 17.842 | 1228 | 1511934 | 1.218 | 812 | 813 |
| Cyclohexanol, 2-methyl-5-(1-methylethen | 18.093 | 1226 | 24260 | 0.020 | 585 | 701 |
| D-Carvone | 18.297 | 1246 | 25085 | 0.020 | 639 | 677 |
| Geraniol | 18.833 | 1255 | 8274151 | 6.667 | 834 | 835 |
| Citral | 19.155 | 1276 | 32537 | 0.026 | 615 | 695 |
| Isopulegol acetate | 19.617 | 1285 | 55110 | 0.044 | 557 | 742 |
| Dihydroedulan | 20.01 | 1293 | 29868 | 0.024 | 591 | 614 |
| (-)-8-p-Menthen-2-yl, acetate, trans | 20.219 | 1300 | 14430 | 0.012 | 540 | 584 |
| trans-Carveol, O-(trifluoroacetyl)- | 20.709 | 1316 | 22866 | 0.018 | 576 | 609 |
| 2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimeth | 21.533 | 1344 | 18722 | 0.015 | 629 | 670 |
| α-Terpinyl acetate | 21.83 | 1350 | 21539 | 0.017 | 734 | 791 |
| Nerol acetate | 22.335 | 1364 | 475875 | 0.383 | 864 | 865 |
| Geranyl acetate | 22.956 | 1382 | 733906 | 0.591 | 880 | 883 |
| 3-Cyclohexene-1-methanol, 5-hydroxy-α,α | 23.226 | 1388 | 36260 | 0.029 | 491 | 563 |
| Tetradecane | 23.469 | 1400 | 13797 | 0.011 | 595 | 812 |
| 1,5-Dimethyl-1-vinyl-4-hexenyl butyrate | 23.825 | 1418 | 106804 | 0.086 | 739 | 838 |
| Linalyl butyrate | 24.717 | 1418 | 10924 | 0.009 | 632 | 702 |
| Nonanoic acid, 9-oxo-, methyl ester | 25.142 | 1436 | 369438 | 0.298 | 535 | 536 |
| 2-Methylbutyl octanoate | 25.342 | 1449 | 150854 | 0.122 | 606 | 660 |
| 2-Dodecenal, (E)- | 25.781 | 1468 | 21283 | 0.017 | 557 | 671 |
| Pentadecane | 26.565 | 1500 | 14194 | 0.011 | 686 | 799 |
| α-Terpinyl isovalerate | 26.927 | 1500 | 106294 | 0.086 | 649 | 656 |
| 2,2,4-Trimethyl-1,3-pentanediol diisobutyr | 29.412 | 1588 | 52664 | 0.042 | 745 | 768 |
| Heptadecane | 32.268 | 1700 | 11756 | 0.009 | 713 | 816 |
| Octadecane | 34.909 | 1800 | 8807 | 0.007 | 701 | 809 |

**Table 2**. Compounds detected in Earl Grey tea after five minutes treatment with 9V electrolysis and electrocoagulation.

