ANTIOXIDANT PROPERTIES IN GREEN TEA AS INFLUENCED BY PROCESSING TECHNIQUES

A Thesis

Submitted to the

Assam Agricultural University

In partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (AGRICULTURE)

IN

FOOD SCIENCE AND TECHNOLOGY PROGRAMME

DEPARTMENT OF HORTICULTURE

By

PARAMITA BHARADWAJ

(Registration No. 574 of 2014)

[Roll No. 2014-AMJ-76]

DEPARTMENT OF HORTICULTURE

College of Agriculture

ASSAM AGRICULTURAL UNIVERSITY

Jorhat-785013

JULY, 2017

ASSAM AGRICULTURAL UNIVERSITY
Faculty of Agriculture

CERTIFICATE – I

This is to certify that the thesis entitled "**Antioxidant Properties in Green Tea as influenced by Processing Techniques**" submitted to the Faculty of Agriculture, Assam Agricultural University, in partial fulfilment for the degree of **Master of Science (Agriculture)** in **Food Science and Technology Programme, Department of Horticulture,** is a record of research work carried out by **Ms. Paramita Bharadwaj, Regd. No.: 574 of 2014, Roll No.: 2014-AMJ-76**, under my personal supervision and guidance.

All help received by her have been duly acknowledged.

No part of the thesis has been reproduced elsewhere for any degree.

Dated, Jorhat
The ……. July, 2017.

_____
(Dr. Rana P. Bhuyan)
Major Advisor & Chairman
Professor
Department of Tea Husbandry and Technology
College of Agriculture
Assam Agricultural University
Jorhat-785013 (Assam)

CERTIFICATE – II

This is to certify that the thesis entitled "**Antioxidant Properties in Green Tea as Influenced by Processing Techniques**" submitted by **Paramita Bharadwaj**, **Regd. No.: 574 of 2014, Roll No. 2014-AMJ-76** to the Assam Agricultural University, in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Food Science and Technology Programme, Department of Horticulture** has been examined and approved by the student's Advisory Committee after viva-voce.

(Rana Pratap Bhuyan
Major Advisor & Chairman

Chairman
Board of Examiner

Members of the Advisory Committee:

1. _____
Ananta Saikia

2. _____
Subhash C. Barua

3. _____
Samindra Baishya

_____
(Madhumita C. Talukdar)
Professor and Head
Food Science & Technology Programme
Department of Horticulture
Assam Agricultural University
Jorhat-785013 (Assam)

_____
(Chandan Hazarika)
Director
Post-Graduate Studies
Assam Agricultural University
Jorhat-785013 (Assam)

Acknowledgement

The authoress, with immense pleasure, takes the privilege to express her deepest sense of gratitude, indebtedness and reverence to her Major Advisor, Dr. Rana Pratap Bhuyan, Professor, Deptt. of Tea Husbandry & Technology, Assam Agricultural University, Jorhat for his valuable guidance, keen interest, inspiring motivation, unparalleled perseverance and thought provoking suggestions in planning, executing the research works and in producing this manuscript.

She also takes the opportunity to express her deep sense of indebtedness to the members of her advisory committee, Dr. Ananta Saikia, Professor, Department of Horticulture, AAU, Jorhat, Dr. S. C. Barua, Professor & Head, Department of Tea Husbandry & Technology, AAU, Jorhat, Dr. Sunayana Rathi, Asstt. Professor, Department of Biochemistry & Agricultural Chemistry, AAU, Jorhat & Dr. Samindra Baishya, Professor, Department of Biochemistry & Agricultural Chemistry for their impeccable advice, help and warm support during the course of investigation.

The Authoress extends her special gratitude and appreciation to Dr. Prasanta Das, Professor, Department of Agricultural Bio Technology, AAU, Jorhat, for taking all the photographs.

The authoress wishes to extend her thanks & gratitude to all the teaching staffs of Department of Horticulture and Department of Tea Husbandry & Technology & Department of Biochemistry & Agricultural Chemistry for their help, support and valuable suggestions during the course of investigation.

The authoress conveys her heartiest thanks to all the Small Tea Growers of Assam & Tea Board of India, from whom the samples of the research has been collected.

The authoress deeply thanks the employees of Deptt. of Horticulture, AAU, Jorhat and the employees of Deptt. of Biochemistry & Agricultural Chemistry, AAU, Jorhat for their necessary helps during the present study.

The author is thankful, obliged to the Director of Post Graduate Studies Dr. C. Hazarika, Assam Agricultural University, Jorhat, Dr. G. N. Hazarika, Director of Research, AAU, Jorhat, and other members of the DPGS office for providing necessary facilities for carrying out the study.

The authoress offers her heartfelt reverence to her beloved Maa-Baba, her elder brother for their immense love and constant encouragement. Their dauntless perseverance endurance and blessing in every possible way have enabled her to attain this stage of achievement in her life.

The Authoress would cherish in her memory the help, co-peration and moral support rendered by her friends Priyam, Alija, Diksha, Shelja, Priyanka, her junios Munmi, Sweety, Tridisha, Priyanka & Porinita & also her classmates Tania, Nandita & Sahu & other batchmates.

At last but not the least, the author express her sincere thanks to Almighty God, the invisible hand that holds and leads her in every step of life.

The Authoress deeply conveys her gratefulness to M/s Anurup Printer's, Jorhat for their selfless sacrifices, patience and taking the pain of making this complete manuscript.

Dated, Jorhat

The …… July, 2017 *(The Authoress)*

ABSTRACT

Tea (*Camellia sinensis*) is primarily processed as black tea or green tea. Tea is one of the most widely consumed beverages worldwide due to the associated health benefits. In the recent past, consumer preference has been shifting towards green tea. The techniques of manufacturing green tea are relatively uncomplicated. Small tea growers in Assam have started manufacturing green tea from tea leaves plucked from their small holdings. However the techniques of manufacture vary among the processing units. Variations also exist in the manufactured tea depending on geographical conditions and planting materials used.

A study was carried out at Assam Agricultural University, Jorhat, to ascertain the variation in antioxidant properties as influenced by processing techniques. Green tea samples were collected from green tea manufacturers with different techniques of manufacture, during different flushes of tea, in April, May, August and October. The experiment was laid out in factorial completely randomized design with six treatments considering the processing techniques and the four different months of manufacture. The data revealed that the moisture content was highest in green tea manufactured during the month of August with 6.76% in treatment T_6 (pan roasting) and low in green tea manufactured during October with 3.30% in treatment T_3 (dipped in boiling water 1 minute). The mean highest moisture content was found to be in the range of 4.47% in green tea manufactured in April to 6.51% in green tea manufactured in August.

