Mathematical model for the reduction of polyphenols in green tealeaf during withering in Low Temperature Nitrogen Plasma (LTNP) environment

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ABSTRACT

Green tealeaf was withered in Low Temperature Nitrogen Plasma (LTNP) environment. The result showed that LTNP decreases its naturally occurring Polyphenol content during withering. The decrease was caused by oxidation due to the presence of oxygen within the LTNP environment. The rate of reduction of polyphenols \( \frac{d P_c}{dt} \) with withering time (t) follows an exponential function and approaches a limiting minimum rate. Similarly \( \frac{d P_c}{dt} \) decreases as the remaining polyphenol content \( (P_c) \) in the green tealeaf decreases and also follows an exponential function relationship. 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 function relationship, such that \( P_c \) decreases with t. 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 4.877mg/h. The model prediction equations for \( \frac{d P_c}{dt} \) in green tealeaf against t, \( \frac{d P_c}{dt} \) against \( P_c \) and \( P_c \) against t were:
\[
\frac{d P_c}{dt} = 94.00 e^{-0.43t}, \quad \frac{d P_c}{dt} = 0.889 e^{0.051 P_c}, \quad \text{and} \quad P_c = -7.552t + 84.48
\] respectively. These model predictive equations can be used for the design and processing of green tea leaf to obtain \textit{Made tea} with specific polyphenol contents.

**Key words:** Green tea leaf, Low Temperature Plasma, polyphenols, reduction, predictive equation

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**Introduction**

**Plasma**

Plasma phase is the fourth state of matter after solids, liquids and gases. It is sometimes referred to as all states [Chen F. F, (1994), Heinlin J., Morfill G. and Landthaler M., (2010)] and comprises of electrons, protons, neutrons, photons, positive and negative ions, neutral molecules, atoms and a variety of other particles all existing in the same environment. Plasma environment is created by ionization of elements and gases among others [40-41]. 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 [Kim D., Gweon B., Moon S. and Choe W., (2010)]. Hence a broad spectrum of plasma types, characteristics and behaviors can be created from different types of atoms. The ratio of ions to neutral particles and the particle energies also determine the usefulness of the specific plasma. Their unique behaviors provide plasmas useful in a large and growing number of applications [Kim D., Gweon B., Moon S. and Choe W., (2010)]. For these reasons, numerous types of plasma can be made and one such type is Low Temperature Plasma (LTP).

**Low Temperature Plasma**

It is a partially ionized, non-thermal and quasi-neutral environment with electron temperature very high \(10^5\text{k}\) compared to the temperature of ions and neutral particles which remains at room temperature (i.e. about 300k) [Chiada B., AL-zubaydib T., Khaalafc M. and Khudiar A., (2009)]. Due to the small masses of electrons as compared to those of ions and neutral particles their collisions with ions and neutral species are elastic [Hippler R., (2008)]. Hence they do not transfer energy gained from the electric field with the ions and neutral particles. However ions and
neutral particles exchange energy due to their higher masses and their collisions are inelastic [Hippler R., (2008)]. LTP electrons have a large mean kinetic energy of up to 0.2 eV to 2 MeV per particle. They are charge carriers and their electromagnetic interactions have a substantial influence on the LTP system properties as a whole. Related to the quasi-neutrality and the presence of free charge carriers, the most fundamental attribute of the LTP state is its tendency to minimize external electric and magnetic fields inside the bulk, in contrast to its behavior in the surrounding sheaths [Hippler R., (2008)].

Applications of LTP

The main uses of LTP has been for: medical treatment especially in sterilization, treatment of malignant melanoma cells [Heinlin J., Morfill G. and Landthaler M., (2010), Sensenig R., (2010)] and surgery, bio-processes in agriculture and food as a non-chemical gas-phase disinfection agent, biomaterials and tea withering to control polyphenol content. It has also been used to study its influence on germination and early growth of wheat and oat seeds [Božena Šerá, Petr Špatenka, Michal Šerý, Nad’ežda Vrchotová, and Iveta Hrušková, (2010)], 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 [Kan Chi-wai, Chan Kwong and Yuen Chun-wah M., (2004)]. 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 and that UV emissions in LTP are negligible that is why can be used in surgery and treatment of food staffs like the current study [Mastwijk, H. and Nierop G., (2010)]. Research findings have found that, LTP 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 [[Chen F. F, (1994)].