| Earlgrey-After5min-ColiEC | | | | | | |
|---|---|---|---|---|---|---|
| Name | Time | Retention index | | | Match | R Match |
| | | Literature | Area | Area% | | |
| Benzaldehyde | 8.649 | 962 | 131507 | 0.194 | 628 | 691 |
| Cyclohexane, 1-methylene-4-(1-methylethenyl)- | 10.872 | 1026 | 37048 | 0.055 | 629 | 694 |
| Benzylamine | 11.221 | 1019 | 18232 | 0.027 | 483 | 569 |
| Santolina alcohol | 11.568 | 1038 | 35963 | 0.053 | 579 | 724 |
| trans-Sabinene hydrate | 12.158 | 1070 | 12339 | 0.018 | 599 | 689 |
| cis-Linaloloxide | 12.369 | 1066 | 199052 | 0.294 | 813 | 887 |
| Linalool oxide | 12.932 | 1074 | 296161 | 0.437 | 787 | 864 |
| Linalool | 13.639 | 1099 | 59358096 | 87.671 | 899 | 899 |
| Linalool | 14.118 | 1099 | 19617 | 0.029 | 626 | 707 |
| 1,2-Dihydrolinalool | 14.557 | 1120 | 1367007 | 2.019 | 888 | 889 |
| (E)-p-2-Menthen-1-ol | 14.732 | 1140 | 15123 | 0.022 | 629 | 716 |
| (+)-2-Bornanone | 14.847 | 1144 | 18289 | 0.027 | 696 | 775 |
| 1R,4R-p-Mentha-2,8-dien-1-ol | 15.001 | 1123 | 5663 | 0.008 | 574 | 665 |
| Isopulegol | 15.253 | 1146 | 6352 | 0.009 | 533 | 685 |
| 2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimet | 15.743 | 1178 | 23079 | 0.034 | 540 | 656 |
| Terpinen-4-ol | 16.018 | 1177 | 243556 | 0.360 | 855 | 877 |
| Benzenemethanol, α,α,4-trimethyl- | 16.34 | 1183 | 17400 | 0.026 | 701 | 726 |
| α-Terpineol | 16.53 | 1189 | 2077538 | 3.068 | 893 | 903 |
| trans-Carveol | 17.146 | 1217 | 24995 | 0.037 | 537 | 570 |
| trans-Carveol | 17.482 | 1217 | 13440 | 0.020 | 565 | 752 |
| cis-Geraniol | 17.816 | 1228 | 737122 | 1.089 | 813 | 822 |
| Neral | 18.209 | 1240 | 16521 | 0.024 | 578 | 712 |
| Neral | 18.777 | 1240 | 2665430 | 3.937 | 881 | 883 |
| α-Citral | 19.23 | 1270 | 18463 | 0.027 | 627 | 663 |
| Isopulegol acetate | 19.783 | 1285 | 3768 | 0.006 | 439 | 640 |
| Piperitenone | 21.534 | 1340 | 9772 | 0.014 | 451 | 586 |
| 8-Hydroxylinalool | 21.839 | 1361 | 4176 | 0.006 | 483 | 682 |
| Nerol acetate | 22.33 | 1364 | 36961 | 0.055 | 786 | 843 |
| 2,7-Octadiene-1,6-diol, 2,6-dimethyl- | 22.698 | 1361 | 7220 | 0.011 | 467 | 671 |
| Nerol acetate | 22.944 | 1364 | 58429 | 0.086 | 774 | 807 |
| Tetradecane | 23.476 | 1400 | 23604 | 0.035 | 500 | 838 |
| Linalyl butyrate | 23.822 | 1418 | 32526 | 0.048 | 700 | 801 |
| Pentadecane | 26.555 | 1500 | 12208 | 0.018 | 732 | 758 |
| trans-β-Terpinyl butanoate | 26.931 | 1514 | 50257 | 0.074 | 547 | 577 |
| 2-[1-Adamantyl]propan-2-ol | 27.769 | 1543 | 10546 | 0.016 | 517 | 707 |
| 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate | 29.414 | 1588 | 42327 | 0.063 | 684 | 731 |
| Heptadecane | 32.256 | 1700 | 12088 | 0.018 | 762 | 822 |
| Carbonic acid, tridecyl vinyl ester | 34.908 | 1804 | 6470 | 0.010 | 612 | 757 |