All the manufactured green tea samples were bright greenish yellow in colour with good cup characteristics. Biochemical analysis of the samples revealed that green tea manufactured by the treatment T_1 (steamed for 30 seconds) was the best among all the treatments and had highest antioxidant properties. Mean total polyphenol content was found to be in the range of 11.61 mg catechol equivalent g^{-1} to 15.42 mg catechol equivalent g^{-1}, with highest polyphenol observed in the treatment T_1 (steamed for 30 seconds) in green tea manufactured during August (20.38 mg catechol equivalent g^{-1}). Mean total flavonoid content was highest in treatment T_1 (steamed for 30 seconds) with 22.62 mg quercetin equivalent g^{-1} and the lowest in treatment T_6 (pan roasting) with 18.23 mg quercetin equivalent g^{-1}.Total flavonoid content was highest in treatment T_1 (steamed for 30 seconds) with 23.91 mg quercetin equivalent g^{-1} in green tea manufactured during August and was lowest in treatment T_2 (dipped in boiling water for 10 seconds) with 13.66 mg quercetin equivalent g^{-1} in green tea manufactured during April. The Total Antioxidant Activity was measured with the help of DPPH and it was expressed in percentage inhibition. Total antioxidant activity was highest in treatment T_1 (steamed for 30 seconds) in green tea manufactured during August (85.03%) and lowest in treatment T_6 (pan roasting) in green tea manufactured during April (61.33%). The mean highest antioxidant activity (78.76%) was recorded in treatment T_1 (steamed for 30 seconds) and mean lowest antioxidant activity (73.37%) was recorded in treatment T_3 (dipped in boiling water for 1 minute). The inhibition percentage was higher in green tea manufactured during August for all treatments.

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	: Per cent
/	: per
°C	: Degree centigrade
µg	: Microgram
µl	: Microlitre
a*	: CIE color scale redness/greenness
AAU	: Assam Agricultural University
b*	: CIE color scale yellowness/ blueness
Ca	: Calcium
CD	: Critical Differences
CIE	: Commission International Del'Eclairage
cm	: Centimetre
conc.	: Concentrated
DPPH	: 1-1, diphenyl-2- picryl hydrazyl
et al.	: *Et alli* (and others)
Fe	: Iron
Fig.	: Figure
g	: Gram
g.mm	: Gram.Milimeter
g.sec	: Gram.Second
hr.	: Hour
i.e.	: That is
L*	: CIE color scale lightness/darkness
lit	: Litre
M	: Molar (concentration)

MC	: Moisture content
mg	: Milligram (s)
min	: Minute
ml	: Millilitre (s)
mm	: Milimeter
mM	: Millimolar
N	: Normal (concentration)
NaOH	: Sodium Hydroxide
nm	: Nanometer
ppm	: parts per million
QE	: Quercetin equivalent
S.Ed	: Standard error deviation
Sec.	: Second
TAC	: Total antioxidant activity
TFC	: Total flavonoid content
TPC	: Total phenol content
TSS	: Total soluble solids
v/v	: Volume/ Volume
v/w	: Volume/Weight
w/w	: Weight/Weight
wt.	: Weight
CE	: Catechol equivalent

CERTIFICATE – II

This is to certify that the thesis entitled "**Antioxidant Properties in Green Tea as Influenced by Processing Techniques**" submitted by **Paramita Bharadwaj**, **Regd. No.: 574 of 2014, Roll No. 2014-AMJ-76** to the Assam Agricultural University, in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Food Science and Technology Programme, Department of Horticulture** has been examined and approved by the student's Advisory Committee after viva-voce.

(Dr. Rana Pratap Bhuyan
Major Advisor & Chairman

Chairman
Board of Examiner

Members of the Advisory Committee:

1. _____
   Dr. Ananta Saikia

2. _____
   Dr. Subhash C. Barua

3. _____
   Dr. Samindra Baishya

_____
(Dr. Madhumita C. Talukdar)
Professor and Head
Food Science & Tchnology programme
Department of Horticulture
Assam Agricultural University
Jorhat-785013 (Assam)

_____
(Dr. Chandan Hazarika)
Director
Post-Graduate Studies
Assam Agricultural University
Jorhat-785013 (Assam)

Acknowledgement

The authoress, with immense pleasure, takes the privilege to express her deepest sense of gratitude, indebtedness and reverence to her Major Advisor, Dr. Rana Pratap Bhuyan, Professor, Deptt. of Tea Husbandry & Technology, Assam Agricultural University, Jorhat for his valuable guidance, keen interest, inspiring motivation, unparalleled perseverance and thought provoking suggestions in planning, executing the research works and in producing this manuscript.

She also takes the opportunity to express her deep sense of indebtedness to the members of her advisory committee, Dr. Ananta Saikia, Professor, Department of Horticulture, AAU, Jorhat, Dr. S. C. Barua, Professor & Head, Department of Tea Husbandry & Technology, AAU, Jorhat, Dr. Sunayana Rathi, Asstt. Professor, Department of Biochemistry & Agricultural Chemistry, AAU, Jorhat & Dr. Samindra Baishya, Professor, Department of Biochemistry & Agricultural Chemistry for their impeccable advice, help and warm support during the course of investigation.

The Authoress extends her special gratitude and appreciation to Dr. Prasanta Das, Professor, Department of Agricultural Bio Technology, AAU, Jorhat, for taking all the photographs.

The authoress wishes to extend her thanks & gratitude to all the teaching staffs of Department of Horticulture and Department of Tea Husbandry & Technology & Department of Biochemistry & Agricultural Chemistry for their help, support and valuable suggestions during the course of investigation.

The authoress conveys her heartiest thanks to all the Small Tea Growers of Assam & Tea Board of India, from whom the samples of the research has been collected.

The authoress deeply thanks the employees of Deptt. of Horticulture, AAU, Jorhat and the employees of Deptt. of Biochemistry & Agricultural Chemistry, AAU, Jorhat for their necessary helps during the present study.

The author is thankful, obliged to the Director of Post Graduate Studies Dr. C. Hazarika, Assam Agricultural University, Jorhat, Dr. G. N. Hazarika, Director of Research, AAU, Jorhat, and other members of the DPGS office for providing necessary facilities for carrying out the study.

The authoress offers her heartfelt reverence to her beloved Maa-Baba, her elder brother for their immense love and constant encouragement. Their dauntless perseverance endurance and blessing in every possible way have enabled her to attain this stage of achievement in her life.

The Authoress would cherish in her memory the help, co-peration and moral support rendered by her friends Priyam, Alija, Diksha, Shelja, Priyanka, her junios Munmi, Sweety, Tridisha, Priyanka & Porinita & also her classmates & other batchmates.

At last but not the least, the author express her sincere thanks to Almighty God, the invisible hand that holds and leads her in every step of life.

The Authoress deeply conveys her gratefulness to _____for their selfless sacrifices, patience and taking the pain of making this complete manuscript.

Dated, Jorhat

The …… July, 2017 *(The Authoress)*

ABSTRACT

Tea (*Camellia sinensis*) is primarily processed as black tea or green tea. Tea is one of the most widely consumed beverages worldwide due to the associated health benefits. In the recent past, consumer preference has been shifting towards green tea. The techniques of manufacturing green tea are relatively uncomplicated. Small tea growers in Assam have started manufacturing green tea from tea leaves plucked from their small holdings. However the techniques of manufacture vary among the processing units. Variations also exist in the manufactured tea depending on geographical conditions and planting materials used.

A study was carried out at Assam Agricultural University, Jorhat, to ascertain the variation in antioxidant properties as influenced by processing techniques. Green tea samples were collected from green tea manufacturers with different techniques of manufacture, during different flushes of tea, in April, May, August and October. The experiment was laid out in factorial completely randomized design with six treatments considering the processing techniques and the four different months of manufacture. The data revealed that the moisture content was highest in green tea manufactured during the month of August with 6.76% in treatment T_6 (pan roasting) and low in green tea manufactured during October with 3.30% in treatment T_3 (dipped in boiling water 1 minute). The mean highest moisture content was found to be in the range of 4.47% in green tea manufactured in April to 6.51% in green tea manufactured in August.