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 [Kushner M. J., (2008)]. One of the challenges recommended for research was how LTPs interacts with organic materials and living tissue since their exposure in LTP environment results in chemical and structural changes both on the surface and inside the material [Kushner M. J., (2008)]. The interactions are said to be complex scientifically and can bring about complex scientific changes deep inside the bulk of the material [Kushner M. J., (2008)]. Investigation on plasma interactions with organic materials and living tissue is said to have been largely empirical
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 [Kushner M. J., (2008)]. 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 [Kushner M. J., (2008)]. 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 [Kushner M. J., (2008)]. 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. Destructive plasma is like sterilization of medical devices, surgery, etc. and non destructive treatment of wheat and oat seeds 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 [Kushner M. J., (2008)]. Hence comprehensive models of LTP on the vast chemistries and reactions occurring within these materials and tissues need to be researched and understood.

In view of these, the current study explores the use of LTP for chemical 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 in this case green tealeaf.

Certain types of LTP can efficiently kill bacteria without harming the cells and also influence the cells without causing cell death [Kushner M. J., (2008)]. 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 [Božena Šerá, Petr Špatenka, Michal Šerý, Nadˇežda Vrchotová, and Iveta Hrušková, (2010)]. It is also from this basis that LTP was used in the current study. 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 [57] to initiate certain reactions.

**Tea withering**
The withering of tea is a major unit operation in Black Tea manufacture. It is done to reduce moisture and to 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 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 non-breakdown of polyphenol constituents resulting in being optimized in Made Tea, which is desirable. Low Temperature Plasma (LTP) environment is known to form a sheath around solid particles and as a result create an anaerobic environment around the solid particles which is suitable for withering tealeaf to maximize the polyphenol content. Made Tea with higher polyphenol content is of better economic value as it would fetch higher prices.

Polyphenol are broken down by Low Temperature Nitrogen Plasma [Kuloba et al 2012]. The presence of oxygen is due to respiration and transpiration of the green tea leaves as a result of metabolic processes [Kuloba et al 2012]. Therefore, Polyphenol reduction in Made Tea at withering stage is the problem this study investigates. The study looked at using LTP technology to create a suitable withering environment essential for withering green tealeaf with the aim of reducing polyphenol oxidation and maximizing its content in Made Tea. Numerous studies have found that Polyphenols are beneficial to human health [Katiyar S.K., Mukhtar H., (1997), Itaro O., (2000), Obanda M. and Owuor P.O., (1992)] for mitigation and treatment of cancer, diabetes, cardiovascular diseases, high blood pressure etc. Hence their availability in Made Tea in sufficient and controlled amounts can greatly assist in the mitigation and treatment of the above health problems.

Polyphenols occur naturally in green tealeaf. The broad objective of this study was to identify the physical parameters which are pertinent to the reduction of polyphenols in Made tea and using the identified parameters and mathematical modeling arrive at the predictive equations for the reduction of polyphenols in Made tea. Polyphenol optimization in Made Tea is currently being done using anaerobic nitrogen rich environment, while the current study investigates nitrogen gas provided in an ionized form i.e. nitrogen plasma state 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. Polyphenols are a group of compounds and the six main ones found in green tealeaf are; Catechins (C), Epicatechin (EC), Epicatechin gallate (ECG), Epigallocatechin gallate (EGCG), Gallocatechin (GC), Epigallocatechin (EGC) [Claudia A, Graciela E. F. and Rosana F., (2008)]. And these are the component constituent compounds found naturally in tealeaf. These compounds are also the ones verified through analysis from the experimental data. The total sum of these constituent compounds is what gives the total polyphenol content in the green tealeaf. During tea processing Polyphenols are known to be catalyzed by the enzymes polyphenol oxidase (PPO) and Peroxidase (PO) to undergo oxidation process in the presence of oxygen [Claudia A, Graciela E. F. and Rosana F., (2008)]. Recent research by scientists from India in 2012 [Tea Research Association, (2012)] indicated that groups of polyphenols
components (catechins) react in pairs to form compounds called theaflavins.