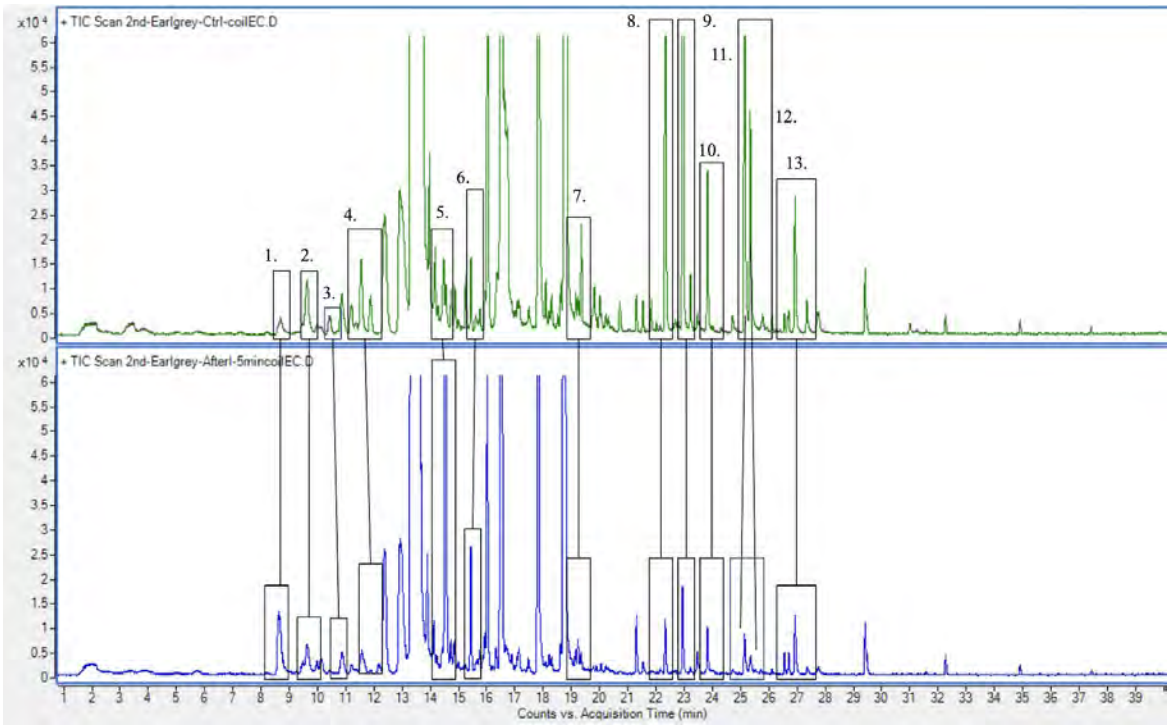


**Figure 17**. Comparison of chromatograms of Earl Grey tea sample before and after five minutes treatment with 9V, 1A electrolysis and electrocoagulation.

**Table 3.** Change in chemical components between Earl Grey samples before and after five minutes treatment with 9V electrolysis.

| No. | Compound | ILit* | Odor Description | % Area in control | % Area after electrolysis |
|---|---|---|---|---|---|
| 1 | Benzaldehyde | 962 | almond-like | 0.031 | 0.194 |
| 2 | β-Myrcene | 991 | woody, citrus fruity | 0.094 | - |
| 3 | 2-Carene | 1001 | flowery | 0.024 | - |
| 4 | γ-Terpinene | 1066 | citrus, terpentine-like | 0.033 | - |
| 5 | 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-,cis- | 1122 | N/A champhor-like | 0.030 | - |
| 6 | Epoxylinalol | 1178 | floral | 0.006 | 0.437 |
| 7 | Isopulegol acetate | 1285 | mentholic, woody | 0.044 | 0.006 |
| 8 | Nerol Acetate | 1364 | citrus, floral | 0.383 | 0.055 |
| 9 | Geranyl Acetate | 1382 | floral, fruity | 0.591 | - |
| 10 | Linalyl butyrate | 1418 | sweet, pear-like | 0.009 | 0.048 |
| 11 | Nonanoic acid, 9-oxo-, methyl ester | 1436 | rancid fruity | 0.298 | - |

| | | | | | |
|---|---|---|---|---|---|
| 12 | 2-Methylbutyl octanoate | 1449 | waxy | 0.122 | - |
| 13 | α-Terpinyl isovalerate | 1500 | floral-incense | 0.086 | - |