All the manufactured green tea samples were bright greenish yellow in colour with good cup characteristics. Biochemical analysis of the samples revealed that green tea manufactured by the treatment T_1 (steamed for 30 seconds) was the best among all the treatments and had highest antioxidant properties. Mean total polyphenol content was found to be in the range of 11.61 mg catechol equivalent g^{-1} to 15.42 mg catechol equivalent g^{-1}, with highest polyphenol observed in the treatment T_1 (steamed for 30 seconds) in green tea manufactured during August (20.38 mg catechol equivalent g^{-1}). Mean total flavonoid content was highest in treatment T_1 (steamed for 30 seconds) with 22.62 mg quercetin equivalent g^{-1} and the lowest in treatment T_6 (pan roasting) with 18.23 mg quercetin equivalent g^{-1}.Total flavonoid content was highest in treatment T_1 (steamed for 30 seconds) with 23.91 mg quercetin equivalent g^{-1} in green tea manufactured during August and was lowest in treatment T_2 (dipped in boiling water for 10 seconds) with 13.66 mg quercetin equivalent g^{-1} in green tea manufactured during April. The Total Antioxidant Activity was measured with the help of DPPH and it was expressed in percentage inhibition. Total antioxidant activity was highest in treatment T_1 (steamed for 30 seconds) in green tea manufactured during August (85.03%) and lowest in treatment T_6 (pan roasting) in green tea manufactured during April (61.33%). The mean highest antioxidant activity (78.76%) was recorded in treatment T_1 (steamed for 30 seconds) and mean lowest antioxidant activity (73.37%) was recorded in treatment T_3 (dipped in boiling water for 1 minute). The inhibition percentage was higher in green tea manufactured during August for all treatments.

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	: Per cent
/	: per
°C	: Degree centigrade
µg	: Microgram
µl	: Microlitre
a*	: CIE color scale redness/greenness
AAU	: Assam Agricultural University
b*	: CIE color scale yellowness/ blueness
Ca	: Calcium
CD	: Critical Differences
CIE	: Commission International Del'Eclairage
cm	: Centimetre
conc.	: Concentrated
DPPH	: 1-1, diphenyl-2- picryl hydrazyl
et al.	: *Et alli* (and others)
Fe	: Iron
Fig.	: Figure
g	: Gram
g.mm	: Gram.Milimeter
g.sec	: Gram.Second
hr.	: Hour
i.e.	: That is
L*	: CIE color scale lightness/darkness
lit	: Litre
M	: Molar (concentration)
MC	: Moisture content

mg	: Milligram (s)
min	: Minute
ml	: Millilitre (s)
mm	: Milimeter
mM	: Millimolar
N	: Normal (concentration)
NaOH	: Sodium Hydroxide
nm	: Nanometer
ppm	: parts per million
QE	: Quercetin equivalent
S.Ed	: Standard error deviation
Sec.	: Second
TAC	: Total antioxidant activity
TFC	: Total flavonoid content
TPC	: Total phenol content
TSS	: Total soluble solids
v/v	: Volume/ Volume
v/w	: Volume/Weight
w/w	: Weight/Weight
wt.	: Weight
CE	: Catechol equivalent

CHAPTER I

INTRODUCTION

The story and history of Indian tea industry are as fascinating as the legends associated with the origin and spread of the tea plant itself (Barua, 2008a). The tea industry in India is more than 170 years old, since the sending of 12 chests of tea, commercially cultivated and manufactured in Assam, for the London auction in 1838. Approximately, 579,000 ha are under tea cultivation in this country and the indigenous varieties of this plant have covered a large part of the tea areas. The major tea growing states are Assam, West Bengal, Tamil Nadu and Kerala. Other states such as Bihar, Himachal Pradesh, Karnataka, Orissa, Tripura and Uttaranchal also grow tea in limited areas. Among the tea growing regions the Brahmaputra basin is the largest one (Hazarika and Muraleedharan, 2011).

It goes to the credit of the Indian tea industry that it had the vision to create, nurture and sustain tea research organizations, from the very beginning of planting. These research organizations, both the Tocklai Experimental Station and the UPASI Tea Research Institute have, in turn contributed significantly to the growth of the tea industry. The biggest research contribution has been the release of new cultivars. In the early nineties, the technique of vegetative propagation was standardized and in 1949, 3 VP clones *viz.* TV I from Assam-China hybrid population, TV2 from Betjan and TV3 from Rajgarh population were developed. Tocklai has so far released 31 TV clones, 151 TRA and Garden series clones and 14 biclonal seed stocks. In South India, the UPASI Tea Research Institute (UPASI TRI) has released 31 VP clones, 5 biclonal seed stocks and 5 clonal graft combinations for commercial planting. Besides these, several clones have been developed by the estates in North East India and a few in South India. Among these, several clones have the potential for high yield, good quality, and tolerance to drought, pests and diseases. Currently, TRA has over 297 clones developed by selection, hybridization and tissue

culture under evaluation from which 10-15 clones are expected to be released (Das, 2011). Clonal populations though have a uniform genetic base, may not be adapted to all types of soil and climatic conditions. The genetic base and long term performance in terms of productivity and quality should be considered for selecting the planting material for any particular region. However, it is suggested that no single planting material should occupy more than 10 percent of the area of the estate (Hazarika and Muraleedharan, 2011).

Tea can be categorized into three main types depending on the level of oxidation: green (unfermented), oolong (partially fermented) and black (fermented) tea. The chemical composition of green tea varies with genetic strain, climatic conditions, soil properties, plucking season, position of the leaf, processing and storage. Some factors are more important than others; for example, the highest quality green teas are plucked during the first flush in late April and early May and quality declines in later harvests. Freely growing tea shoots pass through alternating states of growth and dormancy. The amount of growth made between two states of dormancy is known as a flush. In N.E. India, up to four flushes were recorded on unpruned seed trees in a growing season and up to five flushes on pruned bushes, although the fifth flush was rare and found only in a few genotypes. The first visible sign of growth of a new flush is the unfolding of the outermost janam wrapping the bud; this is followed by the other janams (Barua, 2008b). Usually, the buds and the first two to three leaves are plucked by hand or by machines prior to processing.

These basic types of tea have different quality characteristics, including appearance, flavour, taste, and colour. The relationship between the quality and chemical components in green tea shows that free amino acids, caffeine and polyphenols are qualitatively important components. Especially, catechins, the main component of polyphenols, are well known for their antioxidant properties, which have led to their evaluation in many diseases associated with free radicals, including cancer, cardiovascular and neurodegenerative diseases (Lee *et al.*, 2014).

Green tea is prepared by heating the green leaves so that (unlike black tea), it does not ferment. Heating the tea leaves (by roasting, steaming, or boiling) deactivates an enzyme inside the leaves, preventing the enzyme from causing chemical changes. Therefore, after heating, the tea keeps the green color and the chemical content of the fresh tea leaf.

The steps in processing green tea are plucking of fresh leaves, and storing them until processing, inhibition of fermentation, rolling, drying adequately, grading, final roasting (if needed) followed by packaging and labeling. Such processing technology preserves the natural polyphenols with respect to the health-promoting properties (Singh *et al.*, 2014).