**Factors affecting polyphenol in 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 [Katiyar S.K., Mukhtar H., (1997)]. The health effects of polyphenols depend on the amount consumed and on their bioavailability [Manach C., Scalbert A., and Morand C., (2004)] in foods. Hence the need for maximizing them in Made Tea is essential. Polyphenols content in foods varies according to the following [Manach C., Scalbert A., and Morand C., (2004)]: genetic factors, environmental factors and technological 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 state (conditions), etc. Some of these factors can be controlled to optimize polyphenol content in foods [Manach C., Scalbert A., and Morand C., (2004)]. The reason being that processing has an effect on polyphenol content e.g. fruit peeling, dehulling of legume seeds and removal of surface layer etc [Manach C., Scalbert A., and Morand C., (2004)]. Also the breakup of plant tissues like tea maceration may lead to degradation of polyphenol due the increase in the surface area leading to increased exposure of the polyphenols to oxygen.

**Biochemical reaction mechanism and model equation of Polyphenol in foods**

The overwhelming majority of reactions that occur within plant cells are catalysed by enzymes [Ingalls B. (2012)]. 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. 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 [Ingalls B. (2012)]. This specificity of action allows each enzyme to function with remarkable efficiency increasing reaction rates by as much as $10^7$ times [Ingalls B. (2012)]. 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 [Ingalls B. (2012)]. However in contrast with mass action, the kinetic order of enzyme driven reaction changes as the substrate concentration varies [Ingalls B. (2012)]. Most enzyme-catalysed reactions involve more than one substrate. In order to describe enzyme catalysis of these reactions, Michaelis-Menten [Ingalls B. (2012)] kinetic description is expanded to suit the reaction mechanism [Ingalls B. (2012)]. Catalysis of the irreversible two-substrate reaction e.g. polyphenols and oxygen can be represented in a reaction equation as follows [Ingalls B. (2012)],
**Governing equation**

Rate of reaction of two substrate species is given by;

\[ k_3 k_2 | t \] = \frac{k_3 e^{t} a(t) b(t)}{k_{-1} k_1 + k_{-2} + k_2 a(t) + k_3 b(t) + a(t) b(t)} \]  \[ \text{(2)} \]

\[ k_1, k_{-1}, k_2 \] = kinetic orders of the substrates

\[ e^{t} \] = total enzyme concentration

And can be written more concisely as equation 4 [Ingalls B. (2012)]

\[ v = \frac{V_{\text{max}} a b}{k_{AB} + k_B a + k_A b + a b} \]  \[ \text{(4)} \]

Equation 4 can be expressed as;

\[ v = m b \]  \[ \text{(5)} \]

Where \( m = \frac{V_{\text{max}} b}{k_{AB} + k_B a + k_A b + a b} \), \( v \) = rate of reaction, \( V_{\text{max}} \) = maximum rate of reaction, \( a = \) concentration of substrate Polyphenol, \( b = \) concentration of substrate Oxygen, \( k_A = \) rate constant of substrate polyphenol and \( k_B = \) rate constant of substrate oxygen. The equation validated in the experiments was to whether the relationship between the rates of 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 does not change as the substrate (polyphenol) content varies [Ingalls B. (2012)].

The overall objective was come up with mathematical model predictive equations for the reduction of polyphenols in green tealeaf withered in Low Temperature Plasma environment.

**Materials and methodology**

The materials used for this study was similar to one done by the same authors on a research titled “Investigation into the effect of Low Temperature Nitrogen Plasma on Polyphenol content during tea withering in made Kenyan tea” [Kuloba et al 2012]. A similar type of withering chamber i.e. Dielectric Barrier Discharge (DBD) chamber method was used to create LTNP environment [Kuloba et al 2012]. During the experimentation, three external parameters of
nitrogen plasma namely, electrical power input, temperature and pressure were kept constant and the effect on the content of polyphenols in made tea were studied against withering time. Sample collection and processing were similar to those done by Kuloba et al [Kuloba et al 2012] where fresh green tea leaf samples fifteen years old were collected from a smallholder farmer on different days from Limuru Kenya, a distance of about 50 Km from experimental site. Sampling and experimentation procedure was similar to that carried out by Kuloba et al [Kuloba et al 2012]. The samples were withered in LTNP environments as done by Kuloba et al [Kuloba et al 2012] for various withering time intervals. The samples were then macerated and dried.

Sample Polyphenol content analysis similar to Kuloba et al [Kuloba et al 2012]. Sample preparation for the study was also similar to as done by Kuloba et al [Kuloba et al 2012]. Standard solution preparation, Preparation of stock solution was similar to as done by Kuloba et al [Kuloba et al 2012]. Analysis of standards and calibrations was similar to as done by Kuloba et al [Kuloba et al 2012]. The Chromatographic conditions were similar to those used by Kuloba et al [Kuloba et al 2012].