**Table 4.** Compounds detected in untreated Oolong tea sample (control).

| Name | Time | Retention index | | | Match | R Match |
|---|---|---|---|---|---|---|
| | | Literature | Area | Area% | | |
| Ethyl Acetate | 2.332 | 612 | 283185 | 18.145 | 691 | 790 |
| Toluene | 3.926 | 763 | 385457 | 24.698 | 879 | 884 |
| Carnegine | 4.959 | 1727 | 13276 | 0.851 | 678 | 749 |
| Linoleic acid ethyl ester | 5.719 | 2163 | 16389 | 1.050 | 578 | 596 |
| Pyridine, 2,4-dimethyl- | 7.199 | 930 | 11689 | 0.749 | 543 | 707 |
| Buprofezin | 8.65 | 2195 | 8333 | 0.534 | 487 | 557 |
| Acetic acid, chloro-, isobutyl ester | 9.474 | 976 | 10107 | 0.648 | 419 | 557 |
| 1-Aminoanthraquinone, N-trimethylsilyl- | 9.969 | 2452 | 60176 | 3.856 | 596 | 617 |
| cis-5,8,11,14-Eicosatetraenoic acid, picolinyl este | 10.609 | 3125 | 5024 | 0.322 | 558 | 582 |
| Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S) | 10.887 | 1031 | 8409 | 0.539 | 486 | 623 |
| Oxirane, phenyl- | 11.353 | 1057 | 5206 | 0.334 | 494 | 652 |
| 1H-Pyrrole-2-carboxaldehyde, 1-ethyl- | 11.537 | 1046 | 21312 | 1.366 | 596 | 721 |
| cis-Linalool oxide | 12.351 | 1074 | 24101 | 1.544 | 692 | 772 |
| Pyridine, 2-ethyl-4,6-dimethyl- | 12.563 | 1080 | 7841 | 0.502 | 570 | 685 |
| cis-Linalool oxide | 12.906 | 1074 | 22820 | 1.462 | 654 | 698 |
| Linalool | 13.325 | 1099 | 130966 | 8.392 | 779 | 782 |
| 1,5,7-Octatrien-3-ol, 3,7-dimethyl- | 13.472 | 1107 | 66960 | 4.291 | 819 | 882 |
| 2,4-Dimethylanisole | 13.756 | 1108 | 7514 | 0.481 | 609 | 692 |
| Anthranilic acid, 2TMS derivative | 14.078 | 1612 | 6591 | 0.422 | 609 | 671 |
| Benzyl nitrile | 14.73 | 1144 | 5147 | 0.330 | 556 | 748 |
| Anthranilic acid, 2TMS derivative | 15.429 | 1612 | 126079 | 8.079 | 674 | 794 |
| Chlordiazepoxide | 15.925 | 2800 | 7421 | 0.476 | 604 | 634 |
| Methyl abietate | 16.197 | 2379 | 5968 | 0.382 | 564 | 565 |
| α-Terpineol | 16.483 | 1189 | 2413 | 0.155 | 477 | 646 |
| Dodecane | 16.833 | 1200 | 26463 | 1.696 | 803 | 845 |
| 7-Octen-1-ol, 3,7-dimethyl-, (S)- | 17.011 | 1212 | 3174 | 0.203 | 373 | 615 |
| Folic Acid | 18.732 | 1387 | 9376 | 0.601 | 642 | 645 |
| Tridecane | 20.227 | 1300 | 30221 | 1.936 | 811 | 862 |
| Butanedioic acid | 21.307 | 1321 | 131991 | 8.457 | 458 | 535 |
| Androsta-1,4-dien-3-one, 17-hydroxy-17-methyl- | 22.602 | 2794 | 5926 | 0.380 | 496 | 549 |
| Tetradecane | 23.472 | 1400 | 35381 | 2.267 | 861 | 863 |
| Pentadecane | 26.557 | 1500 | 16658 | 1.067 | 791 | 844 |
| Pyridoxine | 26.712 | 1900 | 25946 | 1.663 | 576 | 639 |
| Hexadecane | 29.487 | 1600 | 18632 | 1.194 | 737 | 833 |
| Heptadecane | 32.268 | 1700 | 14505 | 0.929 | 747 | 807 |