Oxidation refers to a series of chemical reactions that result in the browning of tea leaves and the production of flavour and aroma compounds in the finished tea. Depending on the type of tea being made, oxidation is prevented altogether, or deliberately initiated, controlled and then stopped. Much of the oxidation process revolves around the polyphenols and the enzymes: polyphenol oxidase and peroxidase. When the cells inside the tea leaves are damaged and the components inside are exposed to oxygen and gets mixed, specifically when polyphenols in the cell's vacuoles and the peroxidase in the cell's peroxisomes mix with the polyphenol oxidase in the cell's cytoplasm, a chemical reaction begins. This reaction converts the polyphenols known as catechins into flavanoids called theaflavins and thearubigins (which are also polyphenols). Theaflavins provides the tea with its briskness and bright taste as well as its yellow colour, and thearubigins provides the tea with depth, body and its reddish colour. Also, during the oxidation, chlorophylls are converted to pheophytins and pheophorbides (pigments that lend to the black/brown colouration of the dry and oxidized tea leaves); and lipids, amino acids and carotenoids degrade to produce some of the tea's flavour and aroma compounds. Tea producers use a special methods to initiate, fix, or even prevent oxidation in order to produce different flavours in a finished tea and inherently, different types of tea. Oxidation begins when the cell walls within the tea leaves are

damaged. To achieve cell damage, tea producers macerate, roll or tumble tea leaves to intentionally initiate oxidation. Well macerated leaves exposes much more of the insides of the leaves to oxygen and results in a greater mixture of the chemicals within. Maceration is typically used in the mass production methods to create CTC (cut tear curl) tea or other broken-leaf teas and is achieved using a rotorvane or a CTC machine. While in green tea, prior to rolling, the leaves are treated to stop the polyphenol compounds to react with the enzymes. This makes a green tea high in phenols, beneficial to the human health. Moreover, it even lays back a unique aroma and a colour which is desirable in many nations worldwide.

Most common methods to prevent oxidation include, pan firing, steaming, use of hot tumblers or even baking whereas less common methods are sun drying, microwaving or plunging the leaves in boiling water. When oxidation is prevented altogether, the tea leaves will keep their green color and vegetal characteristics in the cup as the catechins will be left largely intact.

The polyphenols in tea may be subdivided by several chemical backbone structures. Simple tea polyphenols are those that are synthesized during the early stages of polyphenol biosynthesis. The degree of complexity of the polyphenols increases as one progress down the biosynthetic pathway. Green tea polyphenols consist of both simple and complex polyphenols. The large majority of polyphenols in green tea are flavonoid monomers called catechins and flavonols.

Tea consumption in ancient times was regarded as healthy, likely due to the fact that the boiling water used to make tea, kills many of the water-borne pathogens that caused illnesses common in those times (Kursanov and Brovchenko, 1950).

Today, research into the tea chemistry is a fundamental part of studies on the role of tea as a biological antioxidant and in prevention of chronic diseases. There is mounting evidences that substances called free radicals play a role in the development of major human diseases such as heart disease and cancer. Research suggests that antioxidants may help protect against these diseases by minimizing the

detrimental impact of free radical damage to cells and tissues. Much more research is needed to identify the processes involved in free radical-mediated diseases and to help develop the nutritional strategies to optimize the antioxidant status. The role of antioxidants in heart disease is not clear, and their potential role in cancer prevention and the aging process remains speculative (Harbowy and Balentine, 1997).

The most commonly recognized dietary sources of antioxidants are fruits and vegetables, which contain vitamins C, E, and carotenoids. Preliminary research indicates that the f1avonoids, which include the catechins and f1avonols found in both black and green tea, also act as antioxidants. However, additional research is required to determine whether the tea antioxidants substitute for or complement some of the protective functions identified with the more established antioxidant compounds such as vitamins C, E, and beta-carotene. Keeping all the above in mind, the present investigation has been undertaken with the following objectives:

OBJECTIVES:

1. To study the methods of processing Green Tea.

2. To assess anti-oxidant properties as influenced by processing techniques in Green Tea.

CHAPTER II

REVIEW OF LITERATURE

The review of literature pertaining to various aspects of the present investigation is presented below:

.

2.1 Tea – a quality beverage

For tea to be its' best, quality is more important than yield. Soils and climate are two major factors affecting the quality. Even high elevations and cultural practices (including tillage, weeding, fertility management, irrigation, plant protection and harvesting), may also affect the quality of tea (Singh *et al.*, 2014). In the field of food processing, the conditions under which a harvested lot is passed through a series of processes plays a vital role in determining its quality. In case of tea processing, quality of tea is greatly influenced by physical, chemical and thermodynamic conditions of various processes involved in its manufacturing and the time duration for these processes. Different type of tea blends requires different conditions for their processing. The various processes involved in tea manufacturing after plucking of leaves are withering (oxidation), rolling, fermentation, drying and sorting (grading). Out of these, fermentation, also known as enzymatic oxidation, is the most crucial part of tea manufacturing processes. The clone type and flushing time are the most essential factors to produce quality tea, so the quality can be controlled by changing the processing parameters corresponding to the clone type and flushing time. Thus, it is necessary to determine the suitable processing measures for each clone in each flushing time (Ali, 2015).

2.2 Tea processing

Immediately after the harvest, the tea leaves (usually the flush, or first two leaves and the bud of the growing tea shoot) are brought to factories situated

close to the tea gardens for manufacturing. It is the manufacturing process that determines the type of tea produced. There are three general types of manufactured tea: Green (unfermented), Oolong (partially fermented), and Black (fully fermented). The manufacturing processes used to produce each type of tea differ in the degree of enzymatic oxidation or "fermentation". Fermentation refers not to an exogenous, microbial process, as with beer or wine, but the natural browning reaction catalyzed by enzymes endogenous to the plant (Harbowy and Balentine, 1997).

According to Samarasingham (1990), the main group of chemical substances present in the tea flush is polyphenols which undergoes oxidative changes during the stage of fermentation in the manufacturing of black tea. The oxidized polyphenols form coloured substances referred to as theaflavins and thearubigins which are mainly responsible for the colour and character of black tea liquor. Green tea is a non-fermented form of tea. The method of processing is basically different from that of black tea as green tea is not subjected to fermentation which stage is totally eliminated by a process called de-enzyming or inactivation of the enzyme polyphenol oxidase. The processed green tea retains the green colour and the chemical composition of the green leaf without major changes.

Polyphenol oxidases in the tea leaves are more active in the summer season than in the spring season. Enzyme activity in the tea leaves increases gradually in the lapse of the withering period, and higher the withering temperature, faster is the enzyme activity. The enzyme activity increases rapidly by rolling in both the high and low degree withered leaves, and reach about 2 to 3 times as much as that in the fresh leaves. Enzyme activity decreases along with the fermentation process after rolling, and the higher the fermentation temperature, larger is the decrease in activity. It is presumed that the decrease in enzyme activity during the fermentation is caused by the formation of insoluble complexes of the oxidized polyphenols and the enzyme protein (Takeo, 1996).

Many investigators demonstrated that in plant tissues, most of the polyphenol oxidase is present in a soluble form and part of it is in the particulate

fraction. Cellular localization of polyphenol oxidase in the tea leaves was investigated by Kato *et al.* (1976) using Polyclar AT as an adsorbent of polyphenols existing in large amounts in tea leaves. Two polyphenol oxidase fractions from the particulate fraction were separated from each other by sucrose density gradient centrifugation and called the heavy and the light fraction. The centrifugal pattern in the gradient indicated that the position of polyphenol oxidase in the heavy fraction coincided with those of the markers of peroxisomes, catalase and malate dehydrogenase, but not with those of mitochondria and chloroplasts, cytochrome c oxidase and chlorophyll. The peak of activity in the heavy fraction shifted toward lower density concomitantly with the increase of Polyclar AT content in the homogenizing medium. The heavy fraction was broken and disappeared at low pH condition, but the light fraction remained unchanged. Polyphenol oxidase at the higher density position in the heavy fraction is associated with peroxisomes because of the near coincidence of its position with those of catalase and malate dehydrogenase.