**Results and Discussion**

**Observation**

Laboratory analysis gave results for various conditions and durations under which green tea samples were withered. From the Tables 2 indicates that there was a general decrease in the contents of each of the components 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

**Effect of LTNP on Polyphenol Content**

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 Table 2. The results in Tables 2 were statistically analyzed using Microsoft Excel Package and presented in line graphs. The statistical analysis indicated the relationship between the rate of reduction of polyphenols in green tea leaf and withering time follows an exponential function (Figure 4). The equation of the trend line has a negative sign which was an indicator of an inverse relationship. 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
coefficient of determination, indicated the relationship follows also an exponential function, i.e. as the content of polyphenols in the green tea leaf decreased, its rate of reduction decreased too. The reaction rate approaches a limiting minimum rate as the substrate (polyphenol) concentration decreases (figure).

**Discussion**

The decrease in polyphenol content in made tea was as a result of oxidation caused by the presence of oxygen within LTNP plasma environment in the withering chamber. The presence of moisture is as a result of moisture released from green tea leaf due to metabolic processes undergoing partial ionisation. Some of the oxygen ions eventually combined to form neutral oxygen gas in the process.

From table 2, concentration of the constituent compounds of polyphenols in LTNP withered tea shows a decreasing trend with increasing withering time. This observation is as a result of their conversion into other compounds due to oxidation thereby reducing polyphenols in made tea. Withering time in both cases, (LTNP and Non-LTNP) indicate an inversely proportional relationship with polyphenol content in made tea. However LTNP environment withered teas shows 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 a reaction at a faster rate [Mark J. Kushner, 2008] compared to the Non-plasma environments. Other reasons are due to the presence of charged particles (electrons and protons) needed for molecular bonding also enhanced the reaction [Mark J. Kushner, 2008]. 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 increases the reaction potential of the species within the system [Mark J. Kushner, 2008]. On addition, the presence of particles in ionised form within the environment as can be seen from the ionisation reactions above enhances the rate of reaction.

**Conclusion**

The study showed that LTNP green tea leaf withering has an effect on polyphenol concentration in made tea compared with made teas withered using anaerobic technologies. The concentrations of polyphenols in made tea withered in LTNP were less than those of tea withered in Non-LTNP environment. Both LTNP and Non-LTNP withered teas gave higher contents of polyphenols than the existing locally manufactured teas. Compared with existing locally manufactured teas, LTNP withered tea gives higher polyphenol concentration in a shorter withering time. Also observed in the investigation, was that a sample directly macerated and dried gave a higher polyphenol concentration of 111.70mg/g in made tea than the existing locally manufactured and LTNP withered teas.
Ionization density is governed by the strength of the electrical power voltage and the concentration of the gas (nitrogen) inside the DBD chamber. 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 retention time.

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 taking place within and by transpiration of the leaves. The oxygen ions that eventually combined to form neutral oxygen gas in the process are shown by the ionisation reactions occurring within the LTNP chamber. 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 [Kushner M. J., (2008)]. As a result, oxidation of polyphenols occurred, leading to reduction of their content in the green tea leaf. Other reasons are due to the presence of charged particles (electrons and protons) needed for molecular bonding also could have enhanced the reaction [Kushner M. J., (2008)]. 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 increases the reaction potential of the species within the system [Kushner M. J., (2008)]. On addition, the presence of particles in ionised form within the environment as can be seen from the ionisation reactions above enhances the rate of reaction.

Statistical analysis and the prediction equation

For purposes of determining the prediction equations that best fit the relationships, the data was analyzed to determine the rate of polyphenol reduction in the green tealeaf during withering. The equation from statistical analysis using Ms-excel package from the graphs of Figures 1 to 3, gave the following equations in Table 1. The equations were used to calculate the value of the rate of reduction against time, rate of reduction against remaining polyphenol
content in the green tealeaf and the polyphenol content remaining in the green tealeaf against withering time as shown in Table 2. The results were as follows.

- **Rate of polyphenol reduction in green tea leaf against withering time**

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

- **Rate of polyphenol reduction Vs remaining content of Polyphenol in the green tea leaf during withering**

  The relationship 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 provided from the theory [Ingalls B. (2012)].

- **The polyphenol content remaining in the green tealeaf during withering against withering time**

  The relation shows an inverse relationship indicating that, polyphenols were converted into other compounds hence reducing them in Made tea.
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