**Table 5**. Compounds detected in Oolong tea after five minutes treatment with 9V electrolysis and electrocoagulation.

| Oolong-After5min-ColiEC | | | | | | |
|---|---|---|---|---|---|---|
| Name | Time | Retention index | | | Match | R Match |
| | | Literature | Area | Area% | | |
| Benzaldehyde | 8.68 | 962 | 31866 | 1.215 | 778 | 846 |
| 5-Hepten-2-one, 6-methyl- | 9.458 | 986 | 7712 | 0.294 | 494 | 514 |
| cis-Linalool oxide | 12.35 | 1074 | 14632 | 0.558 | 645 | 707 |
| cis-Linalool oxide | 12.901 | 1074 | 12476 | 0.476 | 634 | 743 |
| Linalool | 13.334 | 1099 | 160997 | 6.138 | 604 | 744 |
| Dodecane | 16.837 | 1200 | 179302 | 6.835 | 421 | 756 |
| Tridecane | 20.236 | 1300 | 329859 | 12.575 | 621 | 639 |
| Catechol | 21.3 | 1321 | 22843 | 0.871 | 613 | 641 |
| Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhex | 22.597 | 1373 | 4026 | 0.153 | 520 | 595 |
| Tetradecane | 23.496 | 1400 | 704470 | 26.856 | 601 | 693 |
| Pentadecane | 26.569 | 1500 | 460179 | 17.543 | 559 | 563 |
| Hexadecane | 29.495 | 1600 | 365970 | 13.952 | 626 | 721 |
| Heptadecane | 32.273 | 1700 | 255644 | 9.746 | 747 | 757 |
| Octadecane | 34.914 | 1800 | 73127 | 2.788 | 573 | 632 |

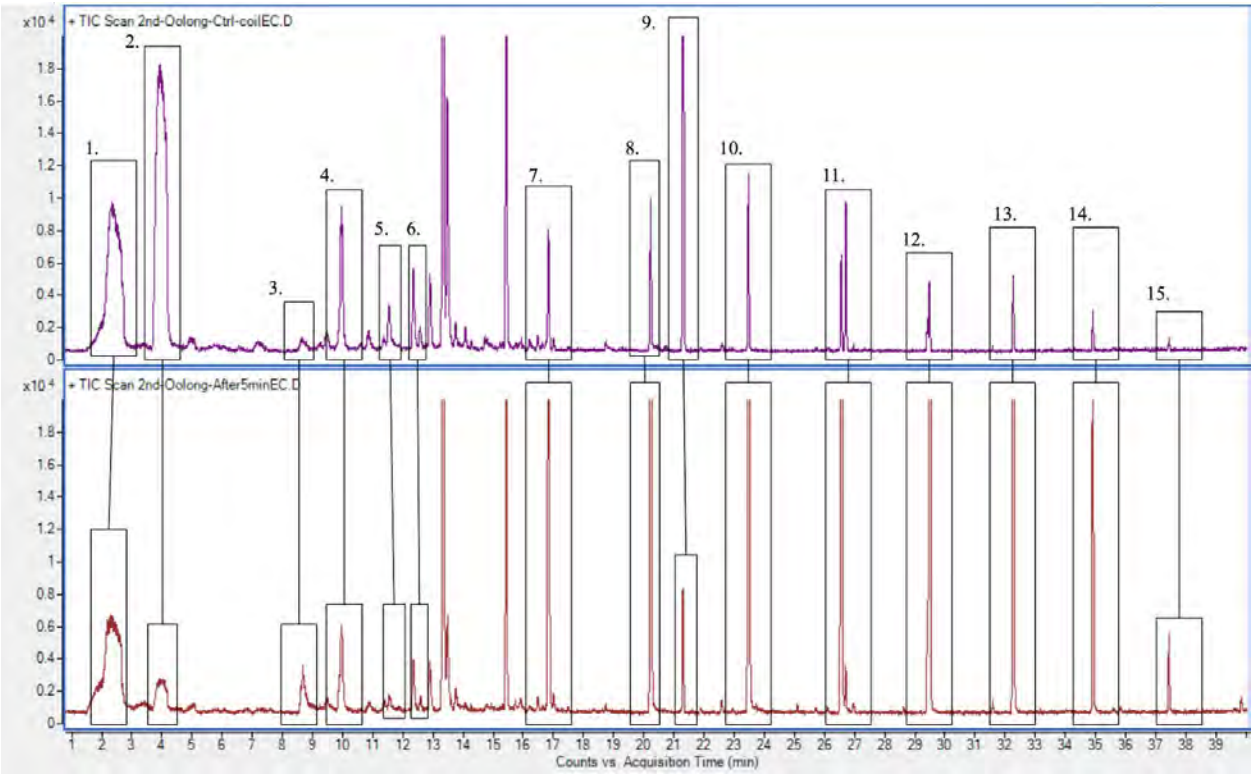


**Figure 18**. Comparison of Oolong tea sample before and after five minutes treatment with 9V electrolysis and electrocoagulation. Peaks **1** and **2** correspond to the solvents used as the TLC mobile phase.