Harbowy and Balentine (1997) reported that the chemistry of green and black tea, therefore, typically centers on the polyphenolic composition of these teas. HPLC techniques, now available for rapidly measuring a number of important tea polyphenols, including the catechins, f1avanols, flavonols, glycosides, and the theaflavins, have dramatically changed the way tea chemistry is studied.

2.2.1 Green Tea Processing

Longo *et al*. (2005) reported that in green tea, during manufacturing, the polyphenols mostly remain intact as enzyme inhibition (steaming or roasting or blanching or pan-frying) of leaves is done after plucking, inactivates polyphenol oxidase. The natural polyphenols in green tea include (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and epicatechin (EC).

While working on green tea, Singh *et al.* (2014) observed that processing technology preserves natural polyphenols with respect to the health-promoting properties in green tea which are dimerized or polymerized to form a variety of theaflavins, such that these teas may have different biological activities.

Generally soon after harvesting, the inactivated enzymes cannot break down the tea tissue chlorophyll, which contributes to the green colour.

It was reported by Wickremasinghe (1978) that there are two popular systems of processing green tea, the Chinese and the Japanese systems; the former utilizes the hot pan method for de-enzyming whereas the latter uses the steam method. For the Assam variety of tea, which contains high amounts of polyphenols, the Chinese method of processing is preferred. The hot pan method employs higher temperatures than the steam method and high temperatures are known to reduce the principal flavanols which are responsible for the bitterness.

Cheng and Fan (2007) conducted a study to investigate the effect of withering time and blanching condition on the catechins content and quality of green tea made from TTES No.8. The experimental results showed that TTES No.8 of large-leafed cultivar processed for 4 hours withering, high temperature (300 ℃) panning conditions, could obtain the best quality, and can maintain a high EGCG content. Thus manufacture process can improve the bitter taste produced from large-leafed green tea, but also retains high EGCG content.

Eguchi and Eguchi (2001) noted that there are two methods of producing green tea in Japan. The most common method used today is to steam the tea leaves, which prevents fermentation and inhibits the activity of oxidation of the enzymes. The other method is to parch the leaves without the steam. This is called the kama-iri method. Over time, the kama-iri method has been gradually replaced by the steam production technology because it could be mechanized more easily. Today, only a few small-scale tea producers still practice the kama-iri method.

For obtaining good quality of tea, uniform and tender two and a bud has to be plucked. Immature shoots and coarse leaves are unacceptable because standard flush is depended upon the standard of the leaves. Coarse plucking reduces the quality of the prepared tea (Singh *et al.*, 2014).

Withering reduces approximately 30 per cent of the humidity content of the leaf within a time of 4 to 12 h. During withering, both physical (leading to a

loss in moisture and changes in cell membrane permeability) (Sanderson and Grahamm, 1973) and chemical (influencing the formation of the tea aroma, which is important for flavour teas) changes takes place (Nagalaksmi, 2003). Several chemical changes occurring during withering operation has been observed by Choudhury and Goswami (1983).

Fresh leaf starts to loose moisture immediately after plucking. In withering process the removal of water occurs with faster rate through the lower surface when stomata of the lower leaf surface begin to close, compared to the upper surface which has no stomata (Kramer and Kozlowski, 1979). As the degree of wither progresses, the permeability of the cell membranes in tea shoots also increases.

Tomlins and Mashingaidze (1997), observed that biochemical changes in withering are the conversion of proteins into amino acids, reduction of lipids and fatty acids, carotenoids and chlorophylls, increment in caffeine content and changes in sugars, organic acids, PPO activity and volatile components.

Wang and Helliwell (2000) reported that steaming causes inactivation of enzymatic activity in freshly picked tea leaves and decreases the oxidation. Fixing operation is performed for reducing or stopping the enzymatic activities in green leaf and retaining the green colour of the leaves. Temperature applied for pan-fixing is more than 180°C but in the case of steam-fixing, temperature is usually 100°C. Leaves are moved through a rotating drum and hot steam is added up to 2 min then the leaves are extracted again. The amount of steam is the deciding factor in this step because too much amount can spoil the leaves and too little initiates the onset of the fermentation. Xua and Chang (2008) found that steam process is beneficial in retaining the antioxidant components, appearance and texture of the cooked product, decreasing the process time.

Water extracts from steamed green tea contains 22-33 per cent phenols and the flavonols along with catechins accounted for 49-87 per cent. The phenolic compounds responsible for effective antioxidant properties, usually, are the most abundant water-soluble components in the tea (Balentine *et al.*, 1997).

Wooden or metal drum can be used for drying in which the leaves are swiveled for nearly 30 min at 55°C warm air. During this process, the leaves lose about 50 per cent of their remaining moisture content. Physical and chemical changes occurring during the drying process affects the final tea quality and that is why a control on a proper drying process is a necessary action to preserve the quality and minimize the energy inputs. Loss of quality and burnt taste is controlled by regulating the drying temperature (Xie *et al.*, 2006).

Drying can be performed with several methods like hot-air drying with a blast oven, vacuum drying with blast oven, vacuum pump and microwave drying and microwave vacuum drying with microwave furnace with/without vacuum. When a microwave energy is applied, water and other polar molecules of tea leaves are induced for a simultaneous high-speed rotation due to microwave irradiation, leading to the surface and interior heating at the same time, and resulting in a large number of water molecules escaping from the tea leaves (Lou, 2002).

Lin *et al.* (2010) showed that the rehydration ratios varies among the different drying methods. Microwave vacuum drying＞ Microwave drying＞ Vacuum drying＞ Hot-air drying. It shows the maximum and minimum rehydration capacity of green tea for microwave vacuum drying and hot air drying respectively. Combination of short drying time and low temperature has least damage on the cell structure of green tea in microwave vacuum drying. Maximum nutrients (like amino acid, protein and caffeine) retains in green tea by microwave vacuum drying and least by hot air drying.

In this processing step, leaves are rolled in a rolling machine for about 10 minutes with differing pressure. Optimum rolling is necessary because less rolling results in uneven crushed particles and more rolling causes loss of chemicals due to improper mixing of chemical with enzyme (Naheed *et al.*, 2007).

Further drying operation follows in which leaves are kept in contact with hot air up to 30 minutes for further drying after that final rolling step has been taken into account by keeping tea leaves between two rotating metal plates of rolling

machine up to 15 minutes then finally leaves are polished with help of polisher by pressing the leaves against a hot plate. This makes the leaves very flat and glowing. For aesthetic point of view this step is important (Singh *et al.*, 2014).

2.3 Physiochemical properties of green tea

2.3.1 Moisture Content

Generally the moisture content varies according to the humidity, cultivar, area and environmental factors. Chen *et al.* (2014) analysed the moisture content of Oolong tea by the equilibrium relative humidity technique. The equilibrium relative humidity and temperature of tea materials were measured by using temperature and relative humidity sensors. Sensors were calibrated, and calibration equations were established to improve accuracy. The moisture content was calculated by using an equilibrium moisture content model. Uncertainty analysis revealed that the performance of the humidity sensor had a significant effect on the accuracy of moisture determination.

