UNIVERSITY OF NAIROBI

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AN INVESTIGATION OF LOW TEMPERATURE PLASMA ENVIRONMENT EFFECT ON THE CONTENT OF POLYPHENOLS DURING MANUFACTURE OF KENYAN TEA

By

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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This thesis has been submitted for examination with our approval as university supervisors.

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Dr. Ayub A. Gitau, Ph.D

DEDICATION

To the Engineering Lecturers in the University of Nairobi who give knowledge to generations of
Kenyan youngsters & to my children, Bill Miranda, Noelle Nasimiyu Kuloba and Hezekiah
Walumbe Kuloba
God bless you all.

ACKNOWLEDGEMENT

ABSTRACT

Low Temperature Nitrogen Plasma (LTNP) was used to process green tealeaf to study its effect on the Polyphenol content in Made Tea. This was carried out using fresh green tea leaves that was collected on different days from the same tea farm. The green tealeaf was grown in the same climatic and soil conditions, cultivated under the same agronomical conditions and was of the same variety and maturity. It was handled from the farm up to the experimental site under similar conditions before it was worked on.

Using a Dielectric Barrier Discharge chamber to create the LTNP environment, seven samples of the green tealeaf were withered for various withering times ranging from 1 - 8 hours. The samples were macerated and dried before analyzing. The analyzed Made Tea samples indicated LTNP had an effect on Polyphenol content. Four constituent compounds forming the polyphenols were analyzed. From the various withering times, the highest polyphenol concentration of 78.56mg/g in Made Tea was achieved in one hour. The analyzed samples also indicated that polyphenol content in Made Tea was inversely proportional to the withering time.

To confirm the LTNP effect on the polyphenol content in the green tealeaf during withering, green tealeaf was also withered in non-plasma environments for comparison. The highest polyphenol content of 133.72mg/g was in a sample withered anaerobically in Nitrogen gas at room temperature and atmospheric pressure for eighteen hours. Another sample where green tea leaf was directly macerated and dried without any withering, no fermentation, had polyphenol content of 101.91mg/g of made tea. A green tealeaf sample withered aerobically, macerated, fermented and dried had polyphenol content of 21.23mg/g in Made Tea. Similarly as for LTNP withered green tealeaf, the polyphenol content also decreased with withering time. The analyzed samples also showed that withering green tea leaf in different conditions in overall resulted in different percentage composition of the constituent compounds and caffeine in the Made Tea. However in both cases the polyphenol content was higher than the currently processed teas available in the Kenyan market.

From the study, it was found that LTNP environment reduces polyphenol content in Made Tea during withering. It was also found that LTNP environment reduces polyphenols faster than

withering green tealeaf in nitrogen rich anaerobically. The decrease was caused by oxidation due to the presence of oxygen within the LTNP environment. The rate of reduction of polyphenols$\left(\frac{dP_c}{dt}\right)$ with withering time (t) follows an exponential function and approaches a limiting minimum rate. Similarly $\frac{dP_c}{dt}$ decreases exponentially with respect to the remaining polyphenol content (P_c) in the green tealeaf. Thus indicating that, the kinetic order of the polyphenol reduction changes with withering time and obeys the mass action law in line with the theoretical prediction. The relationship of P_c and t follows an inverse proportionality relationship. Statistical analysis using MS-Excel of the experimental data showed that the mean rate of change of P_c in green tealeaf withered in LTNP was 7.539mg/h. The model prediction equations for $\frac{dP_c}{dt}$ in green tealeaf against t, $\frac{dP_c}{dt}$ against P_c and P_c against t were determined. These model predictive equations can be used for the design and processing of green tealeaf in order to obtain Made Tea with specific polyphenol contents. In consideration of the chemical composition of Made Tea including polyphenol content, a choice can be made from these withering methods to manufacture Made Tea with specific percentage composition of polyphenols.

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List of abbreviations

LTP	Low Temperature Plasma
LTNP	Low Temperature Nitrogen Plasma
DBD	Dielectric Barrier Discharge
PP	Polyphenol content
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
ECG	Epicatechin gallate
GC	Gallocatechin
EC	Epicatechin
C	Catechin
KIRDI	Kenya Industrial Research and Development Institute
NACOSTI	National Commission for Science, Technology and Innovation

List of symbols

T	Temperature (^{o}C)
P	Pressure (Pa)
α	Ionization density
t	Withering time (H)
T_e	Temperature of the electron (K)
T_h	Temperature of heavy particles (K)
eV	Energies in Electron (eV)
K	Potassium
Ca	Calcium
Ph	Phosphorous
Mg	Magnesium
Mn	Manganese
Fe	Iron
S	Sulphur
Al	Aluminium
Na	Sodium
Si	Silicon
Zn	Zinc
Cu	Copper
O	Oxygen atom
$O_2{}^+$	Positive oxygen ion
O-	Negative oxygen ion
e	Electron charge, stat-coulomb
k_B	Boltzmann constant
N_2	Nitrogen molecule
N	Nitrogen atom
$N_2{}^+$	Positive nitrogen ion
O_2	Oxygen molecule

CHAPTER 1

1.0   INTRODUCTION

1.1   Background

Tea is a major cash crop and foreign exchange earner for Kenya. It is currently contributing between 17% and 20% of Kenya's total export revenue. Its export represents 26% of the total export earnings and about 4% of GDP [1].The export earnings for 2011 stood at 421 million kilograms worth $1.27 billion dollars. The cumulative local tea consumption in 2011 was about 20 million kilograms valued at $128 million, which went up by 7% from 2010, where 18.7 million kilograms of tea worth $119 million was consumed [2].

Three main types of tea products are manufactured from tea plant in countries where it is grown. They include Green tea, Black tea and Oolong tea. Tea plant was introduced in Kenya in 1903 by white settlers and in 2011 Kenya was the third-largest producer in the world (Appendix A) [3]. Small-scale farmers produce more than 80% of the total annual production while large-scale farms produced about 20% [1]. Tea is a tropical evergreen shrub of the *Camellia Sinnesis* species [2]. Two young leaves and a bud are harvested and processed into a finished product or *Made Tea* (Ready for brewing tea beverage) [4]. Brewed tea is a popular beverage and is one of the most commonly consumed all over the world [3]. The demand for tea has continued to increase and its popularity among other beverages is attributed to the many human health-beneficial effects associated with it [4]. The increasing consumption   has resulted in numerous studies on its production and value addition all over the world where it is grown.

1.2   Tea processing

There are different methods of manufacturing *Made Tea.* However, the procedure of manufacture follows the stages shown in Figure 1.1, i.e. withering, maceration, fermentation and

drying stages [5]. Nevertheless different types and forms of **Made Tea** can be manufactured by skipping some of the stages of figure 1.1 [5]. For example *Green Tea* is manufactured by skipping fermentation and withering stages while *Black Tea* is manufactured by following all the stages. The others are *Oolong Tea*, *GABA Tea* etc.

Green Leaf in → | Withering | → | Maceration/ Rolling | → | Fermentation | → | Drying | → *Made Tea*
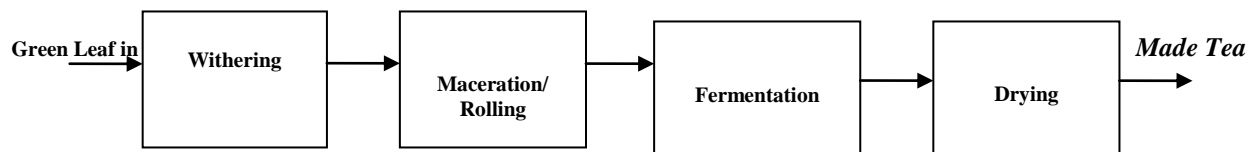
Figure 1.1   **A schematic diagram of tea processing stages**

Studies have shown that the procedure followed during the manufacture of the different types and forms of tea determines the final chemical composition hence quality of **Made Tea** [6].

Some of the key compounds found in tea and which are destroyed by processing are the *polyphenol* **[6]**. Numerous studies have shown that *Polyphenols* are important compounds for nutritional and pharmacotherapeutic benefits to human health [6 - 8]. They are listed as some of the compounds among others used in the mitigation and treatment against cancer by the American Cancer Society [9]. Among the types of tea mentioned above, *Black Tea* has the least percentage composition of *Polyphenols* while *Green Tea* has the highest [4]. This is due to the complete oxidation process in *Black Tea* manufacture that occurs during the withering and fermentation stages leading to formation of other compounds [5].

Since its introduction into date, Kenya has majorly been manufacturing *Black Tea* and minimal quantities of *Oolong* and *Green teas* depending on the customer request [1]. By majoring in *Black Tea* manufacture, the quality of Kenyan **Made Tea** has largely remained the same over the years [10]. For this reason the situation calls for efforts to diversify production of **Made Teas** to different types and forms of different other than *Black Tea*. One way in which further value

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addition can be made is to produce tea with enhanced human health benefits [4] so as to increase the sales and consumption especially in the local market. To achieve this prospect, this study looks at the *polyphenols* in the green tea leaf with a view to retaining its content to the maximum in **Made Tea**. This is because *polyphenols* undergo biochemical reactions in the green tealeaf from the time it is plucked and processed throughout the manufacturing stages shown in Figure 1.1 [5]. In the process, they are destroyed [6]. Though the destruction occurs in all the stages, this study particularly focuses on the **withering** stage [4]. *Polyphenols* occur naturally in tealeaf but are partially destroyed at **withering** stage if the environment is not conducive [5].

Green tealeaf **withering** process is currently done by either using aerobic or anaerobic environments to allow physical and chemical changes to take place [5, 6]. *Polyphenols* are destroyed by **withering** green tealeaf in aerobic environment. And to optimize their content in **Made Tea,** green tealeaf is withered in anaerobic environments. One area of technological advancement with a potential for creating and controlling the **withering** environment hence *polyphenols* in **Made Tea,** is Plasma technology. Plasma technology has been referred to as "one of the key technologies mankind can rely on for innovative long-term solutions in almost all fields of science and engineering" [10]. Plasma can be created in various types and forms with specific characteristics and behaviours to suit particular uses and **Low Temperature Plasma** (LTP) [9 -13] is one such type.

1.3   Statement of the problem

The withering of tea is a major unit operation in *Black Tea* processing in Kenya. It is done to reduce moisture and break down some complex organic compounds including *polyphenols* in the green tealeaf. *Polyphenols* occur naturally in green tea leaves. Their breakdown during **withering** is undesirable as their presence in **Made Tea** is beneficial to human health. Green tealeaf **withering** process as currently practiced in Kenya, is done in an aerobic environment which leads to breakdown of *polyphenols* thereby reducing their content in **Made Tea**. However **withering** of green tealeaf in anaerobic environment leads to increased polyphenol content in **Made Tea [6]**, which is desirable. Low Temperature Plasma (LTP) environment is known to

form a sheath around solid particles [12] and thus would create an anaerobic environment around green tealeaf that would be suitable for *withering* to optimize the *polyphenol* content. ***Made Tea*** with maximized polyphenols is of better economic value to both the farmer and the country at large as it would fetch higher prices than the current *Black Tea*.

Polyphenol breakdown is caused by an oxidation reaction due to the presence of oxygen in the *withering* environment. The presence of oxygen during *withering* of green tea leaves comes from air when it is used for *withering* as currently being done in Kenya. It also comes from respiration and transpiration of the green tea leaves as a result of metabolic processes [6]. Therefore, *Polyphenol* reduction in ***Made Tea*** at *withering* stage is the problem this study intends to improve so as to maximize its content in ***Made Tea***. The study looks at using LTP technology to create a suitable *withering* environment essential for reducing *polyphenol* oxidation and maximizing its content in ***Made Tea***.

1.4   Justification

Numerous studies have found that *Polyphenols* are beneficial to human health [14] for mitigation and treatment of cancer, diabetes, cardiovascular diseases, high blood pressure etc. Hence their availability in ***Made Tea*** in sufficient amounts can greatly assist in the mitigation and treatment of the above health problems currently affecting many Kenyans. Since *Polyphenols* occur naturally in green tea leaf, an appropriate *withering* technology can produce ***Made Tea*** with maximized content. Given that tea is a common beverage used by all people in Kenya, of all ages, rich and poor it would be an opportune time to avail ***Made Tea*** with maximized *polyphenol* content to help the increasing number of Kenyans with the above ailments. It would also be worthwhile to undertake research on a readily available health beneficial drink for posterity.

1.5   Objectives

The broad objective of this study was to investigate the effect of low temperature nitrogen plasma environment on *Polyphenols* in Kenyan **Made Tea** manufacturing.

The specific objectives of the study were:
1. To identify the physical parameters which are pertinent to the reduction of *polyphenols* in **Made Tea**.
2. Using the parameters identified in (1) above and mathematical modeling, to arrive at the predictive equation for the reduction of *polyphenols* in Made tea.
3. Using physical experiments to verify the equation in (2) above.

CHAPTER 2

2.0   LITERATURE REVIEW

2.1   Tea

Tea plant is a tropical evergreen shrub from *Camellia sinensis* species [6]. It is grown in regions with tropical climate. In Kenya it is grown in the Western and Eastern Highlands of the Rift Valley. Its young two leaves and a bud are used for the manufacture of **Made Tea** [4]. Tea is popular in Kenya and is taken by most people on daily basis. It is found virtually in every hotel in Kenya and in most homes where it is used for breakfast and as welcoming refreshment for visitors. It is also taken in offices during tea break in the morning and afternoon hours.

The plant (tea) contains a variety of elements and compounds [6]. In view of the varietal elements and compounds contained in it, the chemical composition of the tealeaf is of much interest. Tea is known to have beneficial effect to human health due these elements and compounds [14, 15]. Numerous reactions are known to take place from the time the leaf is plucked from the mother bush, ruptured and its cells exposed to oxygen or not and then dried. The reactions result in the breakdown of complex organic compounds into simpler ones [7] and the research efforts being made are based on influencing these reactions. Studies done on Kenyan teas have shown it has significant levels of the un-oxidized chemical constituents associated with human-health [7].

Tealeaf chemistry as shown in Tables 2.1 to 2.3 indicates a variety of elements and compounds [6]. The different elements and compounds are interrelated in chemical reaction during processing [6]. Inorganic components mainly found in salts in the cell sap are K, Ca, Ph, Mg, Mn, Fe, S, Al, Na, Si, Zn, and Cu. Also occurring in tea leaf are Nitrogen compounds in which three-quarters of it is in the form of amino acids and the rest comes from the alkaloid caffeine. The leaf also contains carbohydrates and vitamins B and C [6].

Table 2.1: Nitrogen Compounds in tea leaf

Compound	Dry weight % composition
Total nitrogen	4.50
Soluble protein and amino-acid nitrogen	0.92
Insoluble nitrogen	2.51
Caffeine nitrogen	1.07
Caffeine	3.71

Table 2.2: Carbohydrates and associated Compounds in tea leaf

Compounds	Dry weight % composition
Sugars	0.73-1.41
Starch	0.82-2.96
Pectins	6.10

Table 2.3: Inorganic constituents in tea leaf

Constituent	Dry weight % composition
Potassium	1.760
Calcium	0.460
Phosphorous	0.320
Magnesium	0.220
Iron	0.150
Manganese	0.120
Sulphur	0.088
Aluminium	0.069
Sodium	0.030
Silicon	0.024
Zinc	0.003
Copper	0.002

However the major compounds in tealeaf of interest in this study are *polyphenol* and *caffeine*. Others though not of interest in this study are amino acids, vitamins, flavonoids, polysaccharides and fluorine [6]. The percentage compositions of each of these elements and compounds including *polyphenols* in **Made Tea** depend on the effect of several factors. These factors range from differences in tea varieties and clones, climatic and soil conditions where it is grown, methods of agronomical practices and technology used in its processing [7]. All these factors affect the quality of **Made Tea**.

2.2   Tea processing methods

Currently most **Made Teas** on the global market are produced using any one of the following primary processing methods as indicated in Figure 2.1[1].

2.2.1 Black Tea

Green tealeaf is withered, macerated, fermented and dried as shown in Figure 1.1. The manufacture of Black Tea starts with withering green tea leaves where air of temperature up to 38^oC is blown through green tealeaf spread over *withering* racks (beds). Withering of the green tealeaf occur by removing the moisture and eventually reducing its content by about 3% over a period of (18-24 hours) depending on the prevailing weather conditions [6, 7]. After *withering*, green tealeaf is macerated and subjected to a complete oxidation process during the fermentation stage [5] after which it is dried.

2.2.2 Green tea

Green Tea manufacturing is done by first steaming green tea leaf to inactivate enzymes rendering them free from metabolic processes. After which the leaf is macerated and dried skipping the *withering* and fermentation stages [5].

2.2.3 Oolong tea

In Oolong tea manufacturing, withered green tea leaves are rolled in open air using specialized machines after which they are air dried. Fermentation occurs during the rolling and drying in air [4, 5].

2.2.4 Purple teas

It gets its name from the colour of the leaves [7] which are purple in colour. Purple Tea can be processed using any of the above methods to make *Made Tea*.

2.2.5 Special teas

One of the teas under this group is GABA Tea. It is processed by *withering* green tea leaf in anaerobic conditions/environments [6] to enable the compound called Gamma Amino Butyric

Acid to be formed and optimized in the green tealeaf. *Polyphenols* also simultaneously react and optimized in **Made Tea** during this method.

Harvesting

(Green tea leaf)

Withering

Steaming   Maceration

Rolling   CTC

Maceration

                              Fermenting   Fermenting

Drying   Drying   Drying   Drying

Sorting   Sorting   Sorting   Sorting

Packaging   Packaging   Packaging   Packaging

Green Tea   Oolong Tea   Orthodox Black Tea   CTC Black Tea
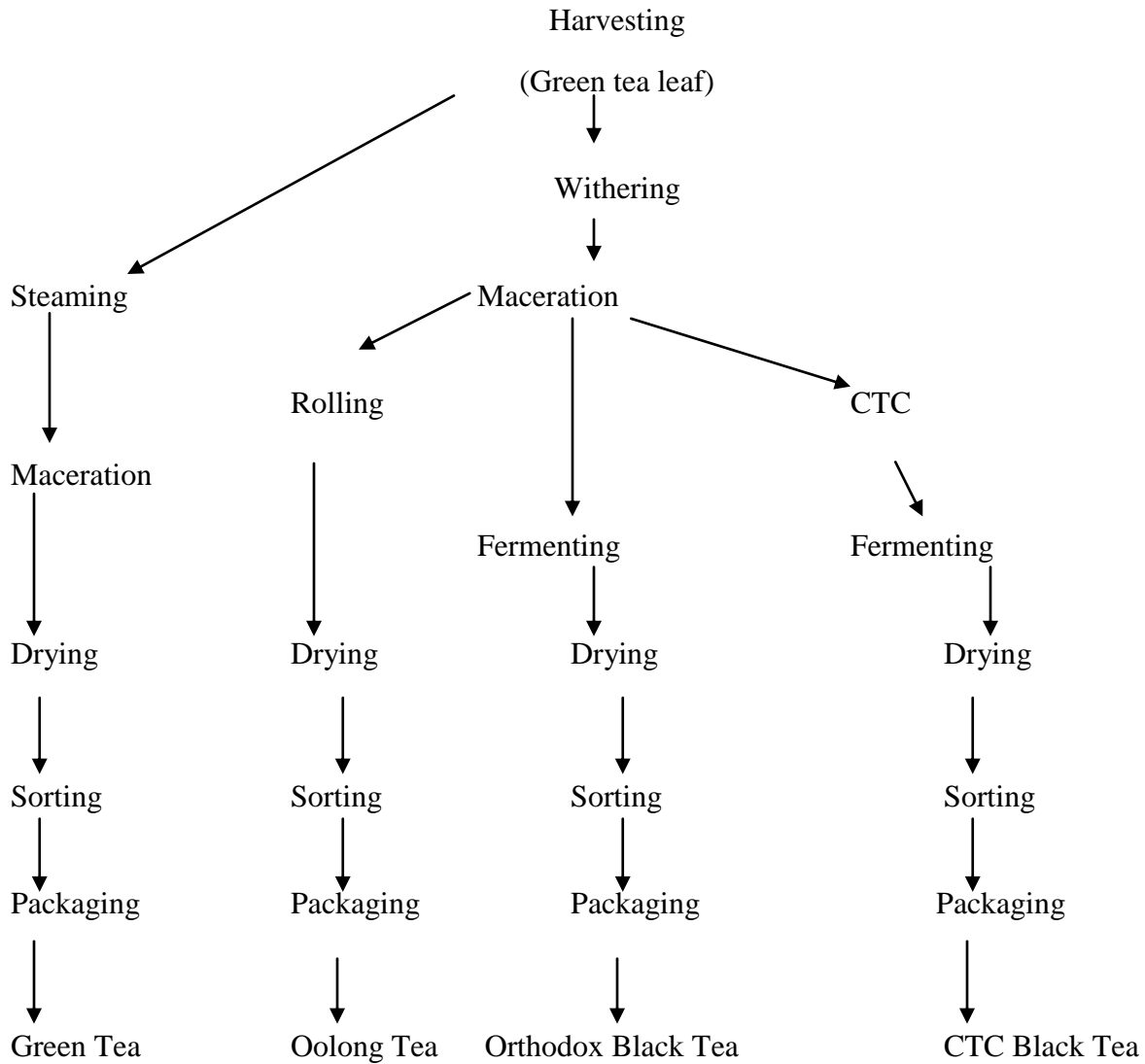
Figure 2.1   Schematic diagram of the technological methods used in tea processing to produce tea of various types [6]

From Figure 2.1, it can be seen that it is possible to manufacture sample *Made Teas* processed from the same pre-harvest and post-harvest factors to have different percentage composition of the elements and compounds due to the processing procedure followed in Figure 2.1above. As a result, can be seen from Figure 2.1, that the percentage composition of the elements and compounds in *Made Teas* depends majorly on the procedure used in processing [4, 5] including that of the polyphenols. From Figure 2.1, tea processing in general may be processed by following or not any of the following procedures namely; *withering* (optional), maceration or rolling, fermentation (optional) and drying (optional) as shown in figure 1.1. *Withering* may be the first stage in tea processing and the biochemical reactions that occur during this stage has a big impact on the quality of *Made Tea* [7].

2.3   Green tealeaf withering process

As mentioned in the introduction physical and chemical changes take place in the green tealeaf. The purpose of physical *withering* is to enable the green tea leaf to wilt by loosing moisture in order to prepare it for the next stage in the processing line [4]. While chemical *withering* is where chemicals in the green tea leaf undergo biochemical changes resulting in the breakdown of some naturally occurring compounds e.g. polyphenols leading to their destruction hence reduced in *Made Tea* **or** the synthesis of other compounds like Gamma Amino Butyric Acid [15]. However, one of the *withering* objectives is to breakdown some complex organic compounds to simpler ones with the aim of producing *Made Tea* of a particular type e.g. in the manufacture of Black Tea. In the process along with other simpler molecules, recombine to form compounds which contribute to the quality attributes of *Made Tea* [6].

Tea *withering* therefore may be used to produce black, Oolong and Gamma Amino Butyric Acid (GABA) teas among others based on the procedure followed together with the *withering* technology. In black and Oolong teas, green tea leaves are exposed to air at moderately low temperature of up to approximately $38^{\circ}C$ [6] to enable green tea leaves to wilt by loosing moisture to become placid. In so doing to a small extend *polyphenols* undergo oxidation resulting in the formation of other compounds [6].

While GABA tea is withered in nitrogen rich (anaerobic) environment [6] at room temperature to enable green tealeaf undergo non-oxidative reaction resulting in the synthesis of GABA from L-glutamate [15]. The breakdown of *Polyphenols* is minimal in this environment.

Chemical withering of green tea leaf on the other hand starts immediately after tea plucking. It is independent of the rate of loss of moisture and is a function of time and temperature [4, 6]. Although the desired moisture level may be reached in a few hours, the catabolic changes, which had been initiated at the time of plucking takes time. It is therefore necessary to give sufficient time for breakdown of large organic molecules to simpler structures [4, 6]. The current **withering** technologies used for producing tea with high levels of *polyphenols* take more than 12hours [6, 7]. And the chemical changes which occur among others during **withering** are [7]:

- Release of carbon dioxide and water due to break down of larger molecules e.g. *polyphenols.*
- Changes in enzyme activity e.g. phenylalanine ammonia-lyase necessary for reactions.
- Partial break down of proteins to amino acids which act as precursors for aroma.
- Increase in caffeine content which contributes to made tea briskness.
- Production of Volatile Flavour Components (VFC).

These chemical changes occur within the cells of the leaf, but the range and the extent of the reactions depend on environmental conditions in which the leaves are subjected to according to several studies [5].

During **withering** just as carbon dioxide in photosynthesis [16], nitrogen or oxygen are absorbed into the molecular cells of the leaf by virtue of changes in osmotic and partial pressure within the green leaves and diffuse into the intercellular spaces/sub-stomatal cavities of the leaves as governed by laws of diffusion i.e. by Fick's law and Graham's law. Once inside the leaf, its movement is governed by Henry's Law, nitrogen/oxygen diffuses from the intercellular air spaces to the *polyphenols* sites inside the cells.

Research carried out by Tsushida [17], found that a large amount of Gamma Amino Butyric Acid (GABA) and *Polyphenols* could be accumulated in the green tea leaves that had been plucked and withered in anaerobic conditions for some time [17]. Optimization of withering time during Black Tea manufacture has been done to determine the quantity of theaflavins which is one of the products of *Polyphenol* oxidation and the highest levels of the theaflavins (TFs) formation ($\geq 1.25\%$) was with the withering time of about 19-20 hrs [18].

Polyphenol optimized **Made Tea** is currently being produced in anaerobic and nitrogen rich environment, the current study investigates nitrogen gas provided in an ionized form i.e. nitrogen plasma state specifically at Low Temperature Plasma (LTP) to provide the **withering** environment. One of the reasons for using it in this form is that LTP is known to influence chemical and biochemical reactions because of its reactive nature [13].

2.4 Polyphenol occurrence in tea

Polyphenols occur in the tea plant as derivatives of Gallic acid (chemical formula: $C_6H_2(OH)_3COOH$) and catechins (chemical formula: $C_{15}H_{14}O_{64}H_2O$). They are therefore naturally found in plants including tea. Catechins are the building blocks of tannins in plants including tea plant. In green tealeaf they are usually referred to as *polyphenols* [19].