**Table 6.** Change in chemical components between Oolong tea sample before and after five minutes treatment with 9V electrolysis and electrocoagulation.

| No. | Compound | ILit* | Odor Description | Area % | % Area after electrolysis |
|---|---|---|---|---|---|
| 1 | Benzaldehyde | 962 | almond-like | - | 1.215 |
| 2 | 1H-Pyrrole-2-carboxaldehyde | 1046 | N/A | 1.366 | - |
| 3 | cis-Linalool oxide | 1074 | woody, floral | 1.426 | 0.558 |
| 4 | Dodecane | 1200 | mild gasoline-like | 1.696 | 6.835 |
| 5 | Tridecane | 1300 | oil-like | - | 12.575 |
| 6 | Butanedioic acid | 1321 | odorless | 8.457 | - |
| 7 | Tetradecane | 1400 | mild waxy | 2.267 | 26.856 |
| 8 | Pentadecane | 1500 | woody, oil-like | 1.067 | 17.543 |
| 9 | Hexadecane | 1600 | mild gasoline-like | 1.194 | - |
| 10 | Heptadecane | 1700 | mild fuel-like | 0.929 | 9.747 |
| 11 | Octadecane | 1800 | N/A | - | 73.127 |

# Chapter 4
# Conclusion

Novel electrolysis on a TLC plate approach has been developed with the application successfully demonstrated for improved analysis of tea samples. With the capacitor design, the approach could also be performed either at the anode or the cathode without additional electrolyte. The method is also fast and simple by application of suitable conditions (*e.g.* using a 2V battery for 2min). After the on-TLC plate electrolysis, the tea fingerprinting can be improved allowing differentiation between the investigated teas that could not be achieved with the conventional TLC technique. The change of TLC fingerprint is also further confirmed by the change of volatile compound profiles after the electrolysis investigated by HS-SPME GC-MS. With further development, the established analytical concept and approach are expected to be very useful in the areas of improved TLC fingerprinting and antioxidant property analysis in the future.

# References

[1] Luczaj, W.; Skrzydlewska, E. Antioxidative properties of black tea. *Prev. Med*. **2005**, *40*, 910-918.

[2] Wang, K.; Liu, F.; Liu, Z.; Huang, J.; Xu, Z.; Li, Y.; Chen, J.; Gong, Y.; Yang, X. Comparison of catechins and volatile compounds among different types of tea using high performance liquid chromatography and gas chromatograph mass spectrometer. *Int. J. Food Sci. Technol*. **2011**, *46*, 1406-1412.

[3] Chan, P. T.; Fong, W. P.; Cheung, Y. L.; Huang, Y.; Ho, W. K. K.; Chen, Z. Y. Jasmine green tea epicatechins are hypolipidemic in hamsters fed a high-fat diet. *J. Nutr.* **1999**, *129*, 1094-1101.

[4] Chen, Z.; Zhu, Q. Y.; Tsang, D.; Huang, Y. Degradation of green tea catechins in tea drinks. J. Agric. Food Chem. **2001**, *49*, 477-482.

[5] Cheng, T. O. Review. All teas are not created equal: The Chinese green tea and cardiovascular health, *Int. J. Cardiol*. **2006**, *108*, 301-308.

[6] Tanaka, T.; Kouno, I. Oxidation of Tea Catechins: Chemical Structures and Reaction Mechanism. *Food Sci. Techol. Res.*, **2003**, *9*, 128-133

[7] Kim, Y.; Goodner, K. L.; Park, J. D.; Choi, J.; Talcott, S. T. Changes in antioxidant phytochemicals and volatile composition of Camellia Sinensis by oxidation during tea fermentation. *Food Chem*. **2011**, *129*, 1331-1342.

[8] Chemoprevention Branch & Agent Development Committee. Clinical development plan: Tea extracts, green tea polyphenols, epigallocatechin gallate. *J. Cell Biochem*. **1996**, *63*, 236–257.

[9] Zhang, Y.; Li, Q.; Xing, H.; Lu, X.; Zhao, L.; Qu, K.; Bi, K. Evaluation of antioxidant activity of ten compounds in different tea samples by means of an on-line HPLC- DPPH assay. *Food Res. Int.* **2013**, *53*, 847-856.