Topuz *et al.* (2014) determined moisture contents of the green tea powders and found to be in the range of 2.79–3.26 g 100 g^{-1}. The results changed significantly with respect to shooting period; however, tea clone and shading level did not affect the moisture content of samples.

Botheju *et al.* (2011) found out in an experiment that the moisture content of tea leaves is reduced to 55 per cent w.b. within 12-16 h during withering in Orthodox black tea. The researchers worked on tea samples of Sri Lanka.

Li *et al.* (2012) studied on the effects of the moisture content of tea on diffuse reflectance spectroscopy were investigated by integrated wavelet transform and multivariate analysis. A total of 738 representative samples, including fresh tea leaves, manufactured tea and partially processed tea were collected, which covers the range of MC values from 3.15 to 71.40 per cent.

Sato *et al.* (2007) demonstrated that when the tea darkens, we need to stop the process by drying at 203°F for 5 min, followed by 140°F for approximately

60 min. And then use a convection oven or a bamboo Chinese dryer with heating coils. It reduces the leaf moisture content to about 5 per cent.

2.3.2 Colour

Colour is derived from the natural pigments in plants many of which change as the plant proceed through maturation. The primary pigments imparting colour quality are the fat soluble chlorophylls (green), carotenoids (yellow, orange, and red), water soluble anthocyanin (red, blue), flavonoids (yellow), and betalains (red). In addition, enzymatic and non-enzymatic browning reactions may result in the formation of water soluble brown, grey, and black coloured pigments. The enzymes involved in browning reactions include polyphenol oxidase, which catalyses the oxidation of Polyphenolic compounds, and phenylalanine ammonia lyase, which catalyses the synthesis of precursors to phenolic substances (Barrett *et al.*, 2010).

Liang *et al.* (2005) investigated the colour differences in terms of ΔL, Δa, Δb and ΔE between distilled water and infusions of 29 green teas, 16 black teas and 13 oolong teas and their correlation with sensory quality attributes of tea were investigated. The regression of total quality score of green tea (TQSg) upon the Δa was TQSg = −2.26_a + 77.2 (R^2 = 0.22, $p < 0.01$), that of total quality score of black tea (TQSb) upon Δb was TQSb = 0.70_b + 44.6 (R^2 = 0.31, $p < 0.05$) and that of total quality score of oolong tea (TQSo) upon ΔL was TQSo = 1.09_L + 94.5 (R^2 = 0.75, $p < 0.01$).

Sharma and Thomas (2013) worked on electronic vision study of tea grains' color during infusion. They stated that RGB (Red Green Blue) color model is a combination of red, green, and blue colors. They analysed the variance of these components over the infusion period. It was found that the intensity of Red color is increased, Green color is decreased and Blue color is increased as the infusion proceeded. It may also be noted that, in general, bigger sized grain requires more time for the color change than smaller sized grain pointing to the fact that the rate of infusion may be low in bigger sized grain.

Infusion chemical composition, colour difference indicators and volatile constituents of seventeen jasmine scented tea samples and their correlation with sensory total quality score given by tea-tasting panel were studied by techniques of HPLC, colour difference meter and gas chromatograph by Liang *et al.* (2007).

Laddi *et al.* (2011) studied on the influence on color attributes of freshly brewed tea with time due to variations in temperature conditions. It was found that there is significant change in the color values due to two factors *viz*. time and temperature. As the variance for CIE L*a*b* values with imposed cooling (L*= 0.64897, a*= 0.083166 and b*=1.644114) were found lower than color values without cooling (L*= 1.074631, a*= 0.412665 and b*=3.445136) due to the reason that change in color was greater under normal conditions with no cooling applied to the samples. Therefore, the change in CIE L*, a* and b* is less significant due to imposed cooling condition.

2.3.3 Phytochemicals and antioxidant activities

Phytochemicals are non-nutritive secondary metabolites of plant that have a protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves or other parts of plants that interplay with nutrients and dietary fibre to protect them. Some secondary metabolites such as alkaloids, flavonoid, phenols, saponins, tannin have antioxidant properties. An antioxidant is a molecule that inhibits the oxidation of other molecules. Phenolic compound of plant act as primary antioxidant or free radical scavenger (Ayoola *et al.*, 2008)

The antioxidant activities of phenolic compounds are related to the acid moiety and the number and the related positions of hydroxyl groups on the aromatic ring structure. Phenolic compounds have hydroxycinnamic acids are more effective antioxidant the hydroxybenzoic acids due to increased delocalization of phenoxyl radical (Seifu, 2012).

It has been reported that the antioxidant activity of plant material are correlated with the content of their phenolic compound (Cakir *et al.*, 2003).

Phenolic compound especially phenolic acids and flavonoids are ubiquitously present in vegetables, fruits, seeds, tea, wines and juices. Polyphenols protect from oxidative damage and play a beneficial role in reducing the risk of coronary heart disease, diabetes, hypertension and cancer (Andjelkovic´ *et al.*, 2006).

It is estimated that more than 5000 phytochemicals have been identified, but a large percentage still remains unknown (Shahidi, 2004) and need to be identified before their health benefits are fully understood. However, more and more convincing evidence suggests that the benefits of phytochemicals in fruit and vegetables may be even greater than is currently understood because oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic disease (Ames and Gold, 1991)

There has been considerable interest in the flavonoid content of foods and plants since the early 1980s. Steinmetz and Potter (1991) demonstrated a relationship between a diet rich in fruits and vegetables and a reduced risk for chronic disease. Because reduced risk did not correlate with traditional nutrients, attention has focused in many non-nutrient, potentially bioactive compounds, of which the flavonoids constitute one family. Flavonoids are naturally occurring Polyphenolic compounds with a C6-C3-C6 backbone. This group of plant pigments which are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and can be chemically subdivided into six structural categories: flavones, flavonols, flavanones, flavononols, flavan-3-ols (catechins) and anthocyanidins. These compounds (aglycones) are commonly glycosylated and may also be alkoxylated or esterified. As a result, over 5000 different flavonoids have been identified in plant materials (Harborne and Williams, 2000). The methods that have been reported for the determination of flavonoids are based on the aluminium chloride complex formation, which is one of the most commonly used analytical procedures applied to the flavonoid content determination in various plants (Grubesic *et al.*, 2007).

There are different methods for measuring antioxidant activity. One such method is DPPH (2, 2 diphenyl-1-picrylhydrazyl) method. It is a common and

frequently used method to determine the free radical compound which forms the free radicals in solvents. The DPPH system is widely accepted tools to determine the free radical scavenging activities of antioxidants; it accepts an electron or hydrogen radical to become stable diamagnetic molecules (Kalaivani and Mathew, 2010). DPPH is a well-known radical and a trap "scavenger" for other radicals. When free radical scavengers are added, DPPH is reduced and its colour is changed to yellow, based on the efficacy of antioxidants. The principle of the reduction of DPPH free radical is that the antioxidant reacts with the stable free radical DPPH and converts it to 1, 1-diphenyl-2-picrylhydrazine (Sreejayan and Rao, 1996).

DPPH method measures electron donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Any molecule that can donate an electron or hydrogen will react with DPPH, thus neutralizing its colour from a deep purple to a light yellow by electrons from the oxidant compounds. The concentration of DPPH at the end of a reaction will depend on the concentration and the structure of the compound being scavenged (Naik *et al.*, 2005).