Polyphenols are a group of organic compounds and the six main ones found in green tea leaves are; Catechins (C), Epicatechin (EC), Epicatechin gallate (ECG), Epigallocatechin gallate (EGCG), Gallocatechin (GC), Epigallocatechin (EGC) [15]. Hence they are the component constituent compounds of polyphenols found naturally in tealeaf. The total quantity sum of all the six constituent compounds are what gives the total polyphenol content in the green tealeaf. Three of these compounds namely, Epicatechin, Epigallocatechin and the Epigallocatechin gallate which form the bulk of the *polyphenols,* shall be verified by experiments in this study. During tea processing *Polyphenols* are catalyzed by the enzymes *polyphenol* oxidase (PPO) and Peroxidase (PO) to undergo oxidation process in the presence of oxygen [15]. Recent research report done by scientists from India in 2012 [19] indicated that groups of *polyphenols* components react in pairs to form compounds called theaflavins.

Polyphenols are phenolic secondary metabolites which plays an important role in plant-derived food quality because they affect quality characteristics such as appearance, flavour and health-promoting properties [15]. Their phenolic content in foods including tea is affected by many factors that influence their stability, biosynthesis and degradation [6, 15]. For example during their biosynthesis, the key enzymes polyphenol oxidase (PPO), Peroxidase (PO) and phenylalanine ammonia-lyase can be influenced by different stress conditions (environmental) [6] e.g. oxidation environment or non-oxidation environment.

2.4.1 General factors affecting polyphenol content in *Made Tea*

Polyphenols are abundant micronutrients found in our diets and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is potent [5]. The health effects of polyphenols depend on the amount consumed and on their bioavailability [4] in foods. Hence the need for maximizing them in **Made Tea** is essential.

Polyphenols content in foods varies according to the following [4];

- Genetic
- Environmental
- Technologic factors

Genetic factors are based on the variety and clone of the plant species, environmental factors are climate and soils while technological factors includes, agronomical practices, handling & storage and processing. The processing factors include temperature, pressure, time, environmental conditions, etc.

Some of these factors can be controlled to optimize polyphenol content in foods. The reason being that processing has an effect on *polyphenol* content e.g. fruit peeling, de-hulling of legume seeds and removal of surface layer etc [4]. Tea processing as in the breakup of plant tissues like maceration may lead to degradation of *polyphenols* due the increased surface area leading to increased exposure to oxygen.

2.4.2 Reaction mechanism and model equation of *Polyphenol* in foods

The overwhelming majority of reactions that occur within plant cells are catalysed by enzymes [20]. Enzymes catalyze reactions by binding the reactants (enzyme substrates) and facilitating their conversion to the reaction products. Enzyme catalysis reduces the energy barrier associated with the reaction. Consequently, while enzyme catalysis increases the rate at which equilibrium is attained, it has no effect on the equilibrium itself.

Enzymes typically bind only a single substrate species and most catalyze only a specific reaction. This specificity of action allows each enzyme to function with remarkable efficiency increasing reaction rates by as much as 10^7 times [20].

Experimental observations of enzyme-catalysed reactions show that they do not obey mass action rate laws, which says *that the rate of reaction of reactants is directly proportional to concentration of the reactants* [20]. In contrast with mass action, the kinetic order of enzyme driven reaction changes as the substrate concentration varies. Most enzyme-catalysed reactions involve more than one substrate. In order to describe enzyme catalysis of these reactions, Michaelis-Menten kinetics description is expanded to suit the reaction mechanism [20]. Catalysis of the irreversible two-substrate reaction i.e. *polyphenols* and oxygen can be represented in a reaction equation as follows Equation 2.1,

Governing equation

$$\text{Rate of } A + B = P + Q = k_3 C_2 (t) \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

Where A and B   =   Substrates

     P and Q   =   Products

     k_3 =   Kinetic order of the reaction

     C_2 =   Product concentration

     t   =   Time taken

Using conservation of enzyme to substitute for the free enzyme concentration and applying a quasi-steady-state approximation to both complexes formed in the subsequent stages of the reaction, gives a reaction rate of Equation 2.1 to Equation 2.2 [20],

$$k_3 k_2(t) \;=\; \frac{k_3 e^T\, a\,(t)\, b\,(t)}{k_{-1}\frac{k_{-2}+k_3}{k_1 k_2}+\frac{k_{-2}+k_3}{k_2}\,a(t)+\frac{k_3}{k_1}\,b(t)+a(t)b(t)} \qquad \cdots\cdots\cdots\cdots\cdots \; (2.2)$$

$k_1 k_{-1}$ and $k_2 =$ Kinetic orders of the substrates

$\qquad e^T =$ Total enzyme concentration

This rate law is the compulsory order- enzyme-catalysed reaction type [20] and can be written as given in Equation 2.3. The reacting substrates content decreases as the products content increases.

$$A\,+\,B \longrightarrow P + Q \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; (2.3)$$
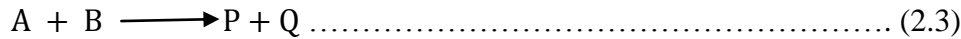
And can be written more concisely as equation 2.4 [22]

$$v \;\;=\;\; \frac{V_{max}ab}{k_{AB}\,+\,k_B a\,+\,k_A b + ab} \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ldots \; (2.4)$$

Equation 2.4 can be expressed as;

$$v \;\;=\;\; ma \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \; (2.5)$$

where $\quad m \;\;=\;\; \dfrac{V_{max}b}{k_{AB}\,+\,k_B a\,+\,k_A b + ab}$

$\qquad\qquad v \;\;=\;\;$ rate of reaction

$\qquad\qquad V_{max} \;=\;$ maximum rate of reaction

$\qquad\qquad a \;\;=\;\;$ concentration of substrate *Polyphenol*

$\qquad\qquad b \;\;=\;\;$ concentration of substrate Oxygen

$\qquad\qquad k_A \;\;=\;\;$ rate constant of substrate *polyphenol*

$\qquad\qquad k_B \;\;=\;\;$ rate constant of substrate oxygen

The equation to validate in the experiments is to whether the relationship between the rates of reaction (reduction) of *polyphenols* follows the mass action law or not i.e. if it follows the mass action, then the kinetic order of enzyme driven reaction changes as the *polyphenols* concentrations varies [20]. This is one of the propositions to be validated in the experiments among others. Transforming the data for clarity by natural log on both sides of equation 2.5 gives;

$$\text{Log } v = m \text{ Log a} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2.6)$$

And the trend line for the relationship of equation 2.6 is a straight line that can be verified by experiment.

2.5   Plasma

Plasma is known to be the fourth state of matter after solids, liquids and gases. It is sometimes referred to as all states. Plasma comprises of electrons, protons, neutrons, photons, positive and negative ions, neutral molecules and atoms and a variety of other particles all existing in the same environment and is formed by ionization of elements and gases among others [21, 22]. The interactions between these charged particles and the neutral particles within the same environment are important in determining the behavior and usefulness of the particular plasma [23].

A broad spectrum of plasma types, characteristics and behaviors can be created from different types of atoms in plasma, the ratio of ions to neutral particles and the particle energies. Their unique behaviors provide plasmas useful in a large and growing number of applications [23].

For example, its utilization in bio-systems, biochemical and biomedical engineering processes is playing a very vital role where conventional methods could not have been possible. In agricultural and food engineering sciences and design, plasma technology is gaining applications. As a result of this reason, plasma chemistry has in recent years been made part of the requirement for training Biosystems Engineering graduates in a number of universities in the

USA e.g. University of Wisconsin. Topics such as plasma enhanced reaction mechanisms, plasma diagnostics and plasma enhanced surface modification of inorganic and organic macromolecular structures and surfaces among others are now being taught [24].

2.5.1 Plasma state of matter

The existence in different physical and chemical properties yet in the same environment makes plasma possible to utilize its varietal properties for many functions. Due to these varieties of species, it is considered an energetic chemical environment that combines particles and radiations of a diverse nature not available in other states of matter. It is widely used in practice due to the following features [23]:

1. Temperatures and energy densities of some plasma components can exceed significantly those in conventional chemical technologies.

2. Plasma systems are able to produce high concentrations of energetic and chemically active species.

3. Plasma systems can essentially be far from thermodynamic equilibrium by providing high concentrations of the chemically active species and keeping the bulk temperatures as low as room temperature.

Plasma phase was discovered and identified as a fundamental state of matter in 1879 by Sir William Crookes, an English physicist. In 1929, Nobel Laureate Irving Langmuir [25] gave this state the name plasma. Plasma is overwhelmingly the dominant constituent of the universe as a whole and comprising more than 99% of the visible universe [25]. In daily life plasma which most people commonly know are the glows from fluorescent lights and plasma TVs.

Additionally, plasmas are conductive assemblies of charged particles, neutrals and fields that exhibit collective effects. Further, they carry electrical currents and generate magnetic fields but with insufficient sustaining power, the particles would recombine into neutral gas [26]. Plasma can be accelerated and steered by electric and magnetic fields which allows it to be controlled

and applied [26]. Research in the field of plasma is yielding a greater understanding of the universe and also provides many practical uses e.g. new manufacturing techniques, consumer products and the prospect of abundant energy [26].

2.5.2   Types of plasma

Plasma can be created in thermal and non-thermal states and further created at low and high temperatures, low and high atmospheric pressures, thermal and non thermal atmospheric plasma etc. Examples of various types of plasma in use are Thermal Plasma, Cold or Non-thermal Plasma, Low Temperature Plasma (LTP) [12, 13, 21, 27], Low Pressure Plasma (LPP), Solution Plasma Processing (SPP), Atmospheric Non Thermal Plasma (ANTP) [21, 28] etc. As a result of being created in many forms and types, plasma finds variety of uses in many fields of science and technology. Plasma temperatures and density, range from relatively cool and thin to very hot and dense [12].

They are further categorized as thermal and non-thermal plasmas [13, 21]. In thermal plasma T_e $\approx T_h$ (where T_e = electron temperature and T_h = heavy particle or sensible temperature) has core gas temperatures above 10,000 K and the gas is significantly ionized. Non-thermal plasmas on the other hand have very high electron temperatures T_e, while the sensible temperatures T_h remain ambient [21].

Non-thermal plasmas have also low degree of ionization hence have low density of charged species. The reason being that electrons and ions never achieve local thermodynamic equilibrium hence the gas as a whole is at room temperature [12, 21]. However, atmospheric non-thermal plasmas have a high density of activated species, i.e. reactive free radicals and excited state atoms and can induce a host of new chemical reactions due to the abundant production of radicals and excited state molecules [12, 21].

In Atmospheric Pressure Plasmas, the mean free paths between electrons and heavy particles are extremely short and therefore it is collision dominated [12, 21]. Under such conditions, local thermodynamic equilibrium (LTE) may prevail. Kinetic equilibrium i.e. $T_e \approx T_h$ as well as

chemical equilibrium (i.e. particle concentrations) in LTE are a function of temperature [12].

Therefore Atmospheric Non-Thermal Plasmas rely on high local electric fields that directly produce energetic electrons. These electrons influence material chemistry, even in the ambient collision dominated regime. The study supposes is because they do not lose much energy in elastic collisions due to their small mass. Instead, they bounce around and transfer most of their energy to molecules by dissociating, ionizing or exciting them. The excitation and radical production cause vast changes in reaction rates [28]. The study also showed that, compared to Low-Pressure Plasmas with longer mean free paths, collisions are much less frequent. Under these conditions, the electron temperature is much higher than the heavy particle temperatures, i.e. $T_e \gg T_h$. Although ionization in Low-Pressure Plasmas is very high, the gas density in this type of plasma is extremely low [28]. Therefore, thermal equilibrium cannot be achieved between electrons and heavy particles during collisions and as a result, the heavy gas particles remain cold after collisions [27].

Atmospheric-Pressure Plasmas have a wide variety of potential industrial applications [27]. They are used in extractive metallurgy, metal recovery, novel nano-material synthesis, refractory and wear resistant coating depositions, chemical synthesis, energy conversion, industrial, medical and nuclear waste destruction as well as engine combustion enhancement and clean up exhaust gas pollutants [27, 28].

2.5.3   Plasma applications

Amongst fields where useful applications have been made include, medical treatment especially in sterilization, surgery etc [22], material treatment/surface coating [13, 23, 26] and waste treatment namely decomposition of compounds containing NO_3, NH_3 or CN_x groups as an environmental management technique [29]. Others are catalytic reactions in chemical processes, bio-processes in agriculture and food as a non-chemical gas-phase disinfection agent [9], nanotechnology and biomaterials [10, 28].

Many products in our daily life depend on successful plasma processes, ranging from computers,

TVs to energy saving lamps. Plasmas are being used for the creation of nanostructures with unrivalled precision and for functional coatings to give products superior surface properties [30]. The newest development is the use of plasmas in life science for sterilization and for production of biocompatible medical devices and materials [30].

All these applications are realized to date, because plasmas are extremely versatile and can be adapted to many different tasks as earlier mentioned. This success story over the past few decades was only possible because many disciplines, ranging from physics and chemistry to engineering and biology are working together to complement each other. Plasma utilization in agricultural products and food processing has been confined on bacterial destruction, sterilization, sanitization and treatment [14].

2.5.4   Plasma technology

Plasma technology is widely used in various aspects of human life due to its effectiveness in areas where conventional methods have not worked. It affects our daily lives is in the form of processes and products. The main guiding factors for its increasing usage has been due to the need in high demand for product quality, productivity, environmental compatibility, precision and flexibility as the principal considerations [11]. Recommendations have been suggested to carry out research using plasma technology in other areas/fields of science [11- 13] because of its versatile nature.

Plasma technology utilization in biochemical and biomedical engineering processes is playing a very vital role where conventional methods could not have been possible [22]. The technology affects our daily lives in the form of processes and products [13]. A lot of research efforts are being made to utilize it in many fields of science and engineering. Other common applications include pretreatment for medical catheters, cardiovascular stents, syringe components, dialysis pump parts and plastic films for drug bags [31]. Plasma is also used to improve bondability on substrates, such as glass, polymers, ceramics and various metals [31].

2.6   The Role of Charge Carriers in plasma state of matter

Charge carriers in plasma are connected with a series of characteristics which are also important in industrial applications. The most active component of a non-thermal Low-Temperature Plasma (LTP) is the hot electron gas [21, 31]. Electrons have high mean kinetic energy and as a result generate electromagnetic radiation (lines and continua) [32]. They also cause production of numerous ionized, excited, and dissociated species of increased chemical activity. Furthermore, their existence exhibits the following among others [32]:

- Electrical conductivity
- Screening of electric fields,
- Multitude of oscillations and waves, typical for the plasma (Langmuir
- Oscillations, ion acoustic oscillations, cyclotron oscillations, drift and surface waves Alfven waves, etc.), as well as corresponding instabilities (plasma turbulence)
- Interaction with magnetic fields an important aspect of modern plasma physics.
- Formation of characteristic boundary sheaths due to the contact of plasmas with solid surfaces. This is of particular importance in the technology of plasma processing.

At ordinary electrical and thermal heating process, the energy levels of electrons are at a fraction of an electron volt. From Pauli Exclusion Principle [31], in this energy state the majority of the electrons cannot receive energy from electrical and thermal heating processes because there are no available energy states for them to occupy within a fraction of an electron volt. However by generating the electrons in plasma state, the majority of electrons are at higher electron volts Table 4, enabling them to take part in the chemical reaction.

2.7   Recent advances in plasma state of matter

In 2008 the American Department of Energy Office of Fusion Energy Sciences, recommended more work efforts in research on utilization of Low Temperature Plasmas (LTP) because of its enormous and vital application potential role. One of the challenges recommended for research

was how LTPs *interacts with organic materials and living tissue* since their exposure in plasma environment results in chemical and structural changes both on the surface and inside the material [12]. The interactions are said to be complex scientifically and can bring about complex scientific changes deep inside the bulk of the material [12]. Investigation on plasma interactions with organic materials and living tissue is said to have been largely empirical [12].

Many unknowns are said to be still unresolved, and range from the interaction mechanisms plasmas modify material structure and tissues, to in depth higher chemistry and physics of the specific plasma systems used for treating such biological materials [12]. From literature, due to large number of possible reactions between organic material and the plasma species, the scientific study of such problem is said to be challenging and depends on the specific organic material and plasma species used. While there has been some progress on the interaction of plasmas with organic materials, the study of plasma/living tissue interaction is said to be almost unexplored field [12]. This emphasis is what formed the basis of this study. Two areas where plasma and living tissue interaction have been exploited are categorized as destructive and nondestructive [14]. Destructive plasma is like sterilization of medical devices, surgery, etc. and non destructive treatment of wheat and oat seeds [32] to enhance their germination and early growth. Others are surface treatments with plasma of materials including bio-materials for implants have been accomplished.

A new area that is beginning to be explored is the use of LTP to modify cell/tissue surfaces [12]. In view of these, the current study explores the use of LTP for biochemical *withering* of green tea leaf which is itself a multicellular organic material. This investigation is essential for understanding LTP environment on living tissue organic materials like tea. Similarly, comprehensive models of LTP on the vast chemistries and reactions occurring within these materials and tissues need to be researched and understood.

Certain types of plasma can efficiently kill bacteria without harming the cells and also influence the cells without causing cell death [12]. As a result it has been used in agriculture and food as an effective, non-chemical, gas-phase disinfectant that can be applied as moderate or low temperature plasma [13].

Low temperature plasma has been used to study its influence on germination and early growth of wheat and oat seeds [32], which improved the former but didn't harm the living cells of the seed. Low temperature plasma has also been used for the treatment of a wool fabric in which the wool characteristics of wettability were changed [33]. Frank Denes, an associate professor of biological systems engineering at the College of Agricultural and Life Sciences University of Wisconsin USA, has previously stated that plasma species interact with inorganic and organic materials and change their structures. It has also been found that UV emissions in Low Temperature Plasma are negligible and that is why can be used for treatment of food staffs like the current study [32]. It has been used for the treatment of malignant melanoma cells [34].

Therefore given the variety of organic and inorganic chemical compounds and elements within the green tea leaf, their interaction in plasma environment is anticipated to result in breakdown of different complex compounds, increased rates of biochemical reactions ending in formation and conversions of new compounds. Research findings have found plasma while influencing biochemical processes is also known to offer new potential for selective application [8]. When used under careful control, has been possible to intensify certain reactions while other reactions are suppressed especially for oxygen potential and temperature [21]. Plasma surface treatment also assists in creating chemically active functional groups, such as amine, hydroxyl and carboxyl groups, to improve interfacial adhesion [21].

The action of electrons in breaking of bonds in chemical reactions has an impact on the rate of chemical reactions [35]. Plasma environment has increased density of electrons and is anticipated to enhance increased chemical reactions within the green tea leaf.

These and many more outstanding properties of plasma technology have been utilized for chemical reactions in controlled atmosphere of both organic and inorganic compounds. By selecting either of reducing or oxidizing conditions and the chemical reaction, plasma can be independently controlled as an energy source [35] to instigate certain reactions. These characteristics of plasma have been used successfully to achieve the following among others [31],

- Complete decomposition of chlorinated hydrocarbons, including decomposition

of compounds containing NO [13, 26].

- Reduction and recirculation of organic iodine in an incinerator.
- Destruction of municipal waste material derived from fuels by conversion into non-hazardous fuel gas and a non-leachable slag.
- Destruction of a waste product from the aluminium smelting industry called Spent Pot Lining (SPL).
- Selective reduction of metal oxides in a slag reduction furnace.

Non-thermal plasma (NTP) is widely used for industrial materials processing. This is largely due to new technologies which easily produce NTPs at normal atmospheric pressure. NTP was originally developed for use on delicate electronics, heat-sensitive medical devices and in biomedical and dental applications, but it is now being investigated for application to foods and beverages as a sanitizing and/or conditioning agent [12] which has recently shown promise as a sanitizing tool. The basis of these successes has been due to the fact that NTP injure and inactivate pathogenic bacteria on inert food and in liquids that are in contact with dirty surfaces. However the antimicrobial efficacy of NTP is related to the specific technology used, the power level used to generate the plasma, the gas mixture used in the plasma emitter and the intensity and length of exposure [12].

2.8   Low Temperature Plasma

It is non-thermal with electron temperature very high (10^5k) compared to the temperature of ions and neutral particles. In this study nitrogen gas was used ionized at a temperature of 300k, which is within the range of low temperature plasma [29]. The temperature of ions and neutral particles remains at room temperature (i.e. about 300k) as shown in table 2.2 [33]. The electrons do not share the energy gained from the electric field because collisions with heavier ions and neutral species are elastic due to their small masses [30].

However ions and neutral particles exchange energy due to their higher masses compared to electrons and their collisions are inelastic [12, 29]. Low temperature nitrogen plasma (LTP) is also a partially ionized state. It is a quasi-neutral particle system in the form of gaseous or fluid-

like mixtures of free electrons, ions and neutral particles (atoms, molecules) with a large mean kinetic energy of the electrons and plasma components of up to 0.2 eV to 2 MeV per particle [29]. The charge carriers and their electromagnetic interaction have a substantial influence on the LTP system properties as a whole. The interactions between the plasma electric charge components show two aspects [29]:

- Coulomb interaction among the charge carriers. Where owing to the long range of the Coulomb force in the case of large charge-carrier densities, each charge carrier interacts simultaneously with many others i.e. collective interaction.
- Formation of macroscopic space charges (in the frame of quasi-neutrality) as a consequence of external influences and modification of charge-carrier movement in the electrical field of these space charges.

Related to the quasi-neutrality and the presence of free charge carriers, the most fundamental attribute of the plasma state is its tendency to minimize external electric and magnetic fields inside the bulk, in contrast to its behavior in the surrounding sheaths [29, 30].

Just like other plasma types, Low Temperature Plasmas can also be provided in thermal and non-thermal plasmas states as shown in Table 2.4.

Table 2.4   Characteristic temperature and pressure of Low Temperature Plasma

Thermal LTP	Non – thermal LTP
$T_e \approx T_i \approx T \leq 2 \times 10^4$ K	$T_i \approx T \approx 300K$
e.g., arc plasma pressure	$T_i \ll T_e \leq 10^5 K$
	e.g. low-pressure at normal glow discharge

Where T $\quad$ = $\quad$ Room temperature

$\quad$ T_i $\quad$ = $\quad$ Temperature of the ion

$\quad$ T_e $\quad$ = $\quad$ Temperature of the electron

2.8.1 Methods of LTP creation

Removing an electron from an atom requires enough ionization energy to break the bond between the negatively charged electron and the positively charged nucleus. Any of the following methods can be used [36],

1. By using high energy radiation such as x rays and ultraviolet light (photo ionization)
2. By bombardment using high energy electrons (electron impact ionization) or small molecular ions (chemical ionization)
3. By exposure to high electric fields (field ionization). And this research employed Field Ionization to ionize nitrogen gas.

2.8.2 Field ionization

It is achieved by exposing a substance to high electric fields which results in removal of electrons from the atomic structure of the substance. Field ionization sources are relatively gentle compared to electron impact sources in that they do not deposit as much excess energy into the parent ion and are usually employed when minimal damage to the ionized specimen is needed. Several methods of field ionization are in existence and one such method is by Dielectric Barrier Discharges [36, 37].

2.9 Dielectric barrier discharge

The Dielectric Barrier Discharge (DBD) principle shown in Figure 2.1 was used to create LTP environment, which was used for withering tea in this study. It works using the following [37],

- Geometry: 2 parallel copper plates, gap 80mm
- External voltage: AC (variable), High Voltage (1-100 kV)

- Gas pressure: Static 101325 Pa or 1.01325 Bar
- Gas temperature: Moderate low temperature approximately $25^{o}C$

DBD is an AC discharge that provides strong thermodynamic, non-equilibrium plasma at atmospheric pressure and moderate gas temperature [37]. It is produced in an arrangement consisting of two (copper blade) electrodes, at least one of which is covered with a dielectric material/layer e.g. glass placed between them. The presence of one or more insulating layer on/or between the two powered electrodes is one of the easiest ways to form non-equilibrium atmospheric pressure discharge [37].