[10] Zeng, A. Z.; Yu, S. J.; Zhang, L.; Chen, X. D. The effects of AC electric field on wine maturation. *Innov Food Sci Emerg Technol*. **2008**, *9*, 463-468.

[11] Cserhati, T.; Forgas, E. Chromatography in food science and technology, 1st ed; CRC Press, 5-6.

[12] Yashin, A. Y.; Nemzer, B. V.; Combet, E.; Yashin, Y. I. Determination of the Chemical Composition of Tea by Chromatographic Methods: A Review. *Journal of Food Research*. **2015**,*4*, 56-87.

[13] Pripdeevech, P.; Wongpornchia, S. Odor and Flavor Volatiles of Different Types of Tea. *Tea in Health and Disease Prevention*. **2013**, 307-322.

[14] Vitzthum, O.G.; Werkhoff, P.; Hubert, P. New volatile constituents of black tea aroma. *J. Agric. Food Chem*. **1975**, *23*, 999-1003.

[15] Selvendran, R.R.; Reynolds, J.; Galliard, T. Production of volatiles by degradation of lipids during manufacture of black tea. *Phytochemistry*. **1978**, *17*, 233-236.

[16] Yamaguchi, K.; Shibamoto, T. Volatile constituents of green tea, Gyokuro (Camellia sinensis L. var. yabukita). *J. Agric. Food Chem*. **1981**, *29*, 366-370.

[17] Kawakami, M.; Ganguly, S.N.; Banerjee, J.; Kobayashi, A. Aroma composition of Oolong tea and black tea by brewed extraction method and characterizing compounds of Darjeeling tea aroma. *J. Agric. Food Chem.* **1995**, *43*, 200-207.

[18] Wu, C. D.; Wei, G. Tea as a functional food for oral health. *Nutrition* **2002**, *18*, 443-444.

[19] Cabrera, C.; Gimenez, R.; Lopez, M. C. Determination of tea components with antioxidant activity. *J Agric Food Chem*. **2003**, *51*, 4427-4435.

[20] Embola, C. W.; Sohn, O. S.; Fiala, E. S.; Weisburger, J. H. Induction of UDP-glucuronosyltransferase 1 (UDP-GT1) gene complex by green tea in male F344 rats. *Food Chem. Toxicol.* **2002**, *40*, 841-844.

[21] Feng, Q.; Kumagai, T.; Torii, Y.; Nakamura, Y.; Osawa, T.; Uchida, K. Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress. *Free Radical Res.* **2001**, *35*, 779- 788.

[22] Xie, M.; Von Bohlen, A.; Klockenka ¨mper, R.; Jian, X.; Gu ¨nther, K. Multielement analysis of Chinese tea (*Camellia sinensis*) by total-reflection X-ray fluorescence. *Z. Lebens. Unters. Forsch.* **1998**, *207*, 31-38.

[23] Amantana, A.; Santana-Rios, G.; Butler, J. A.; Xu, M. R.; Whanger, P. D.; Dashwood, R. H. Antimutagenic activity of selenium-enriched green tea toward the heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline. *Biol. Trace Elem. Res.* **2002**, *86*, 177-191.

[24] Kondo, T.; Ohta, T.; Igura, K.; Hara, Y.; Kaji, D. Tea catechins inhibit angiogenesis endothelial cell growth, migration *in* V*itro*, measured by human and tube formation through inhibition of VEGF receptor binding. *Cancer Lett.* **2002**, *180*, 139-144.

[25] Huang, Y.; Hung, Y.; Hsu, S.; Huang, Y.; & Hwang, D. Application of electrolyzed water in the food industry. *Food Control*. **2008**, *19*, 329-345.

[26] Dilarri, G.; Silva, V. L.; Pecora, H. B.; Montagnolli, R. N.; Corso, C. R.; Bidoia, E. D. Electrolytic treatment and biosurfactants applied to the conservation of Eugenia uniflora fruit. *Food Sci. Technol*. **2016**, *36*, 456-460.

[27] Pintaric, R.; Matela, J.; & Pintaric, S. Suitability of electrolyzed oxidizing water for the disinfection of hard surfaces and equipment in radiology. *J. Environ. Health Sci. Eng*. **2015**, *13*.