The therapeutic potential of medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful (Kanatt *et al.*, 2007). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods and to quantify antioxidants in complex biological systems (Esmaeili and Sonboli, 2010).

2.3.3.1 Total Polyphenol, Total Antioxidant Capacity and Flavonoids

Under field conditions, the phenolic composition of tea shoots varies considerably with seasonal, genetic, and agronomic factors and mechanisms that induce seasonal variations on total phenolic content in tea shoots may include one or all three of the following environmental conditions: day length sunlight, and/or temperature, which vary markedly across seasons (Hilton and Jones, 1973).

Bradfield *et al.* (1947) reported that the natural polyphenols in green tea include (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and epicatechin (EC). The highest concentration is of EGCG followed by EGC, ECG and EC in decreasing order.

According to Mahanta and Baruah (1992) polyphenol concentration in shaded tea flushes are much lower.

Balentine *et al.* (1997) reported that the green tea contains 30 to 42 per cent polyphenols on the dry weight basis and a cup of green tea contains about 300 to 400 mg of polyphenols whereas Obanda *et al.* (1997) showed the level of phenolics in green tea shoots varied among clones. Mou and Xu (2006) found that the shoots of tea which had higher content of tea polyphenol and stronger activity of PPO (polyphenol oxidase) were suitable for producing black tea. Gulati *et al.* (2009) reported that China hybrids produce low level of total catechins compared to Assam and Cambod types.

The four major catechins [(-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epicatechin (EC)], gallic acid (GA), and caffeine were simultaneously determined by a simple and fast HPLC method using a photodiode array detector by Cabrera *et al.* (2003). In all analyzed samples, EGCG ranged from 1.4 to 103.5 mg/g, EGC from 3.9 to 45.3 mg/g, ECG from 0.2 to 45.6 mg/g, and EC ranged from 0.6 to 21.2 mg/g. These results indicated that green tea has a higher content of catechins than both oolong and fermented teas.

Liu *et al.* (2010) found that the lower ratio of polyphenol to amino of tea bud leaves was suitable for manufacturing of green tea. On the contrary, it was suitable for manufacturing black tea. Erturk *et al.* (2010) reported that the great difference of tea shoots in terms of total phenols at different harvest time is supposed to the effect of change of ecological parameters.

Su *et al.* (2007) reported the major polyphenolic components of the aqueous extract of oolong tea were identified as (-)-epigallocatechin (EGC), (-)-

epigallocatechin gallate (EGCG) and (-)-epicatechin-3-gallate (ECG). The two major catechins (EGC and EGCG) in the tea infusion contributed significantly to the investigated antioxidant activities [i.e., DPPH radical scavenging and superoxide radical scavenging activities] with high correlation values in r = 0.9486 and 0.9327 for the EGC and r = 0.9592 and 0.8718 for the EGCG, respectively.

Bharadwaz and Bhattacharjee (2012) reported that polyphenols is a group of compounds in tea leaves as major constituent, is known for its antioxidant property.

Tram *et al.* (2015) showed that the microwave treatment time and water-to-tea ratio had major significant impacts on the yield of polyphenols extracted.

Total phenols were determined by Shonisani (2010) using the Folin-Ciocalteu method and antioxidant activity using Trolox Equivalent Antioxidant Capacity (TEAC) assay. At 90°C for 3 min 7.68mg/100g of total polyphenol and 3.85μmol/g of antioxidants were obtained and this amount decreased to 5.50mg/100mg for total polyphenols and 1.31μmol/g for antioxidant activity at 30°C for 10 minutes.

Vinson and Dabbagh (1998) found that many natural polyphenols and flavonoids were much better antioxidants than vitamins. Of the tea fractions the order of activity/mole phenol was green tea polyphenols > black tea polyphenols > decaffeinated green tea extract - green tea extract > theaflavins > decaffeinated black tea extract – black tea extract. There was no significant difference for the 3 types of teas in any of these antioxidant parameters or in total phenols.

The antioxidant capacity was determined by the ferric thiocyanate method (FTC) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay by Anesini *et al.* (2008). Green tea showed a higher polyphenol content than black tea. The total polyphenol concentration in green tea was found to vary from 21.02 ± 1.54 to 14.32 ± 0.45 per cent of Gallic acid equivalents (GAE), whereas in black tea, the polyphenol content ranged from 17.62 ± 0.42 to 8.42 ± 0.55 per cent of GAE.

Aqueous extracts of green, oolong and black teas tested by DPPH scavenging and α carotene bleaching in comparison with rooibos tea (Gadow *et al.*, 1997). It is in the order green > black > oolong > fermented rooibos > unfermented rooibos > semi-fermented rooibos. However, antioxidant activity as assessed by the DPPH radical scavenging method decreased in the order: green > unfermented rooibos > fermented rooibos > semi-fermented rooibos > black > oolong.

A principal component regression (PCR) model is built for prediction of total antioxidant capacity in green tea using near-infrared (NIR) spectroscopy. The modeling procedures are systematically studied with the focus on outlier detection. Different outlier detection methods are used and compared by Zhang *et al.* (2004).

The antioxidant mechanisms of catechins were studied by investigating products generated by AAPH (2,2-azobis-2-aminopropane hydrochloride) induced radical oxidation by Kondo *et al.* (1999).

Scavenging effects of tea catechins and their epimerized, acylated, and glucostylated derivatives on 1,l -diphenyl-2-picrylhydrazyl (DPPH) radical were evaluated by electron spin resonance spectrometry by Nanjo *et al.* (1996). Tea catechins and their epimers were shown to have 50% radical scavenging ability in the concentration range of 1 to 3 μM. No significant differences were observed between the scavenging activities of tea catechins and their epimers, and, hence, the scavenging effects of catechins are not dependent on their sterical structure.

Alkyl peroxyl radical (ROO) generated from the reaction between 20 mM *t*-butyl hydroperoxide

(t-BuOOH) and 200 μM hematin could kill E. coli. The minimum concentrations of catechins sufficient to rescue the bacteria treated with ROO were found to be 70 μM for (-) epicatechingallate, 100 μM for (-)-epicatechin and 125 μM for (+)-catechin. These values were comparable with the value of α-tocopherol, a typical ROO scavenger. On the other hand, L-ascorbate and β-carotene revealed about one tenth the scavenging activities of catechins. No scavenging activity was found for

superoxide dismutase even at 86 mM. These facts indicate that catechins have high ROO scavenging activity (Nakao *et al.*, 1998).

The relationship between antioxidant activity and anti-mutagenicity of various tea extracts (green tea, pouchong tea, oolong tea, and black tea) was investigated by Yen and Chen (1995). They observed that all the tea extracts exhibited markedly antioxidant activity and reducing power, especially oolong tea, which inhibited 73.6 per cent peroxidation of linoleic acid. Tea extracts exhibited a 65-75 per cent scavenging effect on superoxide at a dose of 1 mg and 30-50 per cent scavenging effect on hydrogen peroxide at a dose of 400 µg. They scavenged 100 per cent hydroxyl radical at a dosage of 4 mg except the black tea. Tea extracts also showed 50-70 per cent scavenging effect on α, α-diphenyl-β-picrylhydrazyl radical. The antioxidant activity and the scavenging effects on active oxygen decreased in the order semi-fermented tea > non-fermented tea > fermented tea. Tea extracts showed strong anti-mutagenic action against five indirect mutagens, i.e., AFB1, Trp-P-1, Glu-P-1, B[α]P, and IQ, especially oolong and pouchong teas. The antioxidant effect of tea extracts was well correlated to their anti-mutagenicity in some cases but varied with the mutagen and antioxidant properties.