In DBDs the electrode and discharge separated by a dielectric barrier material eliminates electrode etching and corrosion. An AC voltage with amplitude of 1-100 kV and a frequency from line frequency to several megahertz is applied to DBD configurations. DBD cold plasma can be produced in various working mediums through ionization by high frequency and high voltage electric discharge [37].
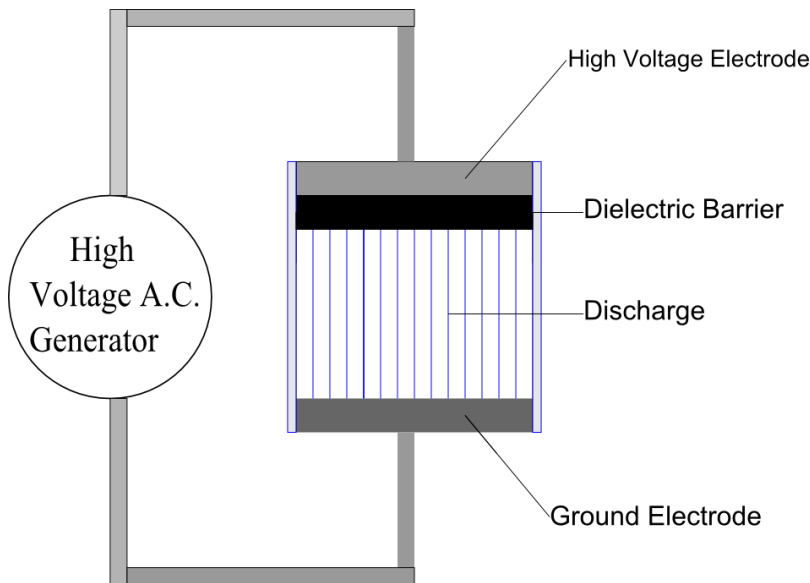
Fig.2.2   Dielectric Barrier Discharge (DBD) principle

2.9.1 Construction

DBD devices can be made in many configurations [37]. Normally planar, using parallel plates, cylindrical materials or using coaxial plates all separated by a dielectric barrier between them. In a common coaxial configuration, the dielectric is shaped in the same form as common fluorescent tubing. It is filled at atmospheric pressure with either a rare gas or rare gas-halide mix with the glass walls acting as the dielectric barrier. Due to the atmospheric pressure level, such processes require high energy levels to sustain [37]. Common dielectric materials include glass, quartz, ceramics and polymers. The gap between the electrodes varies considerably, from less than 0.1 mm in plasma displays to e.g. several millimeters in ozone generators and up to several centimeters in CO_2 lasers [37]. Some of the configurations are shown in Figure. 2.2.
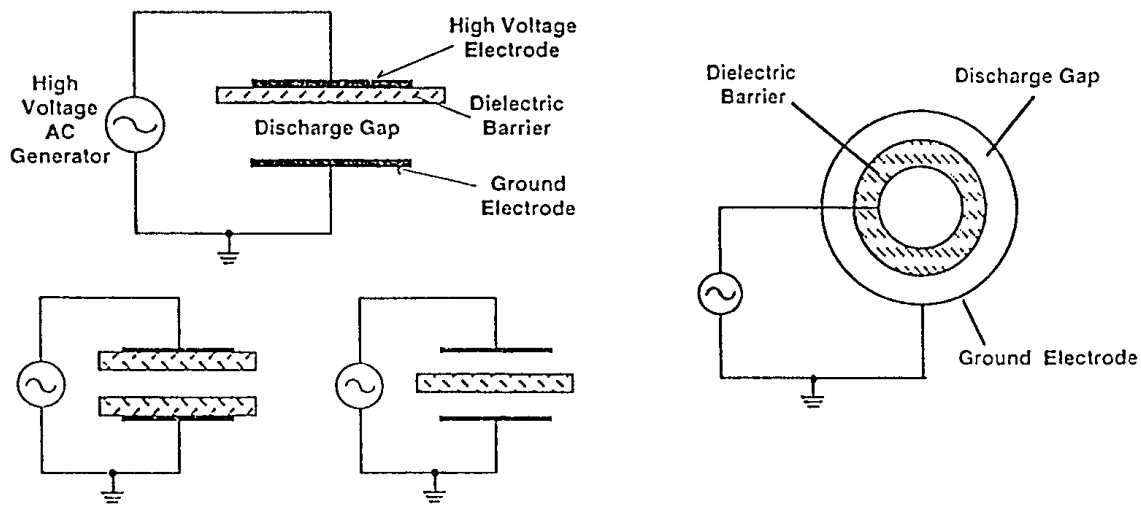
Fig.2.3   Other configurations of Dielectric Barrier Discharge (DBD)

2.9.2 Operation

A DBD device works due to a multitude of random arcs formed between the two electrodes during the operation [40]. As the charges collect on the surface of the dielectric material, they discharge in microseconds (millionths of a second) and reforming elsewhere on the surface [36]. Similar to other electrical discharge methods, the contained plasma is sustained if there is continuous energy source that provides the required degree of ionization and overcoming the

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recombination process that would lead to extinction of the discharge [36]. Such recombinations are directly proportional to the collisions between the molecules and in turn to the pressure of the gas as stated by Paschen's Law, which states that the breakdown voltage of gas between parallel plates is a function of pressure and gap between the electrodes [36]. The voltage necessary to arc across the gap decreases up to a point as the pressure reduces. It then increases, gradually exceeding its original value [36]. Paschen also found that for normal pressure the voltage needed to cause the arc reduces with the gap size but only up to a point. He also stated that as the gap reduces further, the required voltage begins to rise and again exceeding its original value. The discharge process causes the emission of an energetic photon, the frequency and energy of which corresponds to the type of gas used to fill the discharge gap [36]. The gap between the electrodes is gas-filled and voltages of 1–100 kV with frequencies of 50 Hz–1 MHz are used to sustain the discharges. The current is limited by the dielectric materials between the electrodes. The advantage of the dielectric barrier discharge over other discharges is the option to work with non-thermal plasma at atmospheric pressure and the comparatively straightforward scale-up to larger dimensions [36, 37].

2.10   Mathematical modeling

Mathematical modeling of any system involves the formulation of mathematical equations that adequately describe its physical setup into which known inputs (independent variables) are made and outputs realized from which decisions on system design and control are based. An ideal mathematical model should;

- Have a sound mathematical physical basis
- Not have limitations with regard to geometry, shape and physical composition of domain
- Be easy to input parameters which describe the required conditions (environment) have ability to handle transient and buoyant conditions
- Have sufficient accuracy flexible enough to allow the choosing of a desired degree of approximation without reforming the entire problem
- Involve systematic procedure that can be automated for use on digital computers [38-40].

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2.10.1 Importance of theoretical modeling

System modeling/simulation may arise due to one of the following as explained by Smith [40];

a. The need to conduct a low cost study or design of a system whose complex nature precludes development in a laboratory or field experiment or scale model

b. To verify a system of mathematical modeling equations which are to be used in a control system is valid or to gain insight into the system

c. To forecast the response of a system to complex controls or policies as a means of evaluating the consequences of control, design or policy alternatives.

The main stages of modeling real world engineering problems include [40]:

- Formulating real model that describes the system in physical terms
- Making appropriate assumptions about the system hence inputs for the model
- Formulating a mathematical problem/model for the system
- Solving the mathematical problem and getting outputs
- Interpreting the solution that simulated outputs
- Validating the model by its agreement by physical observations
- Using the model to explain, predict, decide or design a better system as explained by Smith [40].

Items 1, 6 and 7 refer to the physical (real) world; 2 and 5 involve the interpretation while 3 and 4 involve the physics or mathematics of the problem. The model is validated by ensuring that the theoretical simulations are in good agreement with observations from the experiments. The seven stages can be summarized in four as follows [39-41];

1. Formulation – the expression of the problem in mathematical language based on the appropriate physical laws governing the process.

2. Solution – appropriate mathematical operations are accomplished so that logical deductions may be drawn from the mathematical model

3. Interpretation – the development of relations between the mathematical simulation/results and their meaning in physical world

4. Refinement – the recycling of the procedures to obtain better predictions as indicated by the experimental checks

The formulation step often results in algebraic, difference, differential, integral equations or combination of these after pertinent variables have been identified and relationships between them postulated. These mathematical models always arise from statements of physical laws such as mass, momentum and energy conservation. Many general equations are expressed by differential equations. Specific phenomena are then singled out from infinite solutions of these equations by assigning the individual boundary or initial conditions which characterize the given problem such as a boundary value for equilibrium or propagation problems respectively. The solution must satisfy the initial or any other related boundary conditions. The problem is described physically (dimensions, observations and what needs to be predicted) and by suitable mathematical equations after making a number of simplifying assumptions which must be as appropriate to reality (boundary conditions) as possible as stated by Smith [40]. These would be considered dynamic models while those developed from experimental data and statistical analysis are termed empirical models [42-43].

2.10.2 The initial boundary conditions

The boundary conditions are defined at the inlet into the control volume in this case the withering chamber in terms of temperature, ionization density and other parameters.

In modeling of Dielectric Barrier Discharge Plasma the following boundary conditions are assumed [41];

- All initial particle concentrations, except those of the electrons and nitrogen are taken to be zero.
- Initial electron density is taken $10^3/cm^3$. Initial electric field potential is also zero.
- All neutral particle concentrations are taken zero at the boundaries of the dielectric
- Normal components of flux are zero at outer boundaries of plasma.
- Normal components of electric field are taken zero on all the outer boundaries of the

domain (dielectric as well as plasma domain).

- No electron and ion current flow inside the dielectric is considered.
- Electric field normal to the dielectric surface is discontinuous by the separated charge.

2.10.3 Model validation

Before a model can be accepted as valid, it must demonstrate that it is accurate and simulates actual events to an acceptable degree as stated by Brugger [38]. It must also prove that can it work for all possible situations and this is only possible when all probable situations are tried though this is unachievable. Therefore a proof of validity consists of proving that the model handles only specific case for which it was developed.

2.11. Summary of literature review

Tea is one of the major cash crops grown in Kenya and is the biggest foreign exchange earner. The consumption of tea locally is about 7% of the total and the rest is exported. Tea has numerous health beneficial effects to human health due to the many compounds associated with it. Its health beneficial effects have attracted many studies on its naturally occurring compounds including polyphenols and are the aim of this study.

Several factors affect polyphenols in made tea from pre-harvest to post-harvest. One of these factors is the environment in which green tealeaf is withered. The withering environment determines the degree of polyphenol conversion to other compounds resulting in its reduction in Made Tea. The reduction is caused by oxidation that forms other compounds. In order to retain maximum amounts of polyphenols in made tea, suitable anaerobic withering environments are required. Hence efforts being made are based on technologies used for creating these types of environments for green tealeaf withering. One such rapidly growing technology and which has been recommended for research is the utilization of Low Temperature Plasma.

Low Temperature Plasma is gaining ground as a key technology for material processing across all fields of science. Methods of its creation are well documented which are suitable for up scaling.

Research findings have shown that LTP enhances biochemical reactions and also creates cover like layer (sheaths) of plasma around solid particles. This can be investigated for creating anaerobic environments around solid matter like tealeaf. Utilization of LTP environment is aimed at facilitating reactions of polyphenols in green tealeaf to occur in anaerobic environment. Green tealeaf is an organic multicellular bio-material and the reactions occurring in it are enzyme driven.

Mathematical models have been used in many cases to predict the mode of reaction. The derived model equations are based on the theoretical reaction mechanisms of the reacting species. These predictive model equations can be used for determination of percentage quantity of a particular compound like polyphenol in the final product during processing.

CHAPTER 3

3.0 THEORETICAL FRAMEWORK

3.1 Factors affecting polyphenol content in Made Tea

Several factors influence the ultimate *polyphenol* concentration in **Made Tea**. These factors range from pre-harvest to post-harvest conditions and include the following [7];

    1.0   Pre-harvest conditions comprising of the following;

- Climatic and soil (C_s)
- Variety and clone (V_c)
- Leaf maturity (L_m)
- Agronomical (A_r)

    2.0   Post-harvest conditions which comprises of the following [7];

- Storage during and after transport leading to fermentation or leaf handling (L_h)

    Technological factors during processing comprising of following;

- Type of environment in terms of Air composition - (F)
- Temperature (T)
- Pressure (P)
- Ionization density (α)
- Withering time (t)

3.2 Discussion of the post-harvest factors that affect polyphenol content in Made Tea before withering

Immediately green tealeaf shoot is detached from mother plant, biochemical degradation start. The rate of degradation is dependent on the following environmental parameters;

- Leave handling

- Temperature
- Humidity
- Air quality/presence of oxygen
- Time (taken to the start of processing)

3.2.1 Leave handling

Storage and transportation are the two main activities done during leave handling. The technology used during storage and transportation to the factory for processing is selected to avoid:

i. High temperature within the green tea leaves as a result of metabolism of the living cells/tissue.

ii. Mechanical damage of the green tea leaf.

3.2.2 Temperature before withering

Green tea leaves are living cells which undergo metabolic processes. The metabolic processes are heat releasing and cause degradation of the green tea leaf. In the process raises temperature within the leaves rapidly leading to leaf reddening if not controlled. Due to this fact and to avoid reddening, green tealeaf withering is also done at low temperature (i.e. $\leq 38^{o}C$) while at the same time blowing fresh air at ambient conditions through the leaves to prevent temperature rise in the leaf leading to its degradation.

Reddening is an oxidation process of compounds inside the green tealeaf. Therefore temperature beyond $38^{o}C$ for *withering* would destroy green tealeaf which is not the function of *withering*. Additionally temperatures greater than $38 \ ^{o}C$ inactivate enzymes.

3.2.3 Humidity influences

It affects the rate and path of degradation process and the more the humidity the faster the degradation. The chemical composition of green tealeaf varies with the level of humidity

conditions present during handling. As a result chemical withering begins before actual withering process.

3.2.4 Air composition

Air composition forms the environment surrounding green tealeaf and affects chemical substances inside the leaf due to chemical withering e.g. *Polyphenols* and Gamma Amino Butyric Acid.

Therefore on the whole post-harvest interventions aim at the control of temperature, humidity and the time to begin processing the green tea leaves.

3.3 Definition of the physical system

This study was aimed at investigating the variation of *polyphenol* content in green tea leaves when withered in a Low Temperature Nitrogen Plasma environment. To achieve this objective the experimental setup was fabricated and used as follows.

3.3.1 Oxidation properties of LTNP environment

The oxygen was availed into the LTNP environment through two methods. First, it was produced during the ionization of water in the chamber as a result of transpiration by the living tissue of green tea leaves and secondly produced as a by-product of photosynthesis due to the metabolic processes occurring within the green tea leaves. It was this oxygen which would oxidize and degrade the *polyphenols* in the green tea leaves.

Additionally during **withering**, green tea leaves being living cells respire and generate heat thereby increasing the temperature within the body of green tea leaves in the chamber thus increasing the rate of oxidation. The quantity of oxygen due to metabolic processes depends on the quantity of the tea leave in the chamber. And the rate of oxidation depends on the quantity of oxygen present in the **withering** chamber.

3.3.2 Non-oxidation properties of the LTNP environment

Non-oxidation properties of the *withering* environment or anaerobic properties are important for retaining maximum polyphenol content in *Made Tea*. It is created by preventing oxygen from the chamber. And it is done using either nitrogen gas as the main and dominant constituent in the *withering* environment or any other medium which prevents oxygen in the environment. In this study Low Temperature Nitrogen Plasma (LTNP) environment was used as the medium for creating the anaerobic environment. It was created due to the plasma sheath which forms around solid matter in this case green tea leaves.

3.4 Physical factors affecting polyphenol content in made tea withered in LTP

Withering stage referred to as chemical withering in tea processing is time dependence. It is done by subjecting green tea leaves to air or nitrogen or any other environment (e.g. LTNP) over a certain period of time. In the process, compounds including *polyphenols* undergo chemical conversions through association, dissociation, detachment attachment, decomposition and recombinations.

  Polyphenols present in the green tea leaf in the final analysis are oxidized and converted to other compounds by oxygen thereby reducing their content in *Made Tea*. In order to circumvent this occurrence, selected technologies can be used to prevent and or reduce oxygen percentage composition in the withering chamber from oxidizing *polyphenols* during *withering*.

In the current study, Low Temperature Nitrogen Plasma (LTNP) was used to create the environment (F) for *withering* green tea leaf. The Physical parameters that would affect the *polyphenol* content in *Made Tea* in this environment are;

- Type of environment (in this case LTNP) or Air quality and composition - (F)
- Temperature of the environment (T)
- Pressure of the environment (P)

- Ionization density in the environment (α)

- Withering time in the environment (t)

3.5   Discussion of the pertinent factors during withering in LTP

The key pertinent factors that have an effect on the content of polyphenols in *Made Tea* are first and foremost the withering environment i.e. with or without oxygen. The others are the temperature (T) inside the chamber, pressure (P) inside the chamber, ionization density (α) by extension the concentration of ions in relation to neutral species inside the chamber, electrical discharge and withering time (t). Amongst these factors and from theoretical analysis time is shown to be important in chemical reaction both at molecular and phenomenological (considering in terms of phenomena) level which is described in terms of rate of change with respect to time (Appendix D) and as a continuum. Equation describing the reactions occurring in LTP as based on Kinetic and Statistical Theory [38] uses time as a determinant of the reactions. However the equation used in this study where LTP is treated as a continuum, determines the macroscopic characteristics (temperature, pressure, etc.). It is based on the balance equations of the number, energy and momentum of particle species [23] causing *polyphenol* reduction. And in the final analysis determines the rate of reaction in the reduction of *polyphenols*. These parameters are functions of state of the LTNP environment and the rate of reaction is used to complete the description of the reaction. However the rate of reduction of *polyphenols* and the thermodynamic variables of particles causing the reduction are functions of time (t).

3.5.1   Temperature

By the nature of the type of environment chosen (LTP), the temperature used was (300K) which was characteristic of LTP environment by its definition. A high temperature reddens the leaf causing degradation of *polyphenols* hence have to be avoided. However, due to the fact that the metabolic processes occurring in the green tealeaf are exothermic, the temperature would normally raise in the bulk of the green tea leaves. To reduce the temperature rise, transportation of the leaf is done using special sacks on a specially designed truck during leaf handling. Whereas during withering air at lower temperature is usually blown through the green tea leaves to control the temperature rise. In black tea manufacture warm air is blown through the leaf to

wilt the leaves and reduce the moisture content by about 3% for purposes of preparing the leaf for the next process of cut, tear and crush popularly known as CTC. During this process spontaneously oxidation of the leaves takes place. While in Green tea manufacture, withering is avoided in order to prevent polyphenol oxidation among other reasons. Thus temperature is a factor on content of polyphenol in made tea. However due to the fact that it reddens/oxidizes green tea leaf thereby destroying polyphenols, was not necessary to vary it to beyond 38 $^{\circ}$C.

A Dielectric Barrier Discharge (DBD) chamber contains a gas that is used for creating plasma environment. Increasing the gas temperature substantially reduces the oxygen generation processes because of formation of ozone as per the rate constant (k_{12}) of Equation 3.10 [45]. The gas temperature T_g, which appears in the rate coefficient of the reactions, is assumed to be the time and space averaged temperature in the DBD discharge space. Studies on the dependence of the rotational temperature in the discharge space as a function of the discharge current have been done [45]. It was found that as the discharge current increases, the temperature in the discharge space also increases [45] which for this study would harm the green tealeaf.

$$k_{12} = 25 \; x \; 10^{-35} \exp\left(\frac{970}{T_g}\right) [cm^6 s^{-1} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \; (3.10)$$

where $T_g = discharge \; space \; (e.g. plasma \; chamber) temperature$

$\quad\quad k_{12} = rate \; of \; reaction \; constant$

It can be seen from the Equation 3.10 that the reaction rate of ozone generation decreases with increasing gas temperature (T_g). Apart from ozone, NOx formation is favored by heat [45]. This is because the ozone destruction reaction is significantly enhanced by an increase in the gas temperature [45].

3.5.2 Ionization density (α)

In chemical reactions, concentration of the reacting species affects the rate of reaction. The ionization density in this research concerns the ultimate concentration of the important species

involved in the *polyphenol* degradation namely oxygen and nitrogen percentage compositions. The percentage composition of oxygen was as a result of ionization of water particles respired from green leaf due to metabolic process occurring.

A high degree of ionization translates to high oxygen particles being produced. At the same time increasing the degree of ionization of nitrogen reduces nitrogen gas in LTNP environment thereby reducing its percentage composition in the withering chamber. Therefore increased amount of oxygen within the withering chamber increases the rate of degradation of polyphenols. And by extension the time taken by the reacting species to completion is reduced. This is as a result of reaction is faster when the density of the reacting species is high and vice versa.

Ionization density with respect to chemical reaction in this study can be seen here as inversely proportional to time taken for withering. Meaning that the higher the density of reacting species the higher the reaction rate and the shorter it takes to completion and the converse is true. Thus experimenting on variation of ionization density would only prove the same. Ionization is directly proportional to the electric field. For this reason, by varying the electric field varies the ionization density and the reaction rate.

Additionally, the degradation of polyphenols is dependent on the amount of oxygen present in the withering environment. This means that rate of ionization that enables formation of neutral oxygen affects the rate of polyphenol degradation. And this would depend on the amount of moisture in green tea leaf prior to withering. A higher ionization density results in a higher oxygen density and hence higher rate of degradation of polyphenols and the converse is true.

LTNP environment is partially ionized, non-equilibrium and quasi-neutral. The ionization is complex as shown in appendix C. In general as a result of ionization, a wide variety of different species were formed. Many particle reaction processes of increasing complexity including dissociation, association, recombination, attachment, detachment and excitation occur. These reactions are of the following nature [30], elastic scattering, excitation, de-excitation, ionization, de-ionization, fragmentation, dissociative ionization, dissociative attachment and volume recombination. The generated radicals join in the addition and substitution reactions causing

chain reactions that produce a variety of species. In view of the numerous types of species produced, it is not possible to state the degree of ionization [30].

3.5.3 Pressure (P)

Pressure in dealing with compressible fluids like LTP implies concentration of plasma species in general. It also by extension means the concentration of the active species involved in reaction with *polyphenols* i.e. oxygen. In view of this, increasing the pressure inside the chamber would result in increased concentration of the particle species including oxygen which reacts with *polyphenols* thereby reducing their content. Therefore higher pressure increases the concentration of oxygen particles hence the rate of reaction increases and the converse is true. Pressure affects *polyphenol* dissociation by either increasing or decreasing the density/concentration of the neutral oxygen particles within the chamber. Experimentation on pressure effect would only prove this inference. The study was done at atmospheric pressure as a requirement for Low Temperature Plasma. Changing the pressure would have totally changed the type of plasma that would not have been suitable for tea withering investigation.

3.5.4 Electrical discharge

The advantage of electrical discharges as a source of non-thermal plasma for oxygen generation is that the plasma parameters can be easily controlled by changing several variables, for instance [45]:

- The power controls the number of ionizations per unit volume per second and hence is an approximate control variable for the plasma density. It is also a possible control variable for the energy of the plasma constituents.
- The operating voltage of the discharge affects the magnitude of the electric field, and hence the energy of the charged particles in the plasma
- The gas pressure controls the electron collision frequency and the mean free paths of all plasma constituents.
- The geometry of the electrodes can affect the energy input by altering the electric field, through changing the geometry of the anode-cathode configuration.

- The type of gas controls the ionization potential, and thus the energy required to produce an electron-ion pair in the plasma
- Finally the cathode characteristics, such as the secondary emission coefficient, or the capability of thermionic emission can also affect the discharge characteristics.

In this study the electrical power was kept constant in line with the type of chamber constructed (Dielectric Barrier Discharge) which works on the principle of a buildup of charge on the dielectric material before discharging into the chamber. Changing the electrical discharge would change the plasma environment. Additionally, changing the electrical discharge would only work to change the ionization in the chamber whose effect changes the oxygen concentration.

3.5.5 Withering Time (t)

Polyphenol molecule dissociation leading to degradation polyphenols in the leaf is a function of time. It occurs due to the presence of Oxygen leading to their reduction in **Made Tea**. The magnitude and rate of degradation increase with withering time. On the other hand the duration of withering time is governed by the **withering** conditions and therefore depends on the type and state of withering environment. This means that the amount of *polyphenols* in **Made Tea** depends on the amount of oxygen in the LTNP environment during **withering**.

Thus short term **withering** with minimum dissociation of polyphenols would be the ultimate aim of **withering** to maximize *polyphenol* content in **Made Tea** or making sure that there is no oxygen in the environment. The time of withering is therefore significant and this is the parameter that was varied in this study.

3.6   The scientific approach model

The main scientific approach used in identifying the pertinent factors which affect polyphenol content during withering in Low Temperature Plasma (LTP) are the model prediction equations governing the reactions of *polyphenols* in Low Temperature Nitrogen Plasma environment considered as a continuum. The *polyphenol* reductions are based on the important species formed

during ionization which participates in the oxidation reaction. This can be represented by the black box containing the environment (LTNP) considered in this study as a continuum and the content of *polyphenols* in the tealeaf before and after **withering** as shown in Figure.3.1.

Polyphenol content
in Green tea leaf ⟶

Withering chamber
(LTNP environment)

Polyphenol content
⟶ in **Made Tea**
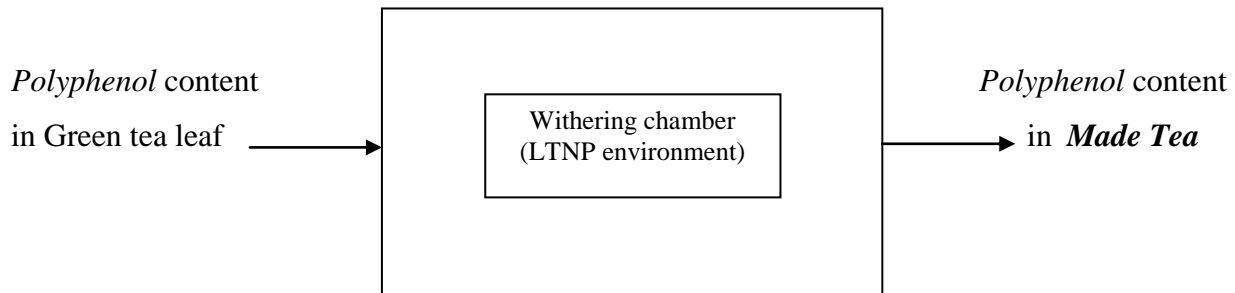
Figure 3.1: **Schematic diagram of the black box representing the input and output of green tealeaf and *Made Tea* samples respectively processed in LTP environment**

All factors that affect *polyphenol* content during the growth of the plant in the farm are held constant at the time of **withering**. For this reason, only technological factors were considered as playing a major role on the content of *polyphenols* in **Made Tea** during **withering** stage. On the other hand most of these technological factors are also LTP characteristic which ends up with only one factor being used as the independent variable. *Polyphenols* in green tea leaves are converted (oxidized) into other compounds by oxygen during **withering**. The conversion leads to their degradation thereby reducing their content in **Made Tea**. The degree of degradation depends on the concentration of oxygen present in the **withering** environment.

From the given pertinent factors in section 3.1, *polyphenol* content in **Made Tea** is a function of both pre-harvest and post-harvest parameters.

The general function representing this relationship is given by the equation 3.1;

$$P_c = f(C_s V_c L_m A_r L_h F \, T \, P \, \alpha \, t) \, \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \, (3.1)$$

Where P_c =   Polyphenol content (mg/g of *Made Tea*)

f   =   function

Removing the function sign in equation 3.1,

$$P_c = k(C_s\ V_c\ L_m\ A_r\ L_h\ F\ T\ P\ \alpha\ t) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\ (3.2)$$

Where k   =   is a constant

Apart from the technological factors all other factors affect the content of *polyphenol* in *Made Tea* during crop cultivation and green tea leaf handling prior to processing in the factory. These factors are therefore held constant when considering factors which affect *polyphenol* content in *Made Tea* during the **withering** stage.

3.7 Mathematical modeling and predictive equation of polyphenol reduction during withering

Using the identified parameters and expressing the relationship of equation 3.2 while keeping all factors before withering stage constant, the polyphenol content in *Made Tea* in mathematical form is given by equation 3.3,

$$P_c = k\ (\overline{C_s}\ \overline{V_c}\ \overline{L_m}\ \overline{A_r}\ \overline{L_h}\ F\ T\ P\ \alpha\ t)\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\ (3.3)$$

Where $\ \overline{C_s}\ \overline{V_c}\ \overline{L_m}\ \overline{A_r}\ and\ \overline{L_h}$ =   are the factors that are kept constant at the time of withering.