[28] Cravero, F.; Englezos, V.; Torchio F.; Giacos, S.; Segade, S. R.; Gerbi, V.; Rantsiou, K.; Rolle, L.; Cocolin, L. Post-harvest control of wine-grape mycobiota using elctrolyzed water. *Innov Food Sci Emerg Technol*. **2016**, *35*, 21-28.

[29] Smolinka, T.; Ojong, E. T.; Garche, J. Hydrogen production from renewable energies-electrolyzer technologies. *Electrochemical Energy Storage for Renewable Sources and Grid Balancing,* 1; Elsevier: Netherlands, 2015; 103-128.

[30] Millet, P.; Grigoview, S. Water electrolysis technologies. *Renewable Hydrogen Technologies*, 1; Elsevier: Netherlands, 2013; 19-41.

[31] Rahman, S.; Khan, I.; Oh, D. Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. *Compr. Rev. Food Sci. Food Saf*. **2016**, *15*.

[32] Wang, M.; Wang, Z.; Gong, X.; Guo, Z. The intensification technologies to water electrolysis for hydrogen production-a review. *Renew. Sust. Energ. Rev*. **2014**, *29*, 573-588.

[33] Sinha, A. K.; Sharma, U. K.; Sharma, N. A comprehensive review on vanilla flavor: extraction, isolation and quantification of vanillin and others constituents. *Int J Food Sci Nutr*, **2018**, *59*, 299-326.

[34] Esposito, L. J.; Kientz, F. G.; Maureaux, M. V.; Truchet, R. F. Vanillin. *Encyclopedia of Chemical Technology*, 5; Kirk-Othmer: John Wiley Sons, New York, 2000; *27*.

[35] Bartnik, M.; Facey, P. C.; Glycosides. *Pharmacognos*y; Academic Press: United States, 2017; 101-161.

[36] Germinario, G.; Garrappa, S.; D'Ambrosio, V.; van der Werf, I. D.; Sabbatini, L. Chemical composition of felt-tip pen inks. *Anal. Bioanal. Chem*. **2017**, *410*, 1079-1094.

[37] Wojtanowski, K. K.; Mroczek, T. Study of a complex secondary metabolites with potent anti-radical activity by two dimensional TLC/HPLC coupled to electrospray ionization time-of-flight mass spectrometry and bioautography. *Anal. Chim. Acta*. **2018**, *1029*, 104-115.

[38] Tranchida, P. Q.; Franchina, F. A.; Dugo, P.; Mondello, L. Comprehensive two-dimensional gas chromatography-mass spectrometry: recent evolution and current trends. *Mass Spectrom. Rev*. **2014**, *35*, 524-534.

[39] Gorecki, T.; Yu, X.; Pawliszyn, J. Theory of analyte extraction by selected porous polymer SPME fibres. *Analyst*. **1999**, *124*, 643-649.

[40] Qiu, F.; Norwood, D. L. Identification of pharmaceutical impurities. *J. Liq. Chromatogr. Relat. Technol*. **2007**, *30*, 877-935.

[41] Camarasu, C.; Madichie, C.; Williams, R. Recent progress in the determination of volatile impurities in pharmaceuticals. *TrAC-Trends Anal. Chem.* **2006**, *25*, 768-777.

[42] Suchatanugal, N.; Chedthanorrakul, P.; Thaveesangsakulthai, I.; Nhujak, C.; Kulsing, C. Thin Layer Chromatography Based Extraction Approaches for improved analysis of volatile compounds with Gas Chromatography- Mass Spectrometry and direct analysis with gas analyzer. *J. Sep. Sci.* **2020**, 1-24.

[43] Thaveesangsakulthai, I.; Wacharasindhu, S.; Vchirawongkwin, V.; Nhujak, T.; Chailapakul O.; Kulsing, C. Development of Electrocoagulation and detection approaches for manipulation of volatile compound profiles in organic medium supported using two phase electrolyte systems. *ChemSelect*. **2020**, *5*, 11630-11636.

[44] Gesser, H.D. *Applied Chemistry*; Springer: Berlin, 2002, 16-55.

[45] Atkins, P.; De Paula, J. *Physical Chemistry*. 9th Edition: Oxford, 2013; 865-869.

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