3.8 Polyphenol prediction equation

The concept of component equation is used to obtain a prediction equation by finding mathematical expressions that fits the data from a set of experiments in which all but one of the

independent terms are held constant and the empirical prediction equation used in this study include those proposed by Gumbe [44]. For example, if

$$P_c = f(C_s V_c L_m A_r L_h F\ T\ P\ \alpha\ t)\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (3.4)$$

Holding all other independent factors constant except withering time (t), Equation 3.4 becomes

$$P_{c1} = f_1(\overline{C_s}\ \ \overline{V_c}\ \ \overline{L_m}\ \ \overline{A_r}\ \overline{L_h}\ \overline{F}\ \overline{T}\ \overline{P}\ \overline{\alpha}\ t_1)\dots\dots\dots\dots\dots\dots\dots\dots \quad (3.5)$$

Where P_{c1} =   polyphenol content in the green tealeaf at time t_1

f_1 =   function of the independent terms at time t_1

Component equation relating P_c and P_{c1} can be obtained by varying t_1 through some selected range of values and observing the values of P_c [44]. If the data for each component experiment plot as a straight line on log-log coordinates transformation, then each component equation has the form [44],

$$P_c = a_1 t_1^{n_1}\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (3.6)$$

Where $a_1 = \overline{C_s}\ \ \overline{V_c}\ \ \overline{L_m}\ \ \overline{A_r}\ \overline{L_h}\ \overline{F}\ \overline{T}\ \overline{P}\ \overline{\alpha}$

Equation 3.6 can be generalized as:

$$P_c = a_i t_i^{n_i}\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (3.7)$$

where, P_c =   Dependent term (in this case polyphenol content)

t_1 =   Independent term (in this case withering time)

$a_i\ and\ n_i$ =   Coefficients

Equation 3.7 is similar to the biochemical enzyme driven Equation 2.1. This is the equation to validate in the experiments and is to whether the relationship between the rates of reaction of

polyphenol reduction by oxidation follows the mass action law or not. If it follows the mass action, then the kinetic order of enzyme driven reaction, changes as the polyphenol content in green tealeaf varies.

The law of mass action

In a fixed volume, under the well-stirred assumption and the continuum hypothesis, a simple description of reaction rates is provided by the law of mass action which *states that the rate of a chemical reaction is proportional to the product of the concentrations of the reactants.* In a case where the reactant(s) describes exchanges with the outside environment like the case of this study, it may appear with no substrate and constants are called Zero-order reactions since they correspond to a rate law with substrate raised to power zero [20].

Before the values of the coefficients in Equation 3.6 are determined, the equations are qualitative. Plotting the various independent terms against the dependent terms on log-log coordinates transformation similar to Equation 3.8, the coefficients of **a** and **n** can be determined.

$$P_c \quad = \quad n_i log t \quad + \quad log a_i \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots.3.8$$

For instance, if data from a set of experiments is fitted in a straight line, then

$$a_1 = Intercept$$

$$n_i = Slope\ of\ the\ line$$

For component experiments which do not fit in a straight line, curve fitting procedures are used for the data.

The general prediction equation for this biochemical enzyme driven reaction is obtained by adding the various qualitative Equations of 3.7 which gives Equation 3.9.

$$P_c \ = \ f(a_1 t_1^{n1} \ + \ a_2 t_2^{n2} + a_3 t_3^{n3} \dots\dots\dots\dots\dots \ a_{i-1} t_{i-r}^{ni-r} \dots\dots\dots\dots\dots\dots\dots. (3.9)$$

where $t_1 = Independent\ term$

$f = Function\ of\ the\ independent\ terms\ e.g. a_1\ a_2\ a_3\ \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots a_i$

$r = number\ of\ primary\ quantities\ such\ as\ temperature, ionization\ density, time$

$i = number\ of\ quantities$

For each observation of p_{c1} as a function of independent term, f can be evaluated from Equation 3.9 and the function would be;

$$f = \frac{P_c}{a_1 t_1^{n1} + a_2 t_2^{n2} + a_3 t_3^{n3} \ldots\ldots\ldots\ldots a_{i-r} t_{i-r}^{ni-r}} \ldots\ldots\ldots\ldots \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.9)$$

From which the mean value of the function (f) can be calculated. The precision of the equation is evaluated by first plotting the observed terms t_{i0} versus the calculated terms (t_{ic}) [20],

where (t_{i0}) =   is the experimentally measured by applying the prediction equation and then calculating the correlation coefficient.

The coefficient of correlation in this case is calculated automatically by the Ms Excel package.

3.9   The generation of oxygen in LTP

Apart from the chemistry of a typical chemical reaction, Low-Temperature Nitrogen Plasma environment is itself an active environment comprising of excited ions, free electrons, radicals and neutral molecules of nitrogen, hydrogen and oxygen. The energies of the reacting species are raised such that their activation energies are lower than in normal and typical chemical reaction. Particles in LTP dissociate and associate, combine and recombine due to an external effect introduced into its vicinity.

Additionally, in conventional chemistry, less amount of energy is transferred to the reactants at a slower rate (e.g., by heating) whereas in plasma chemistry, a comparatively larger amount of energy is transferred to the reactants at a faster rate resulting in formation of intermediate

energetic products.

3.10 Polyphenol Reaction in plasma environment

Polyphenols undergo dissociation reactions in the presence of oxygen when green tea leaf is withered in LTP. The rate of reaction depends on the percentage composition of oxygen in the environment. Factors known for influencing the reaction within the green tea leaf are [16];

1. Type of gaseous environment used for withering (aerobic and anaerobic) environments
2. Period of withering green leaf in the environment (withering time)

The reaction of Polyphenols in green tea leaf to form other compounds during withering process is a biochemical process. And biochemical reactions follow cellular phenomenon in which a form of network interaction of the chemical species change with time as a result of internal and external factors [20]. Additionally, in biochemical set of network reaction connections, the components are molecular species, which may consist of individual ions, molecules, electrons, photons, macromolecules or complexes of macro-molecules. During the reaction, a number of different processes occur such as [20];

a. Chemical association or dissociation (e.g. decarboxylation).
b. Reaction catalysis (enzyme)
c. Promotion or inhibition of the reaction.

The dissociations are caused by the presence of oxygen. Hence the biochemical reactions were either oxidation to convert the existing polyphenols to other compounds or non-oxidative.
The dissociation of polyphenols in green tea leaf is biochemical in nature, and it is catalyzed by enzymes. Enzyme catalysis work by reducing the energy barrier associated with the reaction. Enzyme catalysis increases the rate at which the equilibrium is attained but has no effect on the equilibrium itself.

The interaction rate of the species depends on the abundance of *polyphenol* molecules within the

green tea leaf. These processes in turn would normally result in the oxidation of the polyphenols within the network reaction. The *polyphenols* undergo oxidation in the presence of oxygen to form other compounds thereby reducing their concentration in "made tea" over a period of time during chemical withering process.

The conversion of *polyphenols* into other products is a time varying reaction where the concentration of polyphenols in the leaf decreases with withering time depending on the withering environment.

However when considering chemical reaction of biological nature, a more interesting behaviour occurs and for this reason usually visualized differently [20]. In this reaction the mass action law which states that "the rate of a chemical reaction is proportional to the product of the concentrations of the reactants" is not obeyed [20]. Over time, the changing availability and abundance of concentrations (Polyphenol and oxygen in this case) within the tea leaf results in changes in the rates of reactions in the network [20].

Individual biochemical reactions involve small networks of elementary reactions [20]. To develop rate laws for biochemical reactions, these networks are collapsed into single reaction events, using separation of time-scale methods [20]. The rate laws that described these reaction events are referred to as biochemical kinetics. Because these reactions are cellular in nature, the mathematical models often take the form of network kind of interaction [20] that change with time due to the internal and external factors. In the current study the precise descriptions of reaction or interaction processes of equations 2.18 to 2.23 can take the form of a quantitative or mathematical statement of the process reaction rate where the quality of made tea will depend on the concentrations of *polyphenols* in made tea.

3.11 Reasons for Using LTP

The key advantage of non-thermal plasma or LTP is the efficient energy consumption. In this case the energy delivered to the discharge is mainly used for generating highly energetic electrons and little energy is lost in heating the volume of a gas [39]. Consequently, technologies based on non-thermal plasma can be highly effective in promoting oxidation, enhancing

50

molecular dissociation or producing free radicals to stimulate plasma chemical reactions [39].

The choice of low temperature plasma environment to be used for withering in this regard, was in consideration of the following factors,

1. Plasma environment provides energy for reaction at a faster rate
2. Presence of particles in ionized form within the environment.
3. Density of the ions required for the reaction is higher in plasma
4. Presence of charged particles i.e. electrons and protons needed for molecular bonding
5. The presence of other elements and compounds within the green tea leaf shown in tables 2.1 to 2.3 could generate new compounds under the influence of plasma.
6. Low Temperature Plasma (LTPs) produces unique collective behavior and nonlinear transport not found in other fields of science and plasma physics [36].
7. The ability to change the degree of ionization by many orders of magnitude in a few nano seconds (ns) at temperatures of only a few electron Volts (eV) is a highly nonlinear process [38].
8. Additionally, LTP is easily controlled with regard to ionization density and formation of active species etc [10].
9. The non-equilibrium nature of LTPs with their broad array of positive and negative ions of varying mass, transport coefficients and electrons provides a rich possibility of waves and instabilities that increases the reaction potential of the species within the system [37].

A variety of chemical reactions occur in an LTNP environment. The reactions consist of chemical dissociations and associations, detachments and attachments, excitations and de-excitations, combination and recombination of particle species present in the reaction process [47]. These reactions results in the production of oxygen and nitrogen species. The percentage composition of these two neutral species determines the nature of the *withering* environment i.e. aerobic or anaerobic environment.

A host of other particles present in the LTNP environment also affect the quantity of oxygen and nitrogen in the chamber because of recombination and conversions [47]. The reactions are

continuous such that a non-equilibrium state of matter exists within the environment. It is therefore difficult to predict the other parameters of plasma at any given time due to this non-equilibrium nature of LTP. Especially ionization density, which has a direct effect on the rate of reaction yet, cannot be predicted.

3.12 Physical parameters pertinent to the content of polyphenols in Made Tea

The following are the pertinent parameters which affect the polyphenol content in the green tealeaf during the withering stage of tea processing.

Table: 3.1   Definition of pertinent parameters which affect the polyphenol content

General parameters	Symbol	Parameter definition	Source
Pre-harvest factors	C_s	Climatic and soil	[7, 19]
	V_c	Variety and clone	[6, 7, 15, 19]
	L_m	Leaf maturity	6, 7, 19]
Technological factors	A_r	Agronomical	[6, 7, 19]
	L_h	Storage and handling	[6, 7, 15,19]
	F	Withering environment	[6, 7]
	t	Withering time	[6, 7, 15, 19]
	T	Temperature	[6, 7, 15, 19]
	P	Pressure	[6, 7]
	α	Ionization density	[36, 37]

CHAPTER 4

4.0   MATERIALS AND METHOD

4.1   Withering chamber specifications

Figure 4.1 shows the experimental setup which consists of the various components required to make the LTP chamber. The withering chamber was made up of a one litre glass bottle with a plastic lid. The lid consisted of three holes. One hole at its centre was used to accommodate a 10 mm diameter by 50 mm long copper tube inserted through it. The lower end of this tube was fitted onto a circular copper blade (80mm diameter) through a 10mm hole at its centre as shown in Figure 4.1. This circular blade covering the entire surface area of the lid was used as a high voltage electrode and was fixed on the inner surface of the plastic lid as shown in Figure 4.2. A rubber pipe was fitted on the upper end of the copper tube and connected to the nitrogen gas cylinder as shown in Figure 4.1. A high power voltage cable (30,000V) was fitted on the copper tube near the upper end above the lid as shown in Figure 4.1. The copper tube outside the chamber was completely insulated for safety reasons in view of the fact that the experiment was using a very high electrical power voltage. The lid was tightly fitted on the bottle so as to withstand the pressure inside the bottle during air evacuation using a vacuum pump. A rectangular copper blade 150 x 120 x 1.5mm was used as the lower electrode for the electric field as shown in Figure 4.3 which also acted as earth wire.

The other holes on the lid were used for inserting pressure gauges. The gauges were used for atmospheric and vacuum pressure readings.

Rubber hose pipe to
nitrogen gas cylinder

Copper tube

High voltage power line

Pressure gauges (vacuum and atmospheric)

Circular Copper blade

(High voltage electrode)
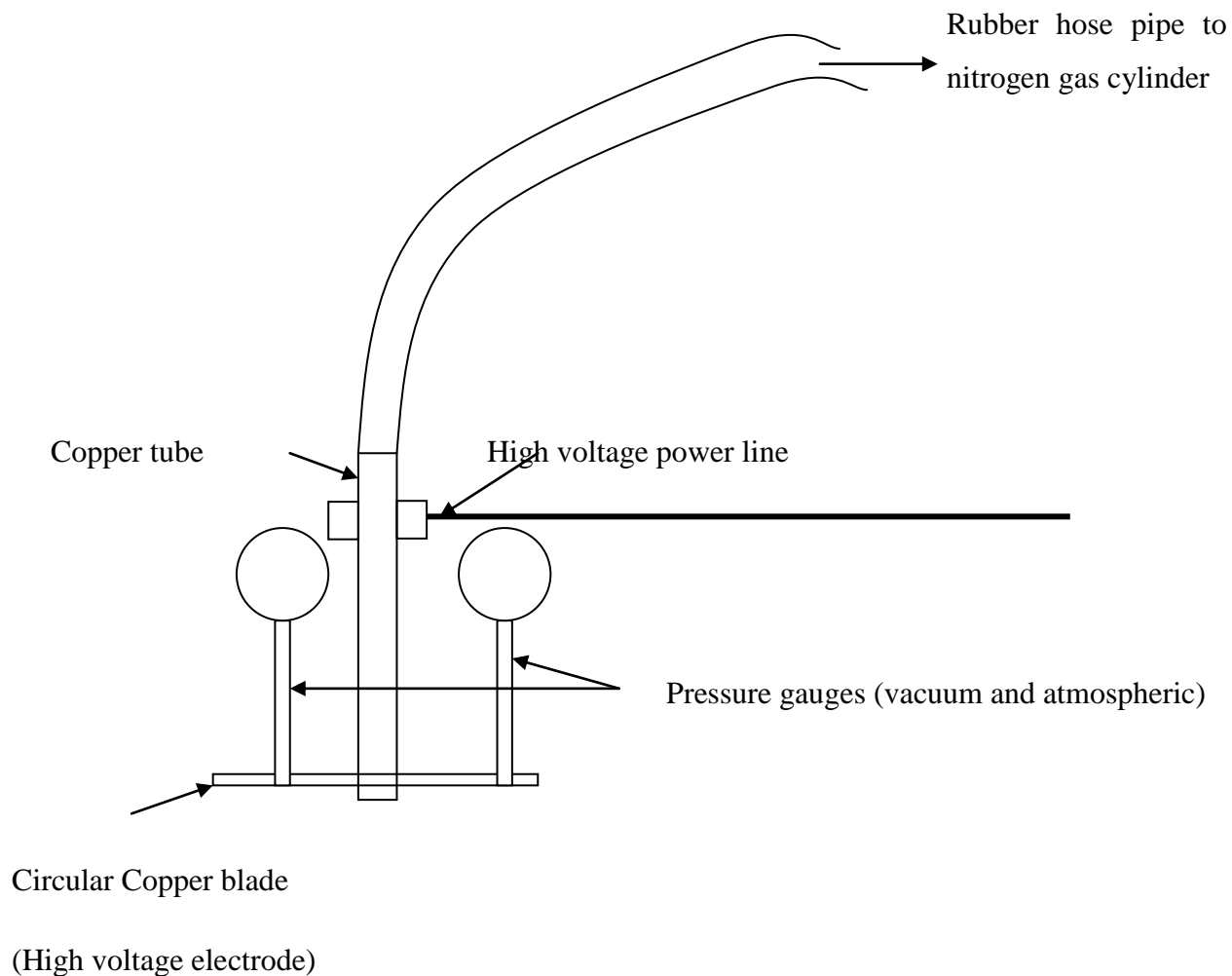
Figure 4.1:   Schematic diagram of copper tube and circular copper blade components for nitrogen gas inlet and high voltage electrode respectively
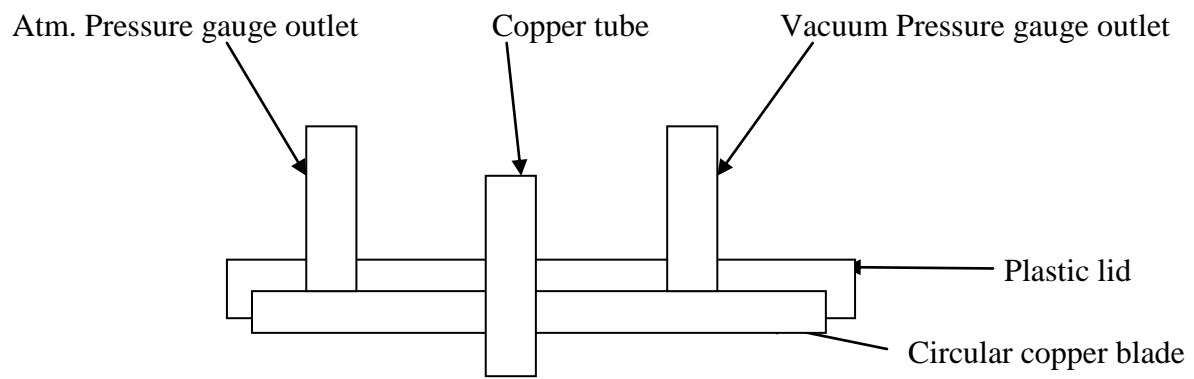
Figure 4.2   **Schematic diagram of circular copper blade fitted on the inner surface of the plastic lid**

4.2   Experimental design

The design setup consists of green tealeaf samples in Dielectric Barrier Discharge chamber which had Low Temperature Nitrogen Plasma.

4.2.1   Experimental layout

Using the Dielectric Barrier Discharge principle, a Low Temperature Plasma (LTP) environment was created inside the chamber. The chamber (here referred to as LTP withering chamber) as shown in figure 4.4 was fabricated using the following;

- Dielectric Barrier Discharge chamber (1 litre glass bottle)
- Nitrogen gas (Gas cylinder)
- High voltage power supply (30KV - color TV mother board)
- Nitrogen gas inlet valve
- Air outlet valve
- Copper blade to earth wire (circular blades)
- Copper wires
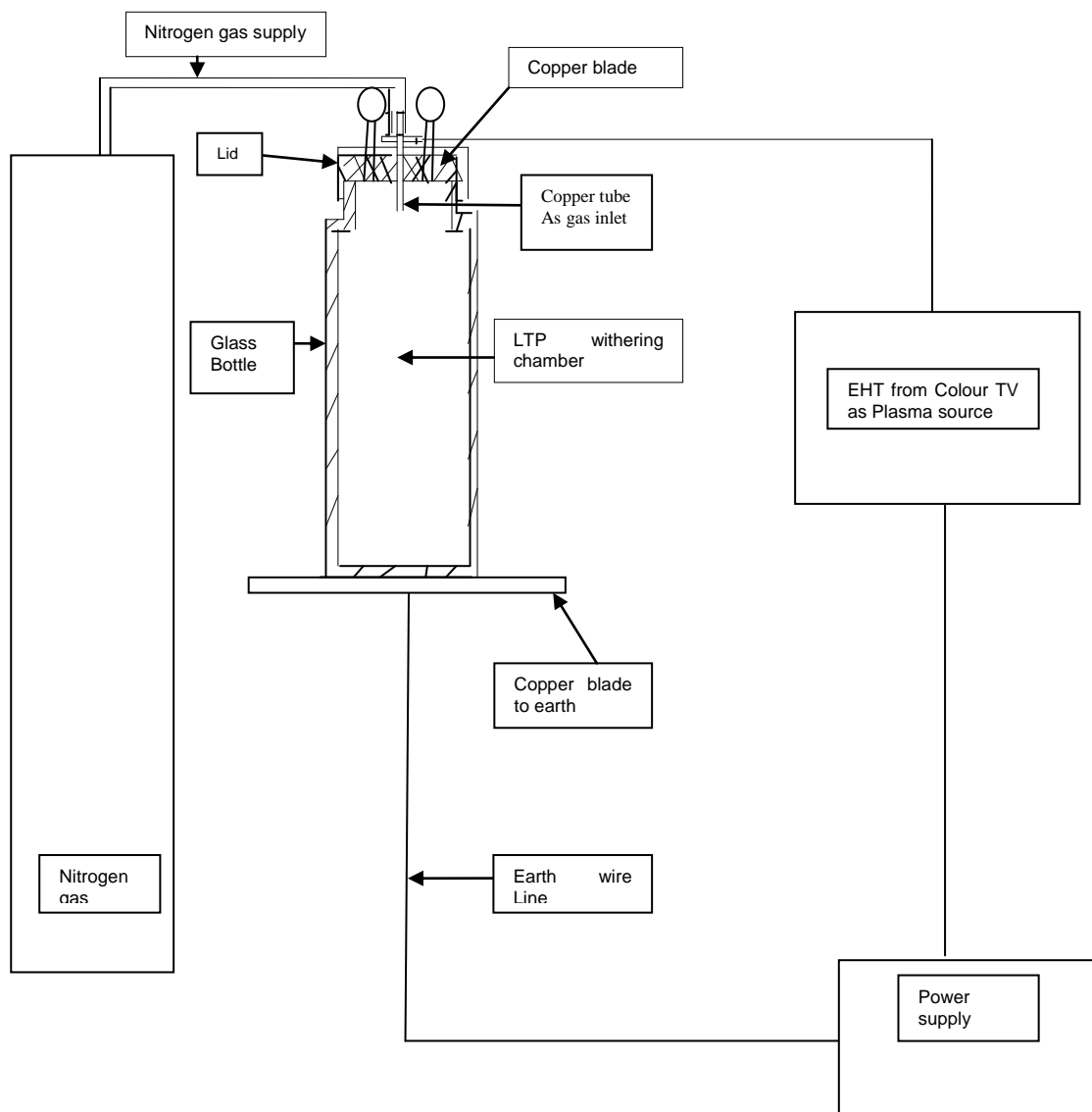
Figure 4.3:   Schematic diagram of the LTP Chamber setup

4.2.2 Instrumentation and data collection

The following instruments were used to run the experiment.

1. Ammeter up to 1000 Amps
2. Voltmeter up to
3. Thermometer
4. Pressure gauge
5. Vacuum pressure gauge
6. Stop watch

4.3 Experimentation and methodology

4.3.1 Sample collection and processing

Fresh green tea samples were collected on different days from Limuru, a distance of about 50 Km from KIRDI, Nairobi.

Scope of sample

Green tea leaf samples used were collected from a tea growing region (Limuru in Kenya). The samples were collected on different days and had the following characteristics;

- Same variety
- Same leaf maturity
- Picked the same two leaves and a bud
- Picked from the same location and farm
- Cultivated using the same agronomical practices
- Grown in the same climatic consisting of deep reddish brown clay soils on steep slopes. The soil pH ranged from 4.3 to 6.7 for the subsoil with a tendency towards strong acidity in the steep areas
- Picked on occasions of similar climate i.e. to avoid leaf with feasible moisture physically (Green leaf without any wetness).

- Average moisture content of leaf was 68% at the time of the year
- For comparison green tea, purple tea and oolong tea were purchased from the local market and analysed

During the experiment 200g of green tea leaf samples were fed into the bottle (LTNP chamber). The experiments were done at same room temperature about 27^0C and atmospheric pressure. The weather conditions in Limuru at the time of picking green tea leave was as shown in table 4.1[46];

Table 4.1:   Average weather conditions in Nairobi during the experiment time

Parameter	Unit value
Pressure:	1022 mbar
Humidity	88%
UV sensibility	1 Low
Dew Point	21°C
Temp	23°C

4.3.2   Experiments

In the first experiments, 200g samples of fresh green tea leaf were withered under numerous environmental conditions as indicated in Table 4.2. This was done by feeding green tea leaves into the Dielectric Barrier Nitrogen Plasma Chamber and left to wither over various withering times. The samples were then macerated and dried in an oven and in a vibro-fluidized bed drier (VFBD).

While in the second set of experiments, 200g samples of fresh green leaf were withered anaerobically in nitrogen gas indicated as in Table 4.3. Similarly the green tea leaves were fed into the chamber and left for various withering times. The samples were then macerated and dried. One green tea sample was directly macerated and dried without undergoing the withering

stage.

The experiments were as follows;

1. Green tea leaf withering time in LTNP environment Vs Polyphenol content
2. Green tea leaf withering time in Non-LTNP environment Vs Polyphenol content

The aim of the second set of experiments was to compare the results and determine if there was any significant difference in *polyphenol* content between LTNP and Non-LTNP environment processed teas.

Table 4.2: **Green tea leaf withered in various Low Temperature Nitrogen Plasma environments**

Sample No.	Green leaf plucked from	Withering time	Fermentation	Withering process conditions
		Hours	Hours	Nitrogen Plasma
Green tea 1	Limuru	1.0	0	Nitrogen plasma withering at room temperature, atmospheric pressure, macerated and dried
Green tea 2	Limuru	3.0	0	……..as above………..
Green tea 3	Limuru	3.5	0	……..as above………..
Green tea 4	Limuru	4.0	0	……..as above………..
Green tea 5	Limuru	4.33	0	……..as above………..
Green tea 6	Limuru	5.0	0	……..as above………..
Green tea 7	Limuru	8.0	0	……..as above………..

Table 4.3: **Green tea leaf withered in various Non-Low Temperature Nitrogen Plasma environments**

Sample No.	Green leaf plucked from	Withering time	Fermentation	Withering process conditions
		Hrs	Hrs	
Green tea 8	Limuru	18	0	Green tea leaf withered in Nitrogen gas at room temperature, atmospheric pressure, macerated and dried
Green tea 9	Limuru	18	0	Green leaf was stored in cool box for 24 hours after which was withered in Nitrogen gas at room temperature, atmospheric pressure, macerated and dried
Green tea 10	Limuru	0	0	No withering, macerated straight away after picking from the farm and dried
Green tea 11	Limuru	24	0	Withered anaerobically in nitrogen gas at room temperature, macerated and dried
Green tea 12	Meru	20	0	Withering anaerobically in nitrogen gas at room temperature macerated and dried
Black tea 13	Meru	48	18.5	Withered anaerobically in nitrogen gas at room temperature, macerated and dried
Green tea 13*	Kericho	0	0	Sample from local manufacturer, no withering, steamed, macerated and dried
Purple tea*	Meru	0	0	Sample bought from the local market in Kenya that was made using Oolong tea technology
Oolong tea*	Meru	0	0	Sample bought from the market in Kenya that was made using Oolong tea technology

ˣ Samples bought from the local market for comparison

4.4　　　　　Laboratory tests

Made tea samples for both LTNP and Non-LTNP experiments were then analyzed for Epigallocatechin, Epigallocatechin gallate Epicatechin gallate and Caffeine contents using High Pressure Liquid Chromatography (HPLC)

4.4.1　Sample Polyphenol concentration analysis

Sample preparation

For each of the samples approximately 200mg were finely ground using a mini pestle and mortar to fine powder. 100mg of the sample was weighed into a 50 ml volumetric flask, 20 ml of a mixture of 80% methanol in distilled water added and ultrasonicated for 30 min while ensuring the water in the ultrasonic bath didn't go beyond 30 ^{0}C (achieved by placing ice blocks in the water bath).

The samples were filtered into a 50 ml volumetric flask using a whattman filter paper number 42. After complete filtration the filter paper was washed with 10 ml of methanol and allowed to drip into the volumetric flask up to complete dryness (done to ensure that no analyte is left on the filter paper). The volumetric flask was toped up to the mark with distilled water.

10 ml of this solution was passed through a micro filter (45µm) so as to remove further tiny micro particles before analysis by HPLC. 50µl of the prepared sample was injected into the instrument for subsequent analysis.

4.4.2　Standard solution preparation

Preparation of stock solution

100ppm standards were appropriately prepared by dissolving the available standards in a solution of 40% acetonitrile in micro filtered distilled water. The standards available were

Epigallocatechin, Epicatechin gallate, caffeine and Epigallocatechin gallate.

Preparation of calibration solutions

From the stock solution calibration standards were prepared from the range of 2.5, 5, and 10 ppm. A multi component mixture of the following standards, Epigallocatechin, Epicatechin gallate, caffeine and Epigallocatechin gallate were prepared by mixing the appropriate (calculated) volumes in 10ml volumetric flask.

Analysis of standards and calibrations

Prior to analysis of the multi component calibration standards, each of the individual standards was injected into the instrument (HPLC) to establish their retention times. After this was achieved the calibration standards were run and their peak areas and retention times noted.

4.4.3   Chromatographic conditions

The following are the specifications of the High Pressure Liquid Chromatography (HPLC) instrument that was used for the analysis of the samples for the contents of Epigallocatechin, Epigallocatechin gallate Epicatechin gallate and Caffeine.

HPLC model type: Knauer
Column type: Vertex column
Column packing material: Eurospher 100-5. C18
Column length ×ID: 250×4.6mm
Mobile phase: solvent A   Solvent B

        Distilled H_2O: 95%   Distilled H_2O: 60%

        Formic acid: 5%   Acetonitrile: 40%

Flow rate: 1ml/min
Detector: Pulsed Diode Array
Wavelength: 280μm

Oven temperature: 30° C.

4.5 Statistical Analysis

4.5.1 Research Questions and Hypotheses

The aim of the experiments was to determine the relationship between independent variable and dependent variable. The research contained more than one independent variable but only withering time (variable) was used. The independent variable was randomly assigned a value and its effect on the dependent variable was determined. Thus experiments were done by varying the independent variable. Green tealeaf being a living tissue was used to find out the effect of Low Temperature Plasma environment on the polyphenol content during the withering stage.

4.5.2 Characteristics of Quantitative Studies

The quantities of polyphenols (dependent variable) were measured and used to construct statistical models to explain what was observed. The goal was to understand the effect of LTNP on polyphenols in green tealeaf during withering and predict the relationship through mathematical modeling based on the theory.

The study experimental layout was designed to collect data with an incline to test the theory (deductive) and to generate theory (inductive) about the effect of LTP on polyphenols. A correlation between polyphenol content and withering time (both quantifiable/numerical) were used to determine whether and to what degree a relationship exists. It was found that the two variables were correlated and used the relationship to predict the value of one variable using the other variable.

The experiments also were to establish the cause/effect of the relationship of the independent variable of the study on the dependent variable. The cause (the independent variable) was controlled by randomly assigning conditions that constituted the independent variable of the study and then measured the effect on the polyphenols i.e. the dependent variable of the study.

Since the experiment was to measure the quantities of both the independent and dependent variables an Ms Excel package was used to analyze the data.

The analyses of the samples were done in triplicate and mean values obtained. The percentage Relative Standard Deviations (%RSD) were also calculated to check the validity of the data collected. From the graphical results, equations representing relationships of the dependent and independent variables were obtained. The graphical results were based on the lines with best coefficient of correlations (R^2) and were able to be used to give predictive equations of the rates of reaction between the variables.

4.6   The physical implications of limiting processes

The mathematical formulation of many important problems in engineering involves the determination of a function satisfying an equation containing derivatives of the unknown function. In mathematics, the limit of a function is a fundamental concept concerning the behavior of that function near a particular input. Such equations are called differential equations.

It is defined as a function f which assigns an output $f(t)$ to every input t. If the function has a limit P_{co} at an input P_c means $f(t)$ gets closer and closer to P_{co} as t moves closer and closer to P_c. More specifically, when f is applied to any input sufficiently close to P_c, the output value is forced arbitrarily close to P_{co}. On the other hand, if some inputs very close to P_c are taken to outputs that stay a fixed distance apart, we say the limit does not exist.

For every input of an independent term (time), the function assigns an output $f(t)$ of the dependent term (Polyphenol content). The function has an output limit say P_{co} at an input t_o. This means $f(t)$ gets closer and closer to P_{co} as t_o moves closer to t value which gives maximum output.

CHAPTER 5

5.0   RESULTS AND DISCUSSION

5.1   General observations

Laboratory analysis gave the following results for the various environmental conditions under which the samples were withered. Tables 5.1 and 5.2 show the laboratory results of for LTNP and Non-LTNP withered teas respectively.

Table 5.1   HPLC peak areas/contents in *Made Tea* of EGC, EGCG, ECG and Caffeine samples withered in LTNP

Sample No.	Withering time	Epigallocatechin		Epigallocatechin gallate		Epicatechin gallate		Caffeine	
	Hrs	Average mg/g of tea							
			% RSD n=3		% RSD n=3		% RSD n=3		% RSD n=3
Green tea 1	1.0	7.11±0.14	1.97	53.66±1.34	2.5	17.79±0.2	1.14	31.61±0.52	1.63
Green tea 2	3.0	6.98±0.12	1.72	48.65±0.87	1.79	8.02±0.19	2.39	38.07±0.37	0.97
Green tea 3	3.5	9.26±0.19	2.05	26.17±0.79	3.01	6.67±0.16	2.43	47.09±0.74	1.56
Green tea 4	4.0	5.72±0.12	2.04	44.31±1.48	3.12	6.55±0.19	2.96	38.45±0.53	1.38
Green tea 5	4.33	6.92±0.11	1.55	28.96±0.75	1.54	5.66±0.14	2.46	28.57±0.24	0.84
Green tea 6	5.0	4.69±0.09	1.90	29.93±0.57	1.89	4.55±0.13	2.80	30.18±0.38	1.26
Green tea 7	8.0	2.96±0.03	1.08	16.42±0.34	2.06	3.88±0.06	1.63	31.62±0.38	1.21

Where RSD   -   relative standard deviation

Table 5.2:   HPLC peak areas/contents in *Made Tea* of EGC, EGCG, ECG and

Caffeine samples withered in Non-LTNP

Sample No.	Withering time	Epigallocatechin		Epigallocatechin gallate		Epicatechin gallate		Caffeine	
Green tea 8	18.0	3.44±0.02	0.55	133.41±4.92	3.69	2.94±0.05	1.67	28.67±0.43	1.49
Green tea 9	18.0	6.36±0.08	1.22	15.31±0.22	1.46	3.82±0.05	1,23	32.56±0.56	1.72
Green Tea 10	0	8.86±0.11	1.26	83.27±3.07	3.70	9.79±0.19	2.00	39.49±0.54	1.36
Green Tea 11	24.0	6.31±0.11	1.66	66.64±2.02	3.03	7.31±0.17	2.32	38.94±0.75	1.93
Green Tea 1 2	20	9.07±0.20	2.18	70.36±2.10	2.18	10.63±0.23	2.13	30.01±0.39	1.30
Black tea	48 .0	5.48±0.10	1.79	12.15±0.35	2.90	3.6±0.02	0.56	27.81±0.35	1.22
Green tea 13*	0	12.43±0.21	1.69	27.59±0.48	1.74	7.02±0.03	0.46	22.33±0.25	1.12
Purple tea*	0	14.26±0.29	2.01	36.85±0.77	2.13	9.61±0.22	2.28	28.77±0.36	1.26
Oolong tea*	0	3.16±0.02	0.73	11.72±0.21	1.7283	3.12±0.03	0.98	25.03±0.28	1.10

* Procured from the local market for comparison purposes

Table 5.1 indicates that there was a general decrease in the contents of each of the *polyphenol* constituent compounds with increasing withering time.

Epigallocatechin gallate had the highest percentage composition amongst the components making up the polyphenols in both LTNP and Non-LTNP withered teas. It was followed by caffeine while Epicatechin gallate and Epigallocatechin showed mixed results in terms of quantities over different withering time scales.

Sample Green tea 12 withered in Non-LTNP environment had the highest amount of Epicatechin gallate of 10.63mg/g in *Made Tea* followed by sample Green tea 10 with 9.79mg/g. Purple tea had Epicatechin gallate concentration of 9.61mg/g in *Made Tea*. Sample Green tea 1 withered in LTNP environment had Epicatechin gallate concentration of 8.87 mg/g in *Made Tea*.

Purple tea had the highest content of Epigallocatechin of 14.3mg/g in *Made Tea* followed by sample Green tea 13 with 12.43mg/g in *Made Tea* and sample Green tea 10 had 8.9mg/g in *Made Tea*. An LTNP withered sample with the highest amount was sample Green tea 3 with 9.3mg/g in *Made Tea*. Sample Green tea 8 withered in Non-LTNP had the highest amount of Epigallocatechin Gallate of 133.4mg/g in *Made Tea* as compared to sample Green tea 10 also withered in Non-LTNP with 83.3mg/g in *Made Tea* while sample Green tea 1 withered in LTNP environment had 53.7mg/g in *Made Tea*. An LTNP withered tea sample Green tea 3 had the highest caffeine content of 47.1mg/g in *Made Tea* followed by Non-LTNP sample Green tea 10 with 39.5mg/g in *Made Tea*.

On overall, *polyphenol* content decreased with increasing withering time. This was observed in both LTNP and Non-LTNP withered teas. However the scale of decrease was faster in LTNP withered tea than in Non-LTNP withered tea. The reason for the observation may be due to the presence of oxygen generated from plasma environment.

All the individual samples were analysed in Triplicates and quantification carried out through external standard calibration. The average concentrations, standard deviations (SD) and Percentage Relative Standard Deviation (%RSD) were calculated using Microsoft Excel Package as indicated in Tables 5.1 and 5.2.

The results given in Tables 5.1 and 5.2 were statistically analyzed using Microsoft Excel Package and presented in line graphs. The statistical analysis indicated the relationship between the rates of reduction of *polyphenols* in green tea leaf and withering time follows exponential (Figure 5.1 to 5.4) functions. The equation of the trend line has a negative sign which was an indication of an inverse relationship, i.e. the content of *polyphenols* in green tea leaf decreases with increasing withering time.

The Rate of change of *polyphenols* with withering time was calculated and plotted on a line graph against remaining content of *polyphenol* in the green tea leaf. The relationship between the rates of *polyphenol* reduction and remaining content of the *polyphenols* indicated also an inverse proportionality. The reaction rate approaches a limiting minimum rate as the *polyphenol* content decreases.

5.2   Data analysis

The data in Tables 5.1 and 5.2 were analyzed using statistical methods of MS Excel packages and are as follows;

LTNP withered tea Polyphenol constituent concentrations

The results given in tables 5.1 were statistically analyzed and presented in line graphs showing the variation of concentrations of epigallocatechin, epigallocatechin gallate, epicatechin gallate and caffeine against withering time as shown Figures 5.1 to 5.4.

$$y = 62.171e^{-0.158x}$$
$$R^2 = 0.6708$$

■ Epigallocatechingallate
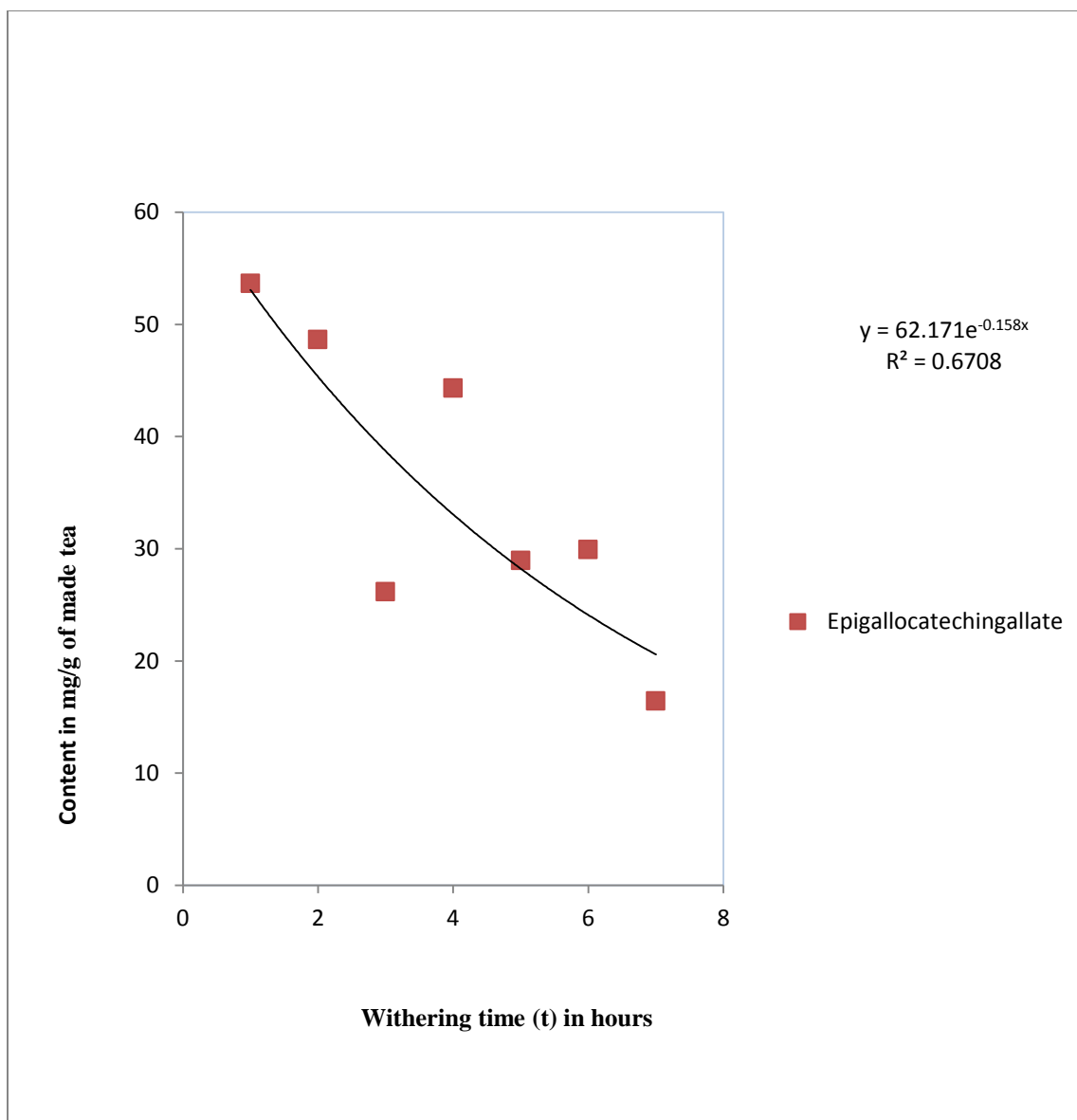
Figure 5.1 **Variation of Epigallocatechin gallate in made tea withered in LTNP against withering time**

The chart shows:
- Y-axis: Content in mg/g of made tea (0 to 10)
- X-axis: Withering time in hours (0 to 8)
- $y = -0.816t + 9.565$
- $R^2 = 0.983$
- ▲ Epicatechin gallate
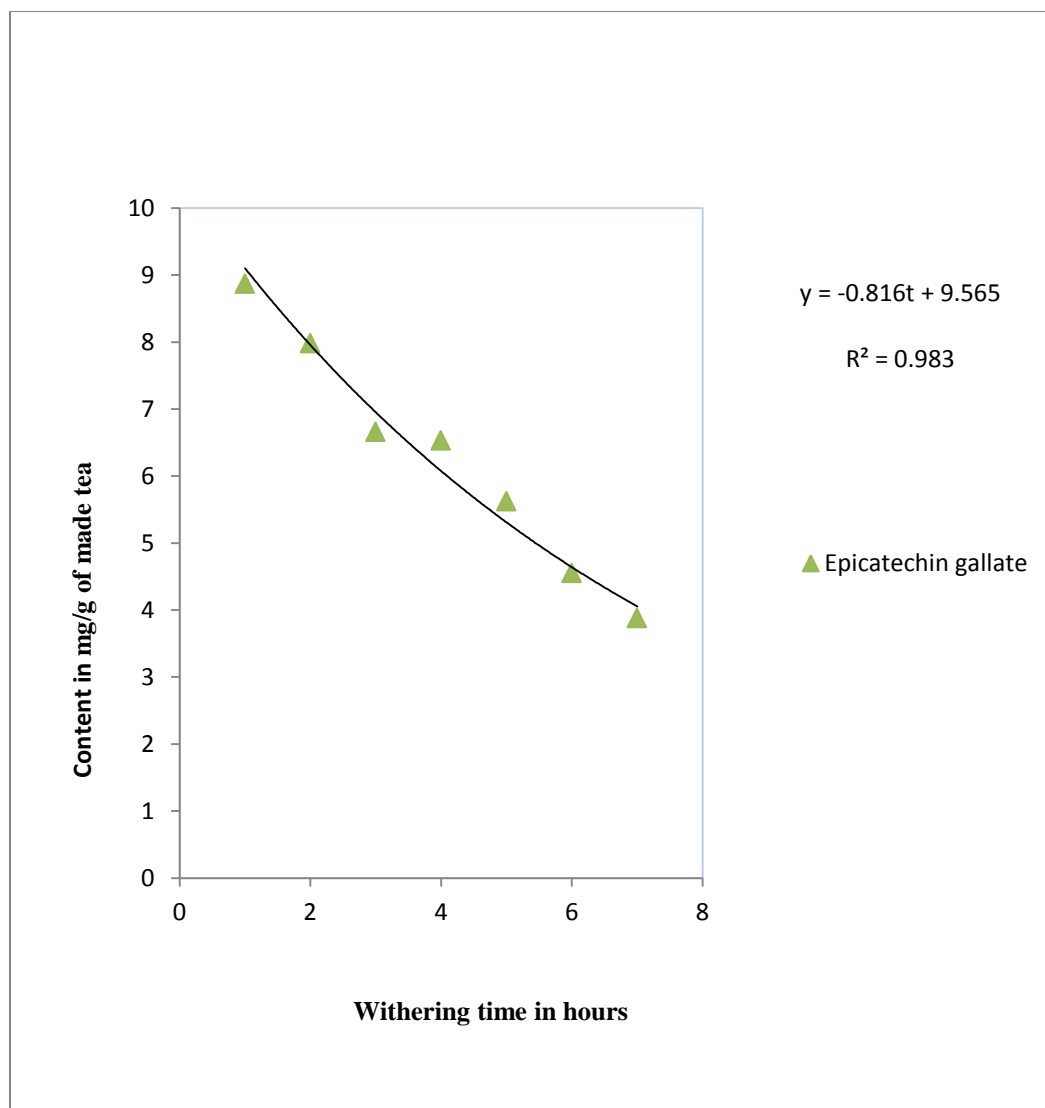
Figure 5.2   Variation of Epicatechin gallate in plasma processed made tea against retention time

The chart contains the following annotations:

$$y = 10.052e^{-0.133x}$$
$$R^2 = 0.6005$$

Y-axis: **Content in mg/g of made tea**

X-axis: **Withering time (t) in hours**

Legend: ◆ Epigallocatechin
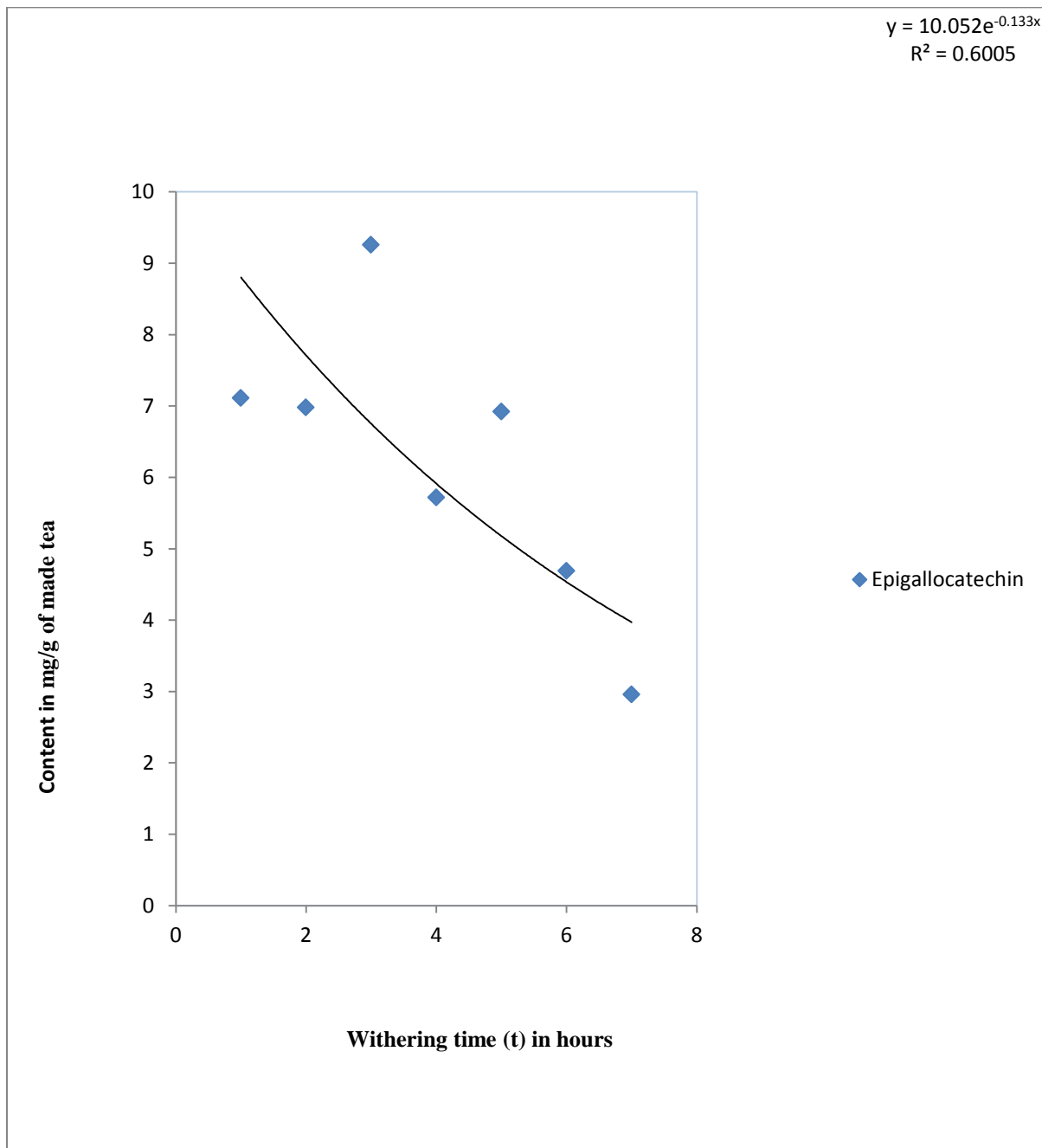
Figure 5.3   Variation of Epigallocatechin in plasma processed made tea against retention time

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$y = 39.708e^{-0.034x}$
$R^2 = 0.1773$

♦ Caffeine
— Expon. (Caffeine)

Content in mg/g of made tea

Withering time (t) in hours

Figure 5.4   Variation of Caffeine in plasma processed made tea against retention time

The coefficient of determination (R^2 = 0.600) Figure 5.3 of the relationship is an exponential type of equation. This means that the other forms of lines are not the best representation of the change of epigallocatechin with respect to withering time. The graph of the polynomial relationship gave a higher coefficient of determination (R^2 = 0.768) however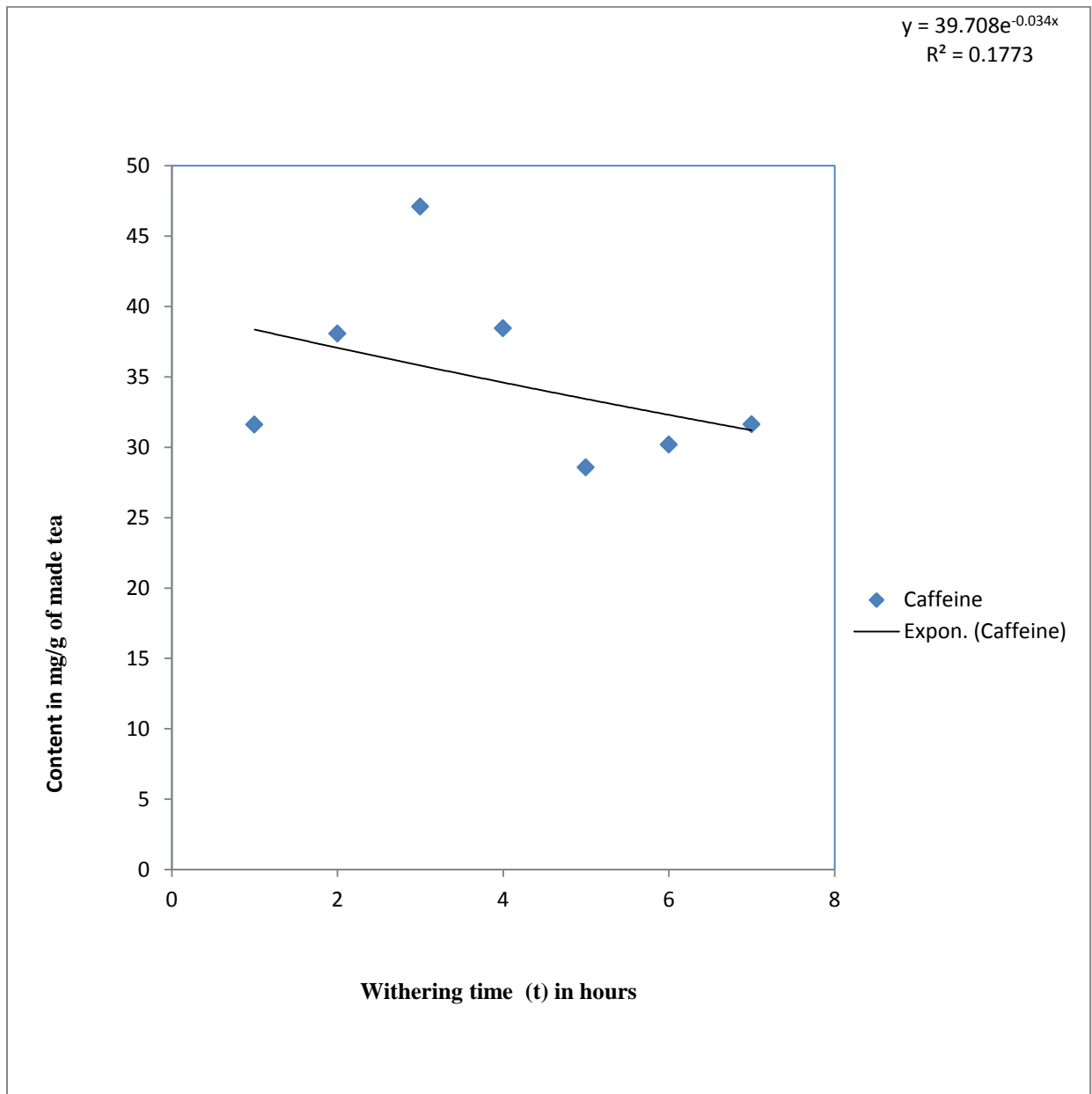 at some point when values of the independent term are substituted in the prediction equation, gives negative values. This should be investigated.

The best coefficient of determination /correlation (R^2) in Figure 5.4 of the relationship was a polynomial type and gave a value R^2 = 0.359. Substitution of values from approximate zero reaches a limit after which starts to give a negative answer. An exponential type of trend line gave R^2 = 177 as shown in the graph. It shows that all the trend lines (exponential, linear, logarithmic, power and moving average) due to the low values of the coefficient of determination (R^2) are not the best representation of the change of caffeine with respect with time. This calls for further research by chemistry experts to find out the reason.

5.3 Polyphenol and caffeine contents

The total *polyphenol* and *Polyphenol*-caffeine contents in each of the samples were plotted on bar charts (Figures 5. 5 and 5.6). The contents were also plotted against withering time as shown in Figure 5.7

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Table 5.3: **HPLC peak areas of Polyphenol and Caffeine concentrations in *Made Tea* samples withered in LTNP and Non-LTNP environments**

Sample No.	Polyphenols	Caffeine	Total Sum polyphenols and caffeine
Average mg/g in LTNP Made Tea			
1	78.56	31.61	110.17
2	63.65	38.07	101.72
3	42.1	47.09	89.19
4	56.58	38.45	95.03
5	41.54	28.57	70.11
6	39.17	30.18	69.35
7	23.26	31.62	54.88
Average mg/g in Non-LTNP Made Tea			
8	139.79	28.67	168.46
9	25.49	32.56	58.05
Green Tea 1	101.92	39.49	141.41
Green Tea 2	80.26	38.94	119.2
Green Tea 3	90.06	30.01	120.07
black tea 5	21.23	27.81	49.04
Green tea 4*	47.04	22.33	69.37
Purple tea*	60.72	28.77	89.49
Oolong tea*	18.00	25.03	43.03

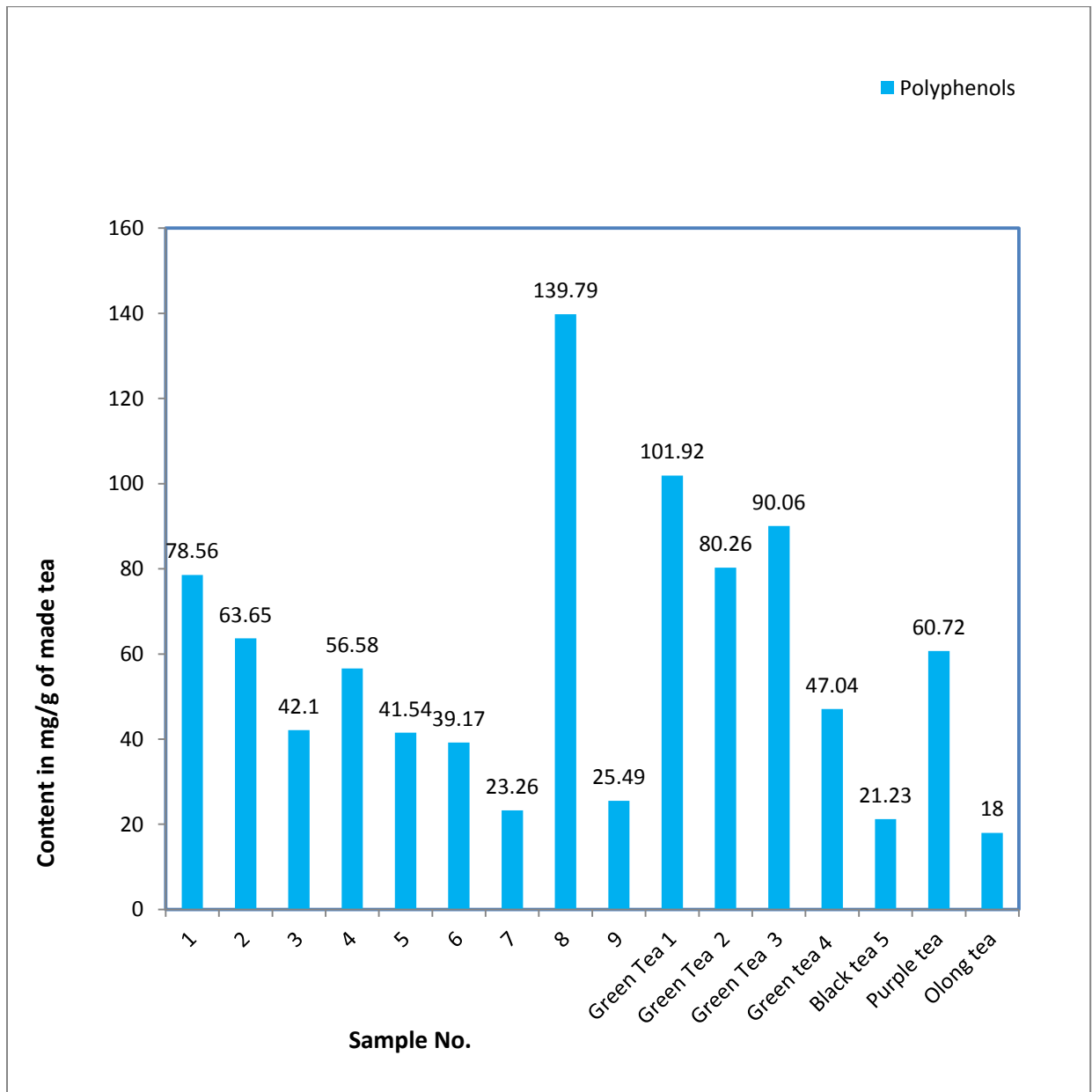* Procured from the local market for comparison purposes

Figure 5.5: **Comparison of Polyphenol content in LTNP and Non-LTNP withered tea samples**
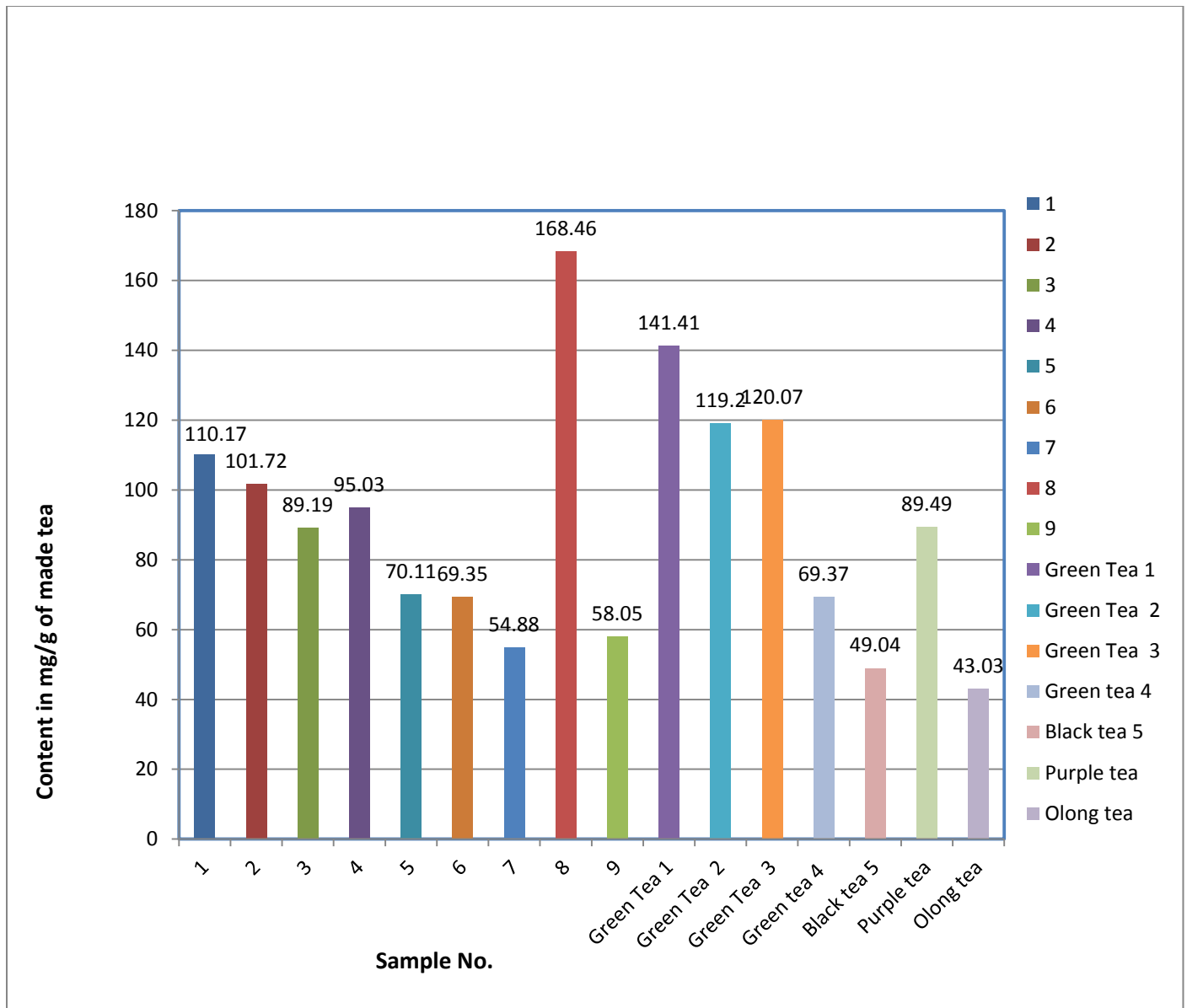
Figure 5.6 **Comparison of sum total polyphenol-caffeine content in LTNP and Non-LTNP withered teas**

5.3.1 Effect of LTNP on Polyphenol Content

Sample Green tea 1 withered under LTNP environment for one hour had the highest average *polyphenol* content of 78.56mg/g in made tea. Sample Green tea 2 had *polyphenol* content of 71.59mg/g in made tea. In general results as given in Table 5.1 indicate a decreasing trend of the *polyphenol* content in made tea (as shown by samples Green tea 1 – 7) with increasing withering time.

5.3.2 Effect of Non- LTNP on Polyphenol Content

Green tea leaf withered in Non-LTNP environments gave average *polyphenol* content of 139.8mg/g in made tea indicated by sample 8. Similarly, samples Green tea 1, Green tea 2 and Green tea 3 withered in Non-LTNP environments as shown in Table 5.3, gave *polyphenol* contents of 101.92mg/g, 80.26mg/g and 90.06mg/g in made tea respectively.

From the statistical analysis of Figure 5.6, can be seen that LTNP processed tea samples shows lower values of *polyphenol* concentrations compared to the non-LTNP processed tea.
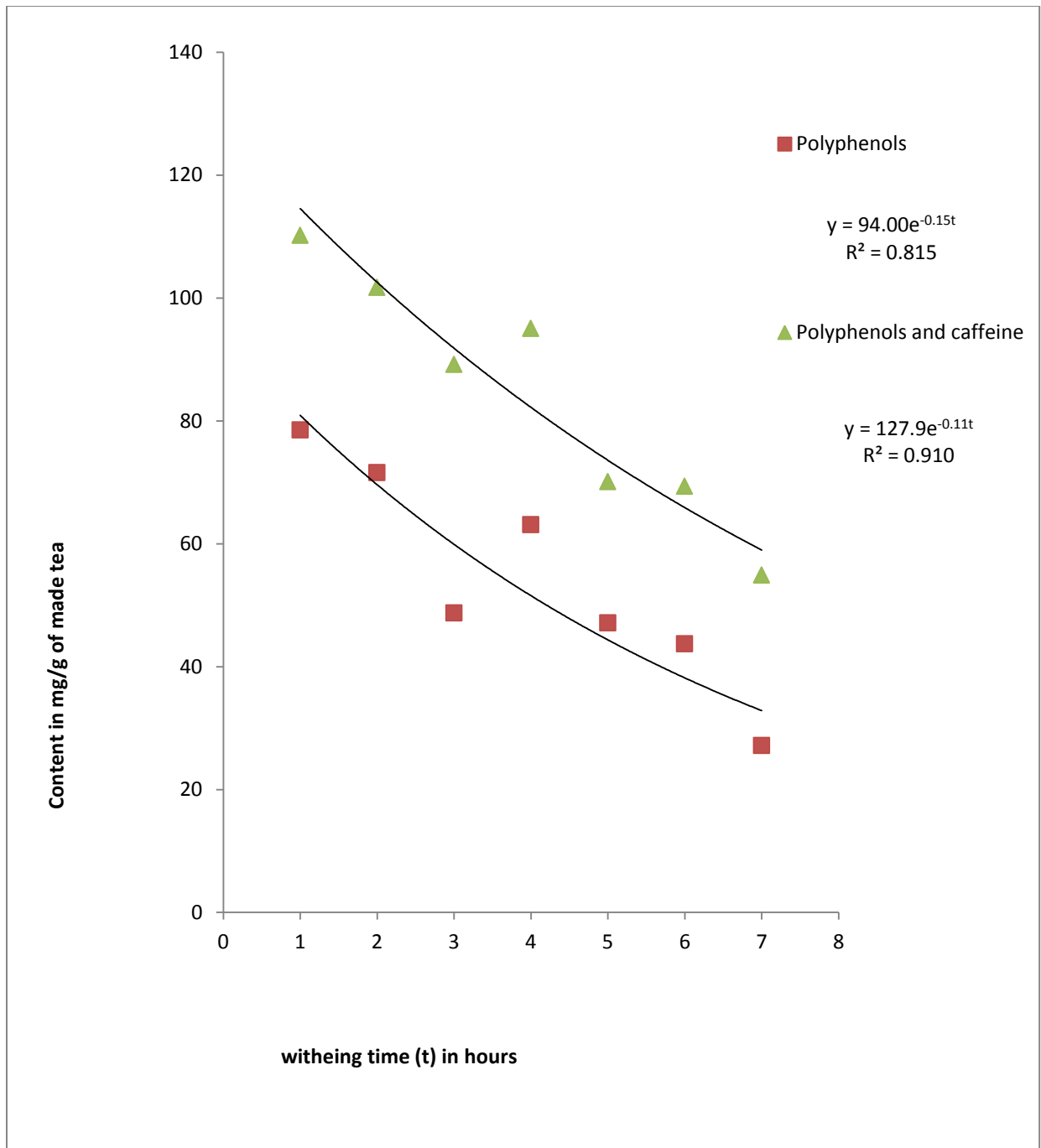
Figure 5.7   Total polyphenol and Polyphenol-caffeine contents

The chart contains the following annotations:

- Polyphenols
- $y = 94.00e^{-0.15t}$
- $R^2 = 0.815$
- Polyphenols and caffeine
- $y = 127.9e^{-0.11t}$
- $R^2 = 0.910$

Axis labels:
- Y-axis: Content in mg/g of made tea
- X-axis: witheing time (t) in hours

5.3.3 Statistical analysis of the rate of reduction of polyphenols

For purposes of determining the prediction equations that fit the relationships, the data in Table 5.1.was analyzed to determine the rate of reaction (Reduction) of *polyphenols* in the green tea leaf with increasing withering time. The calculated data was transformed into log-log relationships between the rate of reduction, withering time and remaining the *polyphenol* content in the green leaf as withering continued. The information was plotted on line graphs as in Figures 5.8 to 5.11 as follows;

Table 5.4   Relationship between polyphenol rate of reaction/rate of reduction and withering time

Withering time (t)	Polyphenols content mg/g in Made tea (P_c)	Rate of polyphenol reduction mg/g in made tea $(\frac{dP_c}{dt})^*$	Log (P_c)	Log $\frac{dP_c}{dt}$	Log (t)
1	78.51	78.51**	4.36	0.00	0.00
3	71.59	23.86	4.27	3.17	1.10
3.5	48.75	13.93	3.89	2.63	1.25
4	63.09	15.77	4.14	2.76	1.39
4.33	47.11	10.88	3.85	2.39	1.47
5	43.72	8.74	3.78	2.17	1.61
8	27.15	3.39	3.30	1.22	2.08

$(\frac{dP_c}{dt})^*$ - rate of change in *Polyphenol* content for ***each specific*** sample on its own measure from time zero

** - it is assumed that for each sample the *polyphenol* content at the commencement of withering was 78.51mg/g in **Made Tea** in line with the scope of samples

Table 5.4 shows changes of polyphenol content (ΔP_c) and withering time (Δt) which were determined for each individual sample from time zero (i.e. at $t = 0$). Therefore rate of polyphenol change ($\frac{\Delta P_c}{\Delta t}$) was for each individual sample from $t = 0$ to the indicated actual time.

Therefore $\frac{\Delta P_c}{\Delta t} \neq \frac{dP_c}{dt}$ because ΔP_c and Δt in Table 5.4 were not continuous for each sample. The reason being practically under the given circumstances it was not possible to draw a sample from the withering chamber and continue with **withering** while keeping LTNP environment. To do that the chamber had to be opened to collect a sample from the green tealeaf undergoing **withering**, which would have destroyed the LTNP environment. The mathematical function of the reduction in polyphenol content follows decay function. Let the mass of polyphenols be P_c in the green tealeaf at any given withering time (t). The rate of change of polyphenols (P_{co}) is given by;

$$P_{co} = \lim_{\Delta t \to 0}\left[\frac{\text{Change in polyphenol content } (\Delta P_c)\text{ in withering time } (\Delta t)}{\Delta t}\right]$$

$$= \lim_{\Delta t \to 0}\left[\frac{(P_{c1} - P_c)}{t - t_0}\right] = \lim_{\Delta t \to 0}\left[\frac{\Delta P_c}{\Delta t}\right]$$

$$= \frac{dP_c}{dt} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (5.1)$$

Separating the variables and integrating

$$\frac{dP_c}{P_{co}} = dt \dots\dots\dots\dots\dots\dots\dots\dots\dots (5.2)$$

$$-kt = \text{In } dP_c + \text{In C}\dots\dots\dots\dots\dots (5.3)$$

At time $t = 0,\ P_c = P_{c1}$

Hence C $= \frac{1}{P_{c1}}$ substituting into Equation (5.3)

$$-kt = \text{In } \frac{P_c}{P_{c1}}$$

$$k = \frac{1}{t}\text{In } \frac{P_c}{P_{c1}}$$

$$\frac{P_c}{P_{c1}} = e^{-kt}\dots\dots\dots\dots\dots\dots\dots\dots (5.4)$$

$$P_c = P_{c1}e^{-kt}\dots\dots\dots\dots\dots\dots\dots (5.5)$$

Equation 5.5 is the theoretical predictive Equation validated in the experiment.

$$y = 88.062e^{-0.438x}$$
$$R^2 = 0.9431$$

Rate of reduction (mg/H) — vertical axis

Withering time (H) — horizontal axis
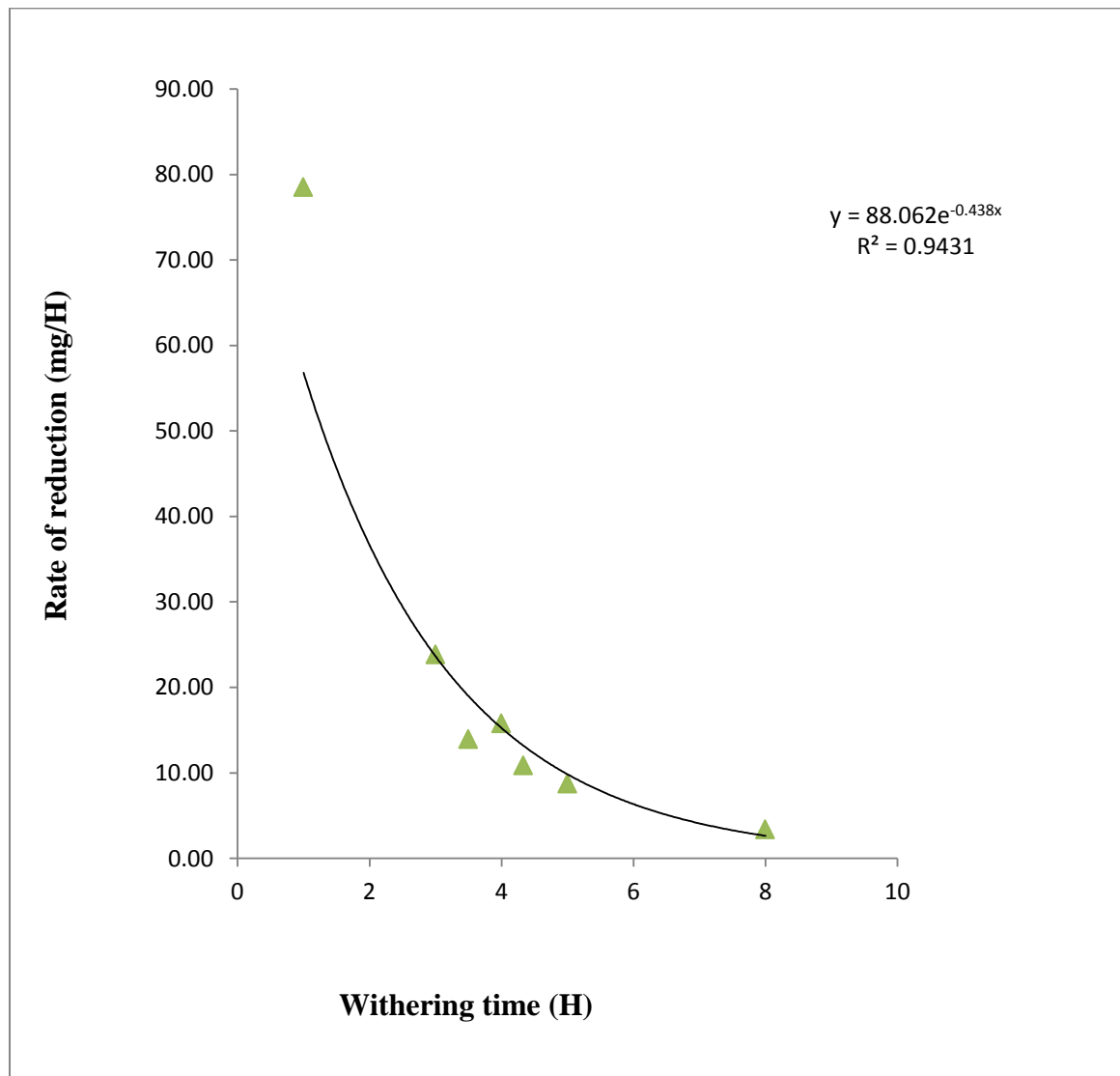
Figure 5.8   **Relationship between the rates of polyphenol reduction Vs withering time in LTNP environment**

The equation from statistical analysis using MS-Excel package from the graph of Figure 5.8 is given by Equation 5.10;

$$y = 88.06e^{-0.43x}$$ …………………………………………………………………… (5.10)

where ,   y   =   represents the rate of reaction/reduction of *polyphenol* $(\frac{dP_c}{dt})$

            x   =   withering time (t)

Expressing in terms of rate of reaction or rate of change of *polyphenols* $(\frac{dP_c}{dt})$ and withering time gives Equation 5.11.

$$\frac{dP_c}{dt} = 88.06e^{-0.43t}$$ …………………………………………………...…… (5.11)
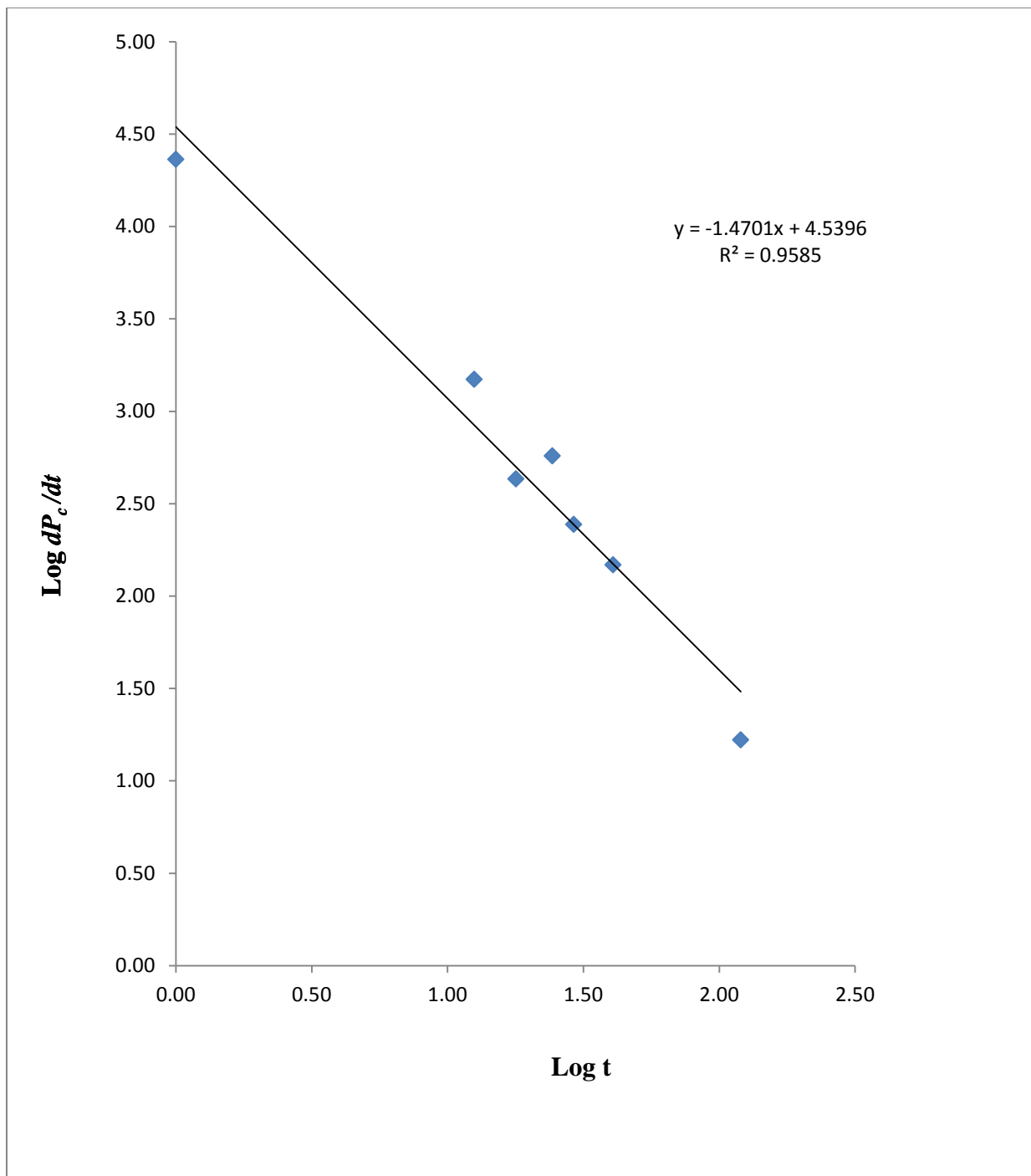
Figure 5.9 **Relationship of rate of polyphenol reduction Vs withering time expressed in Log ($\frac{dP_C}{dt}$) – Log (t) transformation**

Rate of *polyphenol* reduction against withering time as expressed in Log-Log transformation relationship gives the inverse of the exponential form in Figure 5.9.The relationship is given by Equation 5.12 from the graph:

y $\qquad$ = $\qquad$ -1.470x + 4.539 …………………………. (5.12)

Expressing using the formula terms gives Equation 5.13;

$\frac{dP_c}{dt}$ $\qquad$ = $\qquad$ -1.470x + 4.539 …………………………..… (5.13)

The negative sign indicates the inverse relationship, where one of the variables decreases while the other is increasing. In this case the *polyphenol* content is decreasing with increasing time. In overall the negative sign in Equation 5.13 indicates that the rate of *polyphenol* change with change in withering time.

$y = 0.8899e^{0.0514x}$
$R^2 = 0.8998$

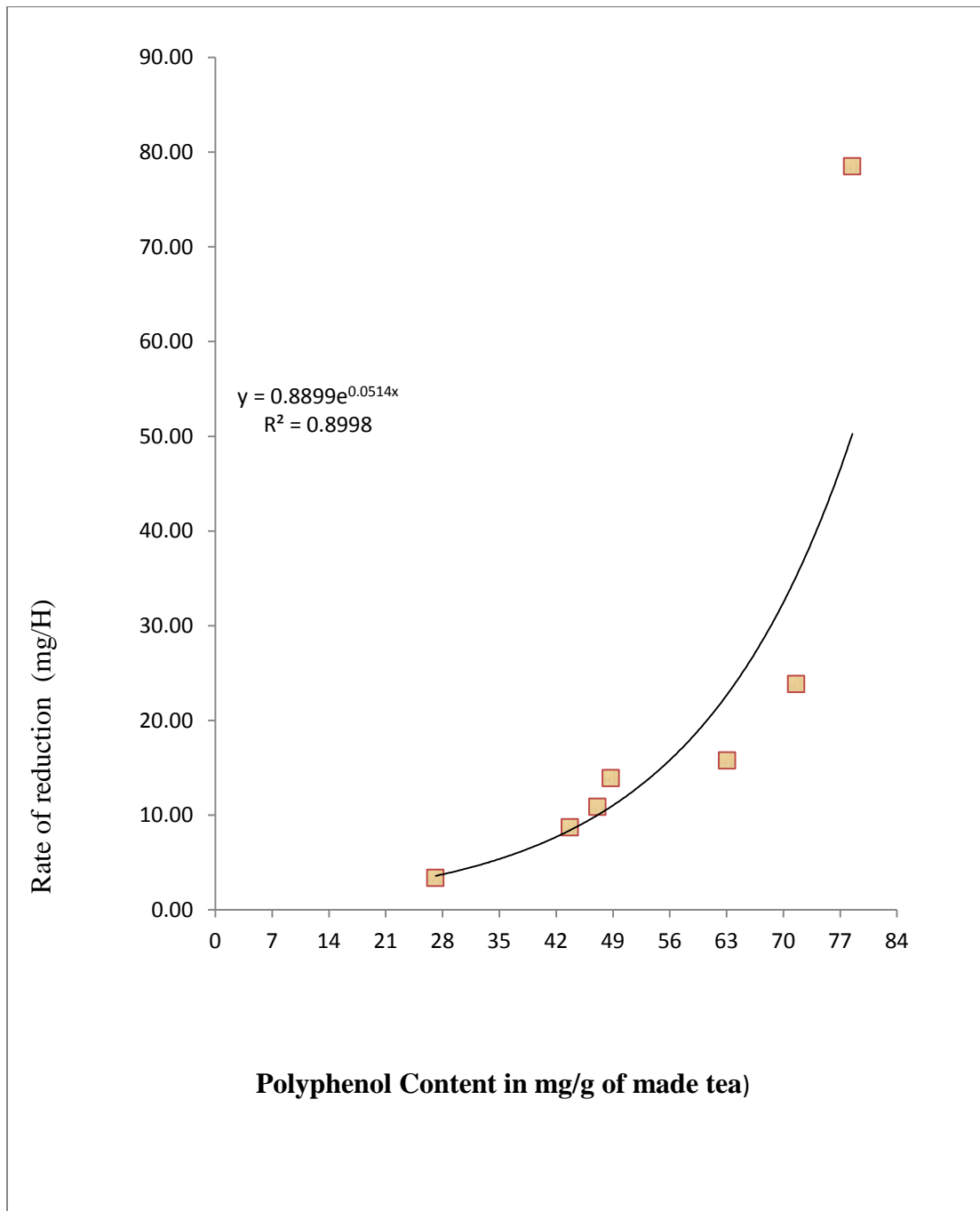**Figure 5.10** Relationship between the rates of polyphenol reduction $(\frac{dP_c}{dt})$ against remaining polyphenol content ($P_{c)}$ in green tea leaf during withering in LTNP environment

The relationship of Figure 5.10 above shows that, the rate of polyphenol reduction decreases with decreasing *polyphenol* content remaining in the green tealeaf during withering and follows an exponential function relationship. It also indicates that the kinetic order of the reaction during the withering process changes with reducing *polyphenol* content in the green tea leaf. The relationship is given by Equation 5.14.

$$y = 0.889e^{0.051x} \quad \text{...} \quad (5.14)$$

Where,   y   =   represents the rate of reaction/reduction of *polyphenol* $(\frac{dP_c}{dt})$

   x   =   content of *polyphenol* remaining in the green leaf (P_c)

Expressing Equation 5.14 in terms of rate of reaction $(\frac{dP_c}{dt})$ against remaining *polyphenol* in the tealeaf can be expressed as in Equation 5.15 below. The equation gave the coefficient of determination (r^2) of fitness of 0.899.

$$\frac{dP_c}{dt} = 0.889e^{0.051\,P_c} \quad \text{...} \quad (5.15)$$

$$y = -7.5521x + 84.483$$
$$R^2 = 0.8432$$
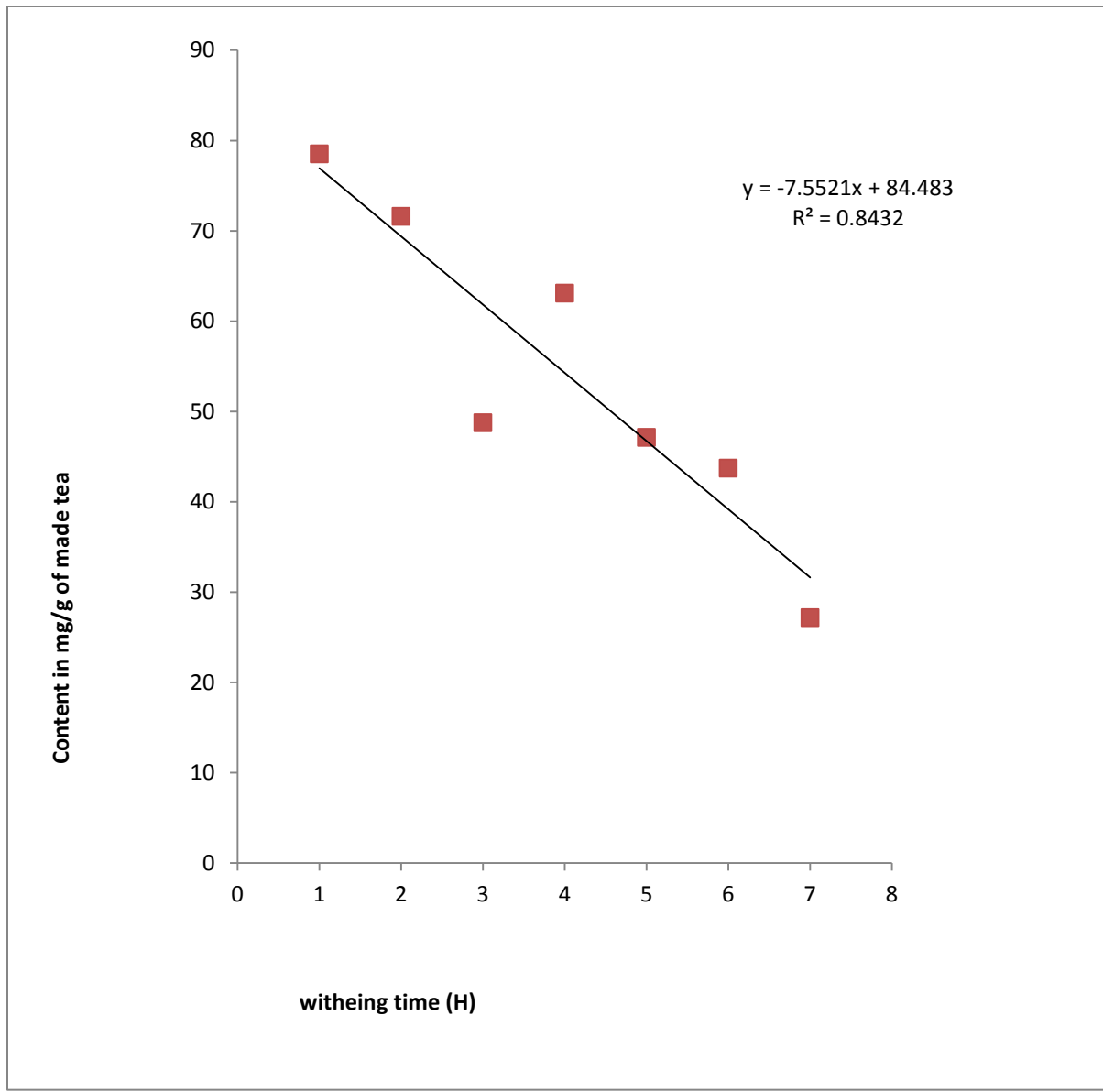
Figure 5.11 **Relationship of remaining polyphenol content in green tealeaf (P_c) against withering time (t)**

From Figure 5.11 above, the mean rate of change of *polyphenols* content in green tea leaf during withering can be predicted from Equation 5.16,

$$y = -7.552x + 84.48 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (5.16)$$

Expressed in terms of *polyphenol* (P_c) and withering time (t) for the case of this study gives Equation 5.17,

$$p_c = -7.552t + 84.48 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (5.17)$$

Table 5.5: Relationship of remaining Polyphenol content in tealeaf against rate of polyphenol reduction

Polyphenols (P_c)	Rate of reaction ($\frac{dP_c}{dt}$)	Calculated $\frac{dP_c}{dt}$ Vs P_c
(Mg/g of *Made Tea*)	Mg/Hour	($\frac{dP_c}{dt} = 0.889e^{0.051} P_c$)*
78.51	78.51	1.256
71.59	23.86	1.151
48.75	13.93	0.810
63.09	15.77	1.039
47.11	10.88	0.779
43.72	8.74	0.713
27.15	3.39	0.289

* From Figure 5.10 the relationship of rate of *polyphenol* reduction was directly proportional to the quantity of *polyphenol* remaining in the tealeaf i.e. rate of reduction decreased as the quantity of *polyphenol* remaining in the leave decreased according to the Equation   5.17.   The negative sign indicates the direction of change (reducing for this case).

Table 5.6: Relationship of remaining Polyphenol content in tealeaf against withering time

Retention time	Polyphenols(P_c)	Calculated P_c Vs t
(H)	(mg/g in *Made Tea*)	($y = -7.552x + 84.48$)**
1	78.51	76.928
3	71.59	61.824
3.5	48.75	58.048
4	63.09	54.272
4.33	47.11	51.780
5	43.72	46.720
8	27.15	24.064

**   From Figure 5.11 and Equation 5.17, at experimental time equals Zero the quantity of *polyphenol* (P_c) was 84.48mg/g in *Made Tea* and was reducing at the rate of 7.552mg/Hour according to Equation 5.17. The coefficient of determination (R^2) for best fit was $R^2 = 0.843$.

Table 5.7: Relationship of rate of polyphenol reduction ($\frac{dP_c}{dt}$) against withering time (t)

Retention time (t) (H)	Rate of reaction ($\frac{dP_c}{dt}$) mg/h	Calculated Log($\frac{dP_c}{dt}$) Vs Log(t) (y = -1.470x + 4.539)*
1	78.51	3.069
3	23.86	0.129
3.5	13.93	-0.606
4	15.77	-1.341
4.33	10.88	-1.8261
5	8.74	-2.811
8	3.39	-7.221

Table 5.7 shows that the rate of *polyphenol* reduction against withering time gives an inversely proportional relationship when values of rate of reaction $\frac{dP_c}{dt}$ against time t were transformed using log – log using equation 5.13.

5.4   Discussion

The decrease in *polyphenol* content in **Made Tea** was as a result of oxidation caused by the presence of oxygen within the LTNP environment in the withering chamber. Oxygen was formed from partial ionization of moisture that was released from green tea leaf due to the metabolic processes and by transpiration of the leaves.

The oxygen ions that eventually combined to form neutral oxygen gas in the process are shown by the ionization reactions occurring within the LTNP chamber as explained in Appendix C of the Oxygen model.

However in general the combination of nitrogen gas and moisture ions gave negatively charged, positively charged and neutrals/radicals particles in the LTNP plasma chamber.

These chemically reactive species initiated and controlled the distributions velocities and energies of the various particles including oxygen. As a result, oxidation of *polyphenols* occurred, leading to reduction of their content in the green tea leaf hence in **Made Tea**. This inference can be seen in samples of Oolong and Black teas (Table 5.2). Oolong and Black tea samples show lower polyphenol contents compared to other samples. The reason was due to fermentation leading to oxidation during their manufacturing process. Oolong tea is normally rolled up leaf, partially fermented and dried. While Black tea gives the lowest concentration due to the fact that it is macerated by cut, tear and crush process that exposes the cells to complete fermentation/oxidation, hence provided mores surface area for oxidation reactions during the fermentation process. Purple tea as given in Table 5.2 was manufactured using the Oolong tea method and as a result also underwent some form of fermentation/oxidation process. It can also be seen that prolonged storage of green leaf before processing drastically reduces the natural *polyphenol* content. The reason to this observation is due to fermentation (oxidation) of the tea leaf as indicated by sample 9 in Table 5.2. Diffusion of the oxygen particles to the *polyphenol* reaction sites is governed by the laws of diffusion. Oxygen ions are the most dominant/abundant from the given ionization particles as shown in appendix D. This may have led to increased

formation of neutral oxygen particles leading to diffusion into the leaf thereby occasioning oxidation of the *polyphenols*.

From Table 5.4, contents of the constituent compounds of *polyphenols* in LTNP withered tea shows a decreasing trend with increasing withering retention time. This observation is as a result of their conversion into other compounds due to oxidation which points to the reducing *polyphenols* in **Made Tea**. **Withering** time in both cases (LTNP and Non-LTNP) indicate an inverse proportional relationship with *polyphenol* content in **Made Tea**. However LTNP environment withered teas show shorter withering times in relation to the *polyphenol* content in **Made Tea**. The explanation to this observation, is due to the fact that, plasma environment provides energy for the reaction at a faster rate [11, 13] compared to the Non-plasma environments.

Other reasons are due to the presence of charged particles (electrons and protons) needed for molecular bonding could have enhanced the reaction [12, 13]. LTPs are non-equilibrium, with extensive range of positive and negative ions of varying mass and transport coefficients. In this nature, they provide a rich possibility of waves and instabilities which increase the reaction potential of the species within the system. In addition, the presence of particles in ionized form within the environment as can be seen from the ionization reactions above enhances the rate of reaction [11 - 13].

5.5   The prediction equation

The prediction equations are gotten from the relationship of Figures 5.8 to 5.11 which relate the following experimental result;

5.5.1   Rate of polyphenol content reduction in green tea leaf and the withering time

Equation 5.11 shows that the relationship between the rate of reduction of *polyphenols* and withering time follows an exponential function. The negative sign is an indicator of an inverse relationship, whereby the rate of *polyphenol* reduction is inversely proportional to the withering

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time. The coefficient of determination (0.9431) suggests the relationship between the Rate of reaction (reduction of *polyphenol* content in green tea leaf) and the withering time is 94.3% valid i.e. goodness of fit.

The reaction rate approaches a limiting minimum rate as the *polyphenol* concentration decreases. Similar to mass action, this kinetic order changes as the substrate concentration varies and the reaction rate is not constant but decreases with decreasing concentration of the reactants.

From the coefficient of determination of the relationship, the proportion of the variance rate of *polyphenol* reduction t is predictable from the given ***withering***. This measure of 0.943 can allow us to determine how certain in making the predictions from the graph Figure 5.8 and model Equation 5.11. The coefficient of determination above, which is also the ratio of the explained variation to the total variation, can be used to predict the validity of model Equation 5.11. This implies that 94.3% of the total variation in the rate of reduction of *polyphenol* can be explained by the relationship between $\frac{dP_c}{dt}$ and t as described by the coefficient of determination (R^2). The other 5.7% of the total variation in $\frac{dP_c}{dt}$ remains unexplained.

5.5.2 Rate of polyphenol reduction ($\frac{dP_c}{dt}$) Vs remaining Polyphenol content (P_c) in the green tea leaf during withering

From the coefficient of determination of the relationship the proportion of the variance rate of reduction is predictable from the given content of *polyphenol* remaining in the green tealeaf being withered. This measure of goodness of fit (0.899) can allow us to determine how certain in making the predictions from the graph of Figure 5.10 and model Equation 5.13. The coefficient of determination above, which is also the ratio of the explained variation to the total variation, can be used to predict the validity of model Equation 5.13. This implies that 90% of the total variation in the rate of reduction of *polyphenol* can be explained by the relationship between $\frac{dP_c}{dt}$ and P_c as described by the regression equation. The other 10% of the total variation in $\frac{dP_c}{dt}$ remains unexplained.

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The relationship also indicates an exponential function, whereby as the concentration of *polyphenol* remaining in the leaf decreases, the rate of reduction decreases too. This explains that the kinetic order of the reaction changes with withering time and obeys the mass action law as predicted in section 24.2.

5.5.3 Polyphenol content remaining in green tealeaf (P$_c$) Vs withering time (t)

Again the negative sign indicates the inverse proportionality, such that *polyphenol* content decreases with increasing time.

When Equation 5.13 is differentiated with respect to withering time gives the mean rate of change of *polyphenol* as shown in Equation 5.18.

$$\frac{dP_c}{dt}[-7.552t + 84.48]dt = 7.552mg/h] \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (5.18)$$

This indicates that the mean rate of change in *polyphenol* content in green tea leaf withered in LTNP is 7.552mg/h.

NB: *Polyphenol* concentration in green tea leaf picked from Limuru changes at the rate of 7.552 mg/h during green leaf withering in Low Temperature Nitrogen Plasma environment according to the mathematical prediction Equation 5.17**.**

5.6   Summary of contribution to knowledge

- Low Temperature Nitrogen Plasma (LTNP) environment can be used for withering green tealeaf.

- Green tealeaf withering in Low Temperature Nitrogen Plasma (LTNP) environment reduces *polyphenols* content.

- The rate of reduction is faster than when green tealeaf is withered in anaerobic nitrogen rich environment.

- The reduction is due to oxidation to other compounds as a result of the presence of oxygen. Oxygen was generated from water molecules produced from metabolic and transpiration processes taking place in the green tealeaf and or any moisture present on the green tealeaf. The oxygen was synthesized in the LTNP environment from the excitation, de-excitation, ionization, de-ionization, fragmentation, dissociative ionization and dissociative attachment processes occurring in the LTNP environment. The generated radicals joined in the addition and substitution reactions causing chain reactions that produced a variety of species amongst them oxygen.

- Kenyan green tealeaf from the Limuru area withered in LTNP for one hour gave a *polyphenol* content of more than 78mg/g in **Made Tea** while the same tea withered in Non-LTNP environment for eighteen hours gave a *polyphenol* content of more than 133mg/g in **Made Tea**.

- The experimental results through mathematical modelling of the statistical data gave the following predictive equations shown in Table 5.1. The predictive equations were for the relationships of the rate of *polyphenol* reduction ($\frac{dP_c}{dt}$) against withering time (t), rate of *polyphenol* reduction ($\frac{dP_c}{dt}$) against remaining *polyphenols* content (p_c) in the green tealeaf and remaining *polyphenol* content against withering time all during withering.

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Table 5.8: Prediction equations

Relationship	Prediction equation
Rate of *polyphenol* content reduction ($\frac{dP_c}{dt}$) in green tea leaf against withering time (t)	$\frac{dP_c}{dt} = 88.06e^{-0.43t}$
Rate of *polyphenol* content reduction ($\frac{dP_c}{dt}$) in green tea leaf against remaining *Polyphenol* content (P_c) in the green tea leaf during withering	$\frac{dP_c}{dt} = 0.889e^{0.051\ P_c}$
Remaining *Polyphenol* content in green tea leaf (P_c) against withering time (t)	$p_c = -7.552t + 84.48$

CHAPTER 6

6.0   CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The study showed that withering green tealeaf in Low Temperature Nitrogen Plasma reduces *polyphenol* content in **Made Tea** with increasing withering time. When compared to green tealeaf withered using Non-LTNP environment, the contents of *polyphenols* in **Made Tea** withered in LTNP were less than those of teas withered in Non-LTNP environment (Tables 5.1 and 5.2 respectively).

Both LTNP and Non-LTNP withered teas gave higher maximum contents of *polyphenols* than the existing locally manufactured teas in Kenya i.e. Green tea, Purple tea, Oolong tea and Black tea. The rate of *polyphenol* reduction is faster in LTNP than in Non-LTNP withered green tealeaf.

Also observed in the investigation, was that a sample directly macerated and dried gave a maximum higher *polyphenol* concentration of 111.70mg/g in **Made Tea** than the existing locally manufactured and LTNP withered teas. However this method could only be adopted if other compounds formed simultaneously during withering are not considered e.g. Gamma Amino Butyric Acid also synthesized in anaerobic [4]environments. The investigation also found that, Kenyan green tealeaf (From Limuru) can be used for production of Made Tea with relatively high content of *polyphenols* (up to 133mg/g in made tea) using Non-LTNP withering environments which can be used for health beneficial effects for mitigation and cure of cancer, diabetes, high blood pressure as per the literature.

The ionization density of the LTP environment is governed by the strength of the applied electrical field, moisture released and the concentration of the plasma gas (nitrogen) inside the DBD chamber. It is therefore possible that the ionization density in LTNP environment can be controlled and used in withering green tea leaf for purposes of controlling *polyphenol* degradation during withering stage. These can be achieved by varying and controlling the electrical power voltage, nitrogen gas concentration within the DBD chamber and the withering

time.

The predictive equations of the various relationships indicated that the rate of reduction of *polyphenols* during withering in LTNP with respect to withering time follows exponential function relationship.

6.2 Recommendations

There are several factors which affect polyphenol content in **Made Tea** as mentioned in this study. This study only focused on the withering environment using Low Temperature Nitrogen Plasma. Therefore the following recommendations are suggested for further studies:

- The effect of Leaf maturity, Variety and Clones, Agronomical practices, Climatic and soil conditions on the content of *polyphenols* in **Made Tea**.

- Due to the fact that there are other varieties and clones of tea grown in other parts of Kenya, more research should be carried out to find out the contents of *polyphenols* and other compounds using similar plasma and non-plasma technologies.

- Clinical studies should be done using the researched tea samples to find out their efficacy in the treatment and medication of cancer, diabetes, high blood pressure and other ailments which *polyphenols* have been proved to aid.

- The analysis of the green tealeaf samples withered in LTNP to ascertain for any new compounds that would have been formed in the process in view of the fact that, this study was one of the pioneering works of using LTP on tea processing.

- The use of LTP for treatment of the other organic living tissue material and analysis to ascertain for any new chemical compounds beneficial to humans that would have been formed in the process

- LTNP should be used to study its effect on treatment of other agricultural produce (maize, wheat, rice, coffee, etc) for purposes of preservation e.g. for the case of maize the destruction of the mold that produces afflatoxin.

- Treatment of seeds using LTNP of the many agricultural produce for purposes of increasing their germination rate in this part of the world.

- LTNP should also be used to study its effect on chemical compositions of other compounds in organic living tissue agricultural produce.

- LTNP should be used for the treatment of locally produced effluents from e.g. tea factories for purposes of waste management.

- More efforts in utilization of plasma types (technology) in other fields of science e.g. in generation of energy.

- Extensive study and manufacture of teas with different quality attributes based on various quantities of bioactive compounds found in tea and other locally available agricultural produce that are beneficial to human health.

- Processing of herbal medicinal plants in LTNP with a view of retaining compounds which would have otherwise been lost during drying.

- Etc

NB. Introduce the teaching of plasma science and engineering in colleges and universities because of its gaining importance in human life and by the fact that it has been regarded as the technology of the future.

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APPENDICES

Appendix A: **Ten largest Tea producers in the world**

Item No.	Country	Production 2011 (metric tons)	% of World Total	% Change from 2010
1	China	1,640,310	35.13%	+ 11.77%
2	India	966,733	20.70%	- 2.46%
3	Kenya	377,912	8.09%	- 5.28 %
4	Sri Lanka	327,500	7.01%	- 1.17 %
5	Turkey	221,600	4.74%	- 5.70 %
6	Vietnam	174,900	-	-
7	Indonesia	150,851	-	-
8	Japan	110,000	-	-
9	Argentina	76000	-	-
10	Iran	60,000	-	-

Sources: FAOSTAT data: 2011 (last accessed May 2013)

Appendix B: HPLC peak area Chromatograms
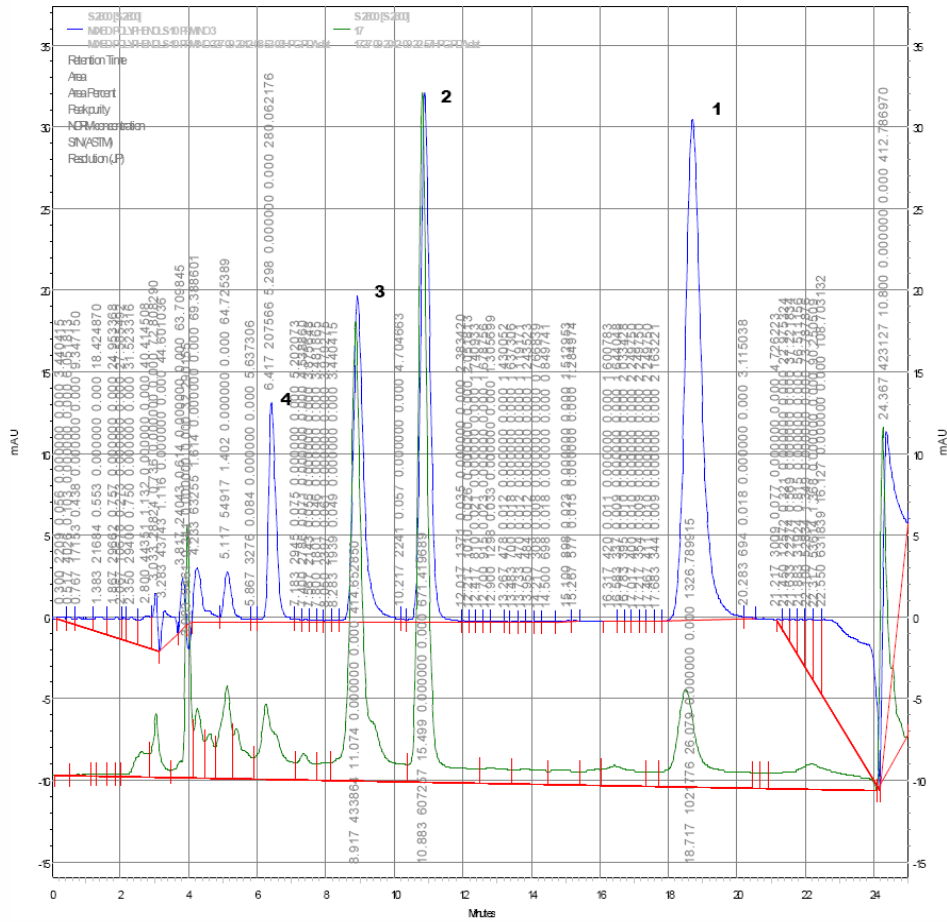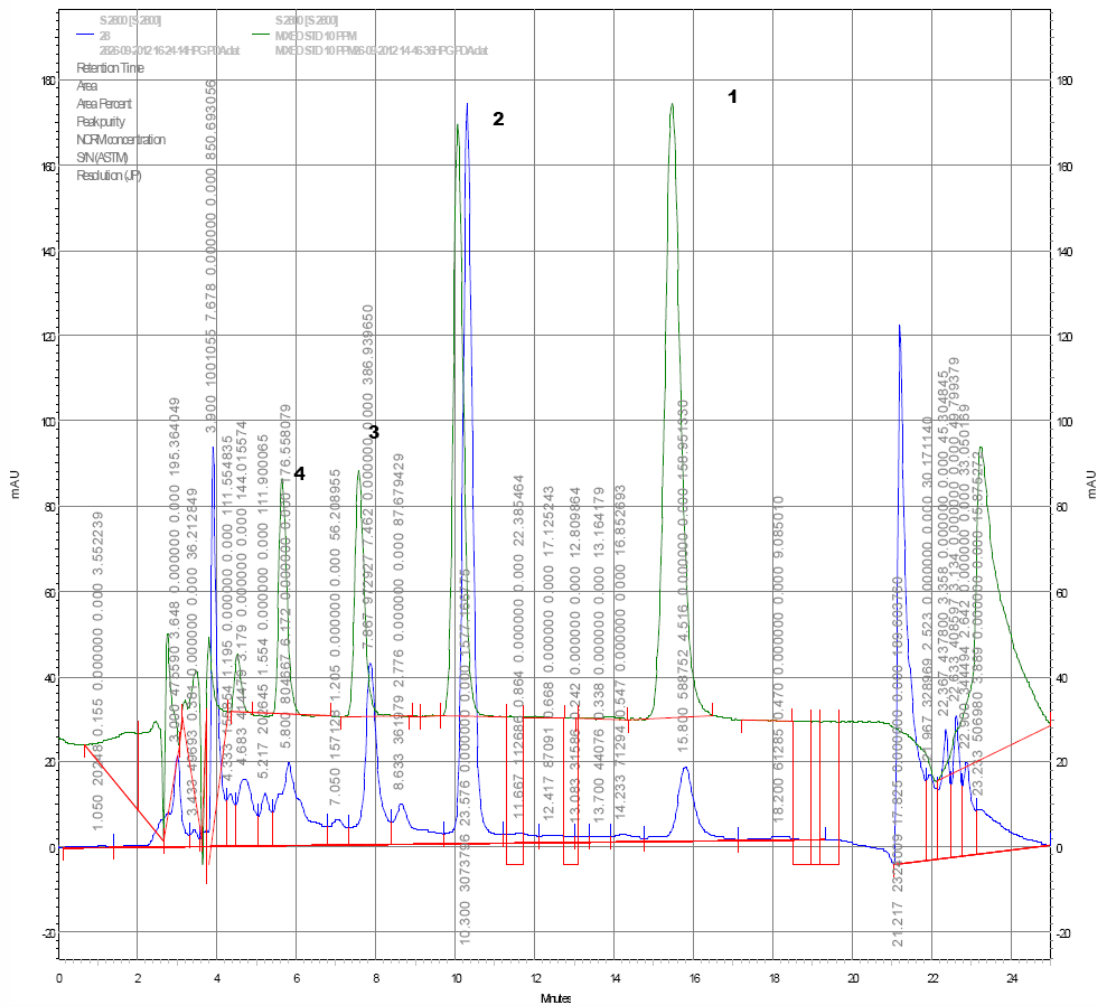
1- epigallocatechin gallate

2- Caffeine

3 - Epicatechin gallate

4 -Epigallocatechin

NB: upper chromatogram is the standard while lower is for the sample

Chromatogram 1: Nitrogen plasma withered tea Polyphenol and caffeine concentrations

1-epigalocatechin gallate

2 caffeine
3 epicatechin gallate
4-epigallocatechin

NB: upper chromatogram is for the standard while lower is for the sample

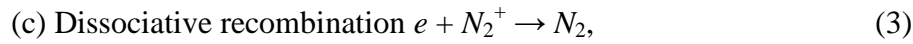**Chromatogram 2: Non-plasma withered tea Polyphenol and caffeine concentrations**

Appendix C: **Modeling of Dielectric Barrier Discharge Plasma**

The value of separated space charge after ionization has been shown to follow a path with electron deposition downstream forming near the negative electrode. A time averaged force, predominantly downstream with a transverse component towards the wall, acts on the plasma in forward direction.
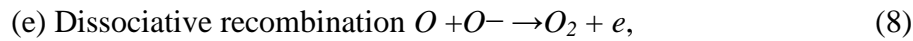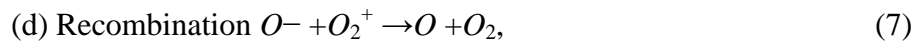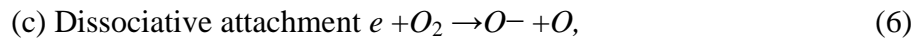
The equations governing chemistry of the reaction are as follows;

The metastable species including N_4^+ and O_4^+ are neglected due to their fast recombination rates. Also, NO is not included to reduce the numerical complexity in the first principles model at this stage.
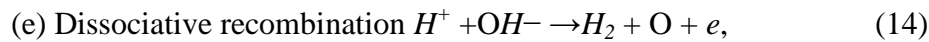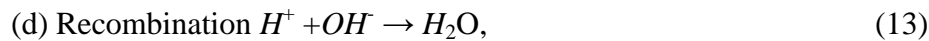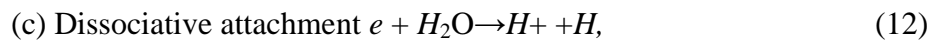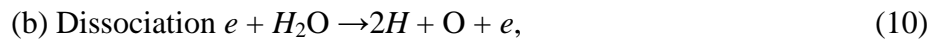
(1) Nitrogen model

(a) Ionization $e + N_2 \rightarrow N_2^+ + 2e,$ (1)

(b) Dissociation $e + N_2 \rightarrow 2N + e,$ (2)

(c) Dissociative recombination $e + N_2^+ \rightarrow N_2,$ (3)

(2) Oxygen model

(a) Ionization $e + O2 \rightarrow O_2^+ + 2e,$ (4)

(b) Dissociation $e + O2 \rightarrow 2O + e,$ (5)

(c) Dissociative attachment $e + O_2 \rightarrow O- + O,$ (6)

(d) Recombination $O- + O_2^+ \rightarrow O + O_2,$ (7)

(e) Dissociative recombination $O + O- \rightarrow O_2 + e,$ (8)

(3) Water model

(a) Ionization $e + H_2O \rightarrow H^+ + OH^- + 2e,$ (9)

(b) Dissociation $e + H_2O \rightarrow 2H + O + e,$ (10)

(c) Dissociative attachment $e + H_2O \rightarrow H+ +H,$ (12)

(d) Recombination $H^+ + OH^- \rightarrow H_2O,$ (13)

(e) Dissociative recombination $H^+ + OH- \rightarrow H_2 + O + e,$ (14)
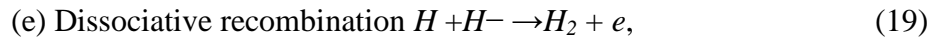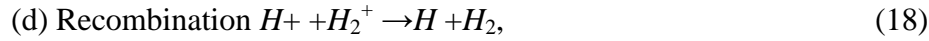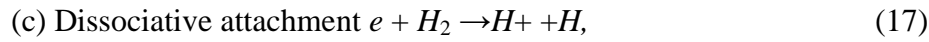
In the collision-induced dissociation environment, the primary interest is large charge transfer between two atoms. Ion separation occurs in the time scale of tens of femto-seconds (i.e. 10^{-15} of seconds), whereas the response from the sample molecules due to a changing electric field takes more than hundreds of femto-seconds. The charge exchange between hydrogen and oxygen atoms, therefore, proceeds practically instantaneously within one molecule. [40]

(4) Hydrogen model

(a) Ionization $e + H_2 \rightarrow H_2^+ + 2e,$ (15)

(b) Dissociation $e + H_2 \rightarrow 2H + e,$ (16)

(c) Dissociative attachment $e + H_2 \rightarrow H+ +H,$ (17)

(d) Recombination $H+ +H_2^+ \rightarrow H +H_2,$ (18)

(e) Dissociative recombination $H +H- \rightarrow H_2 + e,$ (19)

The derived and simplified drift-diffusion form of continuity and Poisson's equations for the neutrals of oxygen and nitrogen are as follows,

$$\frac{\partial n_e}{\partial t} + \nabla(n_e + v_e) = k_1 n_e n_{N_2} - k_3 n_e n_{N_2} + (k_5 - k_7)n_e n_{O_2} + k_9 n_e n_{O_2}$$

         -    Neutral oxygen and nitrogen   (20)

$$\frac{\partial n_N}{\partial t} = 2k_2 n_e n_{N_2} - \nabla(n_{N_2} v_{N_2})$$ -   Neutral nitrogen   (21)

$$\frac{\partial n_{N_2}}{\partial t} = -(k_2 + k_1 + \nabla)n_e n_{N_2}$$ -   Neutral nitrogen   (22)

$$\frac{\partial n_{N_2^+}}{\partial t} = -k_1 n_e n_{N_2} - \nabla(n_{N_2})$$ -   Neutral nitrogen   (23)

$$\frac{\partial n_{O_2}}{\partial t} + \nabla(n_{O_2} v_{O_2}) = (k_4 + k_5 + k_6)n_e n_{O_2}$$ -   Neutral oxygen   (24)

$$\frac{\partial n_O}{\partial t} = (2k_2 + k_6)n_e n_{O_2}$$ -   Neutral oxygen   (25)

$$\frac{\partial n_{O^-}}{\partial t} = k_6 n_e n_{O_2}$$ -   Neutral oxygen   (26)

$$\frac{\partial n_{O_2^+}}{\partial t} = k_4 n_e n_{O_2}$$ -   Neutral oxygen   (27)

∇ -   Curl operator representing the non-linear velocity of the species in the ensemble at any particular point P(x, y, z).

N_2 -   Neutral nitrogen

O_2 -   Neutral Oxygen

The derived and simplified drift-diffusion form of continuity and Poisson's equations for the neutrals of oxygen and nitrogen indicates that the production of neutral oxygen and nitrogen are a function of time.

Above, $1 k$ ......$k19$ are various rate coefficients related to Equations (20) through (27) obtained from Kossyi et al.

Appendix D: **Definition of terminologies**

Green tealeaf	-	Fresh green tealeaf plucked from mother bush for processing
Made tea	-	Processed and finished tea that is ready for brewing tea beverage
Withering	-	Can be physical withering in which moisture content in the leaf is reduced by approximately 3% or chemical withering in which complex organic compounds are broken down to simpler compounds.
Maceration	-	It is a process in which withered green tealeaf is cut, crushed and torn to smaller particle.
Fermentation	-	It is a process in which oxidation of the compounds takes place resulting in change of colour of the macerated leaf from green to dark brown and when dried leads to black tea.
Catechins	-	Are the building blocks of tannins in plants
Polyphenols	-	Are naturally occurring organic compounds which are beneficial to human health for mitigation against cancer, diabetes, cardio-vascular diseases etc.
Black tea	-	It is made tea which has undergone complete fermentation/oxidation during processing
Oolong tea	-	Made tea which has undergone partial fermentation/oxidation during processing
Green tea	-	Made tea which did not undergo any form of oxidation during processing.
Purple tea	-	Derives its name from the colour of its leaves and can be processed using any of the above three procedures i.e. Black, Oolong and Green tea .
Special teas	-	Are teas along the stages of Figure1 processed under specific conditions e.g. GABA tea

GABA tea	-	Withered in nitrogen rich environment, macerated and dried
Plasma	-	It is known as the fourth state of matter after solids, liquids and gases created by ionization of elements and compounds.
Low Temperature Plasma	-	Partially ionized and non-equilibrium medium/phase comprising of ions, neutral atoms, electrons and other particles.
Low Temperature Nitrogen Plasma	-	Partially ionized and non-equilibrium phase comprising of nitrogen ions, neutral atoms, electrons and other particles.
Biomaterials	-	Are materials which are compatible with living cells e.g. implants in human bodies or biocompatible medical devices
Bio-Systems Engineering	-	Engineering systems relating to biological processes
Biochemical Engineering	-	Chemical processes relating to biological process
Biomedical Engineering	-	Medical systems relating to biological processes
Nanotechnology	-	The science of materials/items whose thickness is in the range of nanometers i.e. 10^{-9}m
Nanostructures	-	Are structures whose basic units are in the sizes of nanometers.
Nano-material	-	Material whose basic unit is in the size of nanometers
Thermal state	-	Hot state in which the electron temperature (T_e) is approximately equal to heavy particle or sensible temperature (T_h)
Non-thermal states	-	Cold state in which the electron temperature (T_e) is much higher than the heavy particle or sensible temperature (T_h)
Living tissue	-	Materials whose cells undergo metabolic processes

Field ionization	-	The use of electric field to ionize a substance normally from one Kilovolt and above
Dielectric material	-	Materials which do not conduct electricity but allows electrons to pass through them, an example
	-	is Glass
Mathematical modelling	-	The formulation of mathematical equations that adequately describe a physical setup involving independent variables and outputs realized from which decisions on system design and control are based.

High Pressure Liquid Chromatography (HPLC) – Is an instrument used for analysis of compounds/elements through separation and derivatization

Photo 1: Picture of nitrogen gas, plasma source and Dielectric Barrier discharge chamber
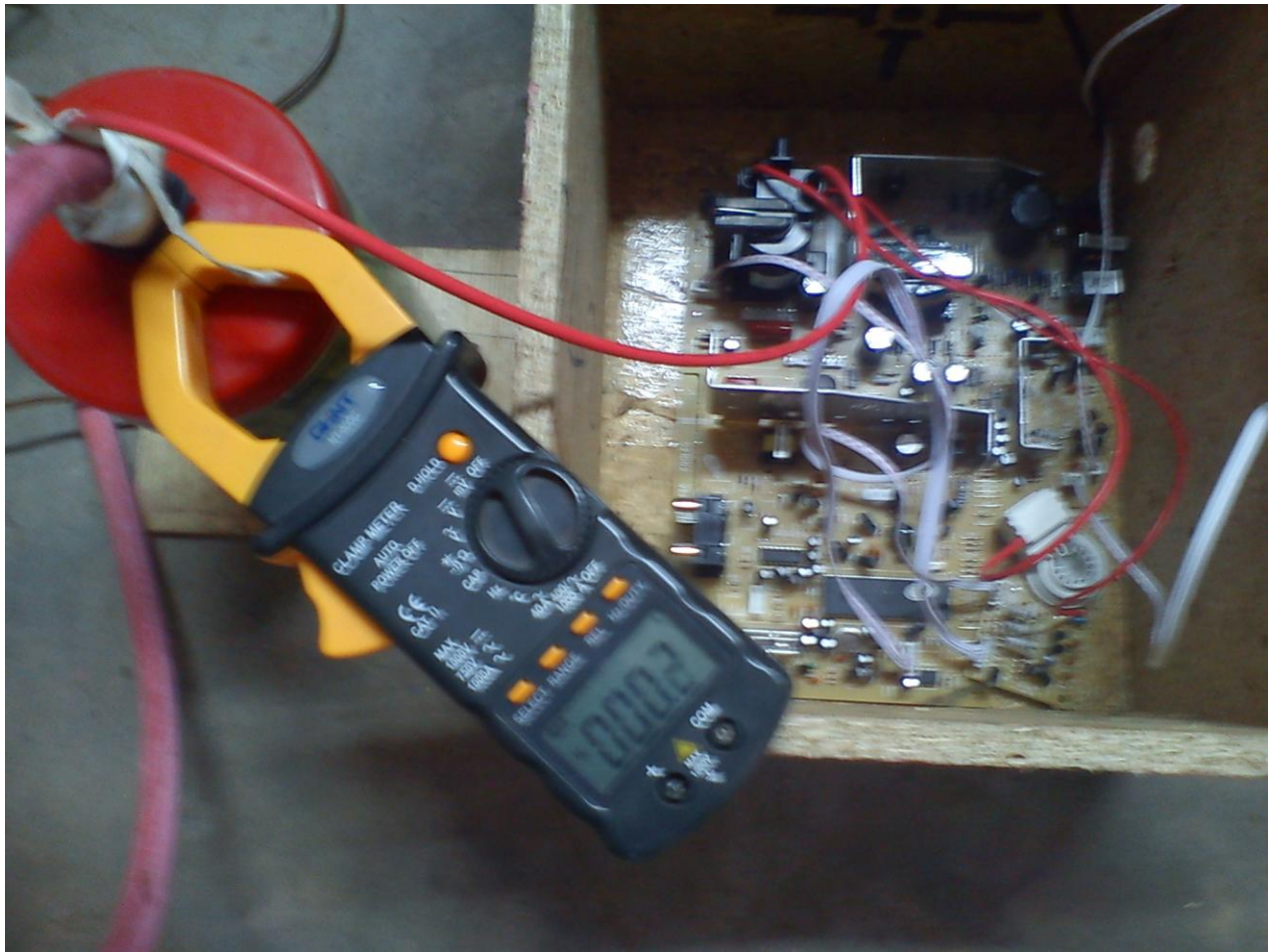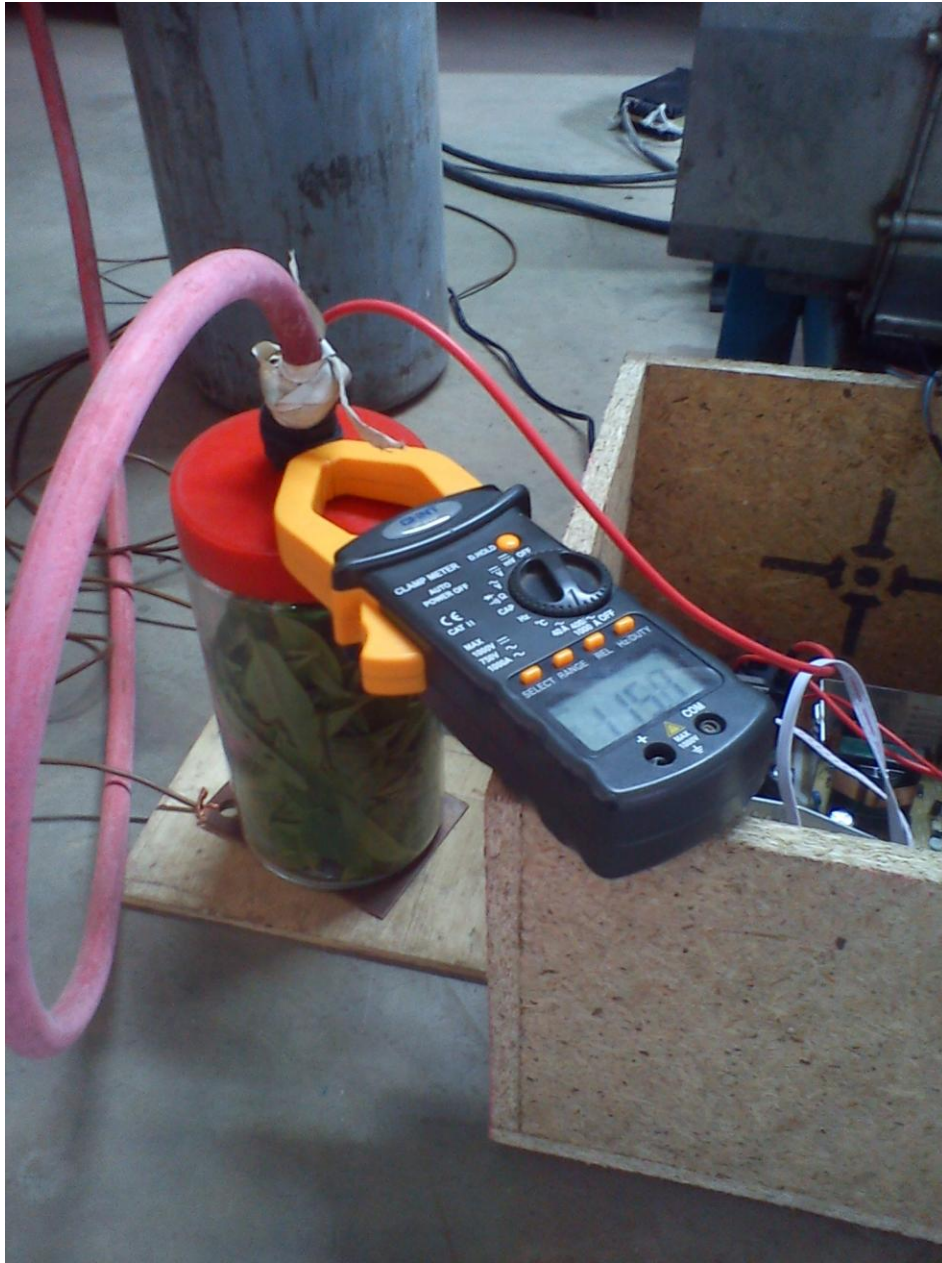
Photo 2: Picture of LTP source and Multimeter

Photo 3: Picture of nitrogen gas LTP plasma source