Guo *et al.* (1999) examined the relationship between the free radical scavenging activities and the chemical structures of tea catechins ((-)-epigallocatechin gallate (EGCG), (-) epigallocatechin (EGC) and (-)-epicatechin (EC)) and their corresponding epimers ((-)-gallocatechin gallate (GCG), (-)-gallocatechin (GC) and (+)-catechin ((+)-C)). The scavenging effects of galloylated catechins (EGCG and GCG) on the four free radicals were stronger than those of nongalloylated catechins (EGC, GC, EC, (+)-C), and the scavenging effects of EGC and GC were stronger than those of EC and (+)-C.

The antioxidant activity and the total phenolics content of various tea extracts were analysed. For measuring the antioxidant activity, the Trolox equivalent antioxidant capacity (TEAC) test and the low density lipoprotein (LDL) oxidation test were used by Liebert *et al.* (1999). In all cases the antioxidant activity as well as the

content of the phenolics increased with the brewing time. In black tea (brewed without stirring or chopping), the total phenolics increased from 33.8 mg/100 ml after 0.5 min up to 68.4 mg/100 ml after 10 min brewing time. Stirring during brewing led to higher phenolic yields in the extract. Thus, phenolics in stirred black tea ranged from 44.5 mg/100 ml (0.5 min) to 96.7 mg/100 ml (10 min). Chopping the tea leaves resulted in the highest content of phenolics. Antioxidant activity was well correlated with the corresponding total phenolics content. The results of the LDL oxidation test varied more than those of the TEAC test.

Yoshioka *et al.* (1991) investigated four catechins, (-)-epicatechin (EC), (-)-epigallocatechin (EG), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGG), extracted from tea were investigated by with respect to the formation of radicals and chemiluminescence during autoxidation. An alkaline solution (0.05 N NaOH) of these catechins indicated ESR absorption accompanying a browning reaction.

Relative antioxidant activities of green tea catechins were measured against radicals generated in the aqueous phase and against propagating lipid peroxyl radicals by Salah *et al.* (1995).

Effects of (+)-catechin, (-)-epigallocatechin gallate and gallic acid on the growth of *Escherichia coli* K-12 under oxidative conditions were examined by Inoue *et al.* (1996). (+)-Catechin did not protect the bacterial growth which was inhibited by H_2O_2 lipid hydroperoxide, paraquat, $Cu2^+$, H_2O_2 + $Cu2^+$ and lipid hydroperoxide + $Cu2^+$. Both (-)-epigallocatechin gallate and gallic acid could restore the growth inhibition of *E. coli* K-12 under the same conditions. Radical scavenging activity of (-)-epigallocatechin gallate, which is one of the most abundant components in Japanese green tea *(Camellia sinensis* L.), is likely to be dependent upon the gallic acid group of the compound.

Gramza *et al.* (2005) measured the antiradical activity with the use of two different methods of scavenging the stable free radicals ABTS and DPPH. Examined tea extracts showed different antiradical activity. Best activity in

scavenging ABTS expressed as TAA (total antioxidant activity) showed black tea aqueous and ethanol extracts. Green tea extracts were four times less effective. Antiradical activity showed that the lowest concentration needed to scavenge the 50 per cent of initial DPPH radical (EC_{50}) was green and black tea ethanol extracts. Aqueous extracts showed 50 per cent lower activity than equivalent ethanol extracts. Research proved that antiradical activity of plant extracts is dependent on mechanisms of oxidative activity of free radicals used and the chemical structure of contained antioxidants.

Mahanta and Baruah, (1998) opined that higher amounts of terpenoid improved the Flavour characteristic of second flush tea of North-East India in general and Darjeeling in particular.

Flavonoids are 2-phenyl benzopyran based compounds that are subdivided into six classes: flavones, flavanones, isoflavones, flavonols, flavanols, and anthocyanins. Flavanols and flavonols, the main classes found in tea, are 30 per cent of the dry weight of fresh leaf. Catechins (flavan-3-ols), the predominate form, are characterized by di- or tri-hydroxyl group substitution of the B ring and the meta-5,7-dihydroxy substitution of the A ring. Flavonol glycosides make up 2 to 3% of the water-soluble extract solids of tea. The flavonol aglycones are not found in significant quantities in tea beverage due to their poor solubility in water (Balentine *et al.*, 1997).

Tea flavonoids can be analysed by various state-of-the-art analytical techniques, however, as the structures of TRs have not yet been fully elucidated, quantitative analysis of TRs in black tea remains elusive. This is possibly one of the major challenges in tea analysis. Methods for analysing flavonoids in tea and tea extracts vary greatly between laboratories and between commercial sources. Uniform analytical methods are needed for clinical studies, stability studies, active ingredient analysis and manufacturing control, and to allow data from different sources to be compared (Huafu *et al.*, 2000).

According to Punyasiri *et al.* (2004), the enormous content of flavan-3-ols is the most striking feature of tea leaf chemistry, it is up to 20 per cent (w/w, dry)

and depends mostly on tea variety. These flavan-3-ols are presented by or derived from catechin (3 β-hydroxyl group), epicatechin (3α-hydroxyl group) or the respective 3'4'5' trihydroxyflavan-3-ols, gallocatechin and epigallocatechin. Demonstration of flavonoid enzymes with their respective substrate specificities and substrate conversions of the cytosolic enzymes allows us to outline the most important branches of pathways in tea flavonoid biosynthesis.

The mineral and flavonoid contents of commercially available different types of teas (premium black, flavored black, green, and fruit tea) and the infusions produced from them were determined by Pekal *et al.* (2013). Studied teas differed in the contents of elements between their raw materials and infusions. Iron and copper exhibited the lowest efficiency of extraction by hot water. For the most popular black tea brand (Lipton Yellow label), the efficiency of metal extraction decreased in the order: Co>Mn>Ni>Zn>Cu>Fe. Flavoured black and citrus tea infusion had the highest content of Co and Ni, while yellow label and green Indonesia are a good source of Fe, Mn, and Zn. Flavonoids were predominantly present as glycosides. Rutin was present at much higher levels in black and green teas. Significant amounts of naringin and hesperidin were determined in tea infusions with citrus aromas or fruits.

2.4 Tea and health

Recent studies have investigated that after tea consumption; break down of flavonoids into smaller phenolic acids takes place within the colon from bacterial degradation, absorption occurs through the small intestine. These phenolic acids can be absorbed in the circulatory system (Fatima and Rizvi, 2011)

Green tea in its purest and most unadulterated form has always influenced human health from generations and day by day scientific evidences throughout the world are making people aware of health benefits associated with this herbal drink. The catechins epigallocatechin gallate (EGCG) is found in the greatest concentration and mostly studied for its health benefits (Anand *et al.*, 2012).

Tachebele *et al.* (2014) gave the opinion that both green and black teas possess anti-diabetic activity, and are effective in the prevention and treatment of diabetes.

Caffeine (1,3,7-trimethylxanthine) is associated with stimulatory effects on central nervous system and cause increase in basal metabolic rate (BMR) by about 3-4 per cent (Fatima and Rizvi, 2011).

Sinija and Mishra (2008) reported that tea helps in prevention of Lung cancer, Pancreatic cancer, Prostrate cancer, Breast cancer, Esophageal cancer, Colorectal cancer, Stomach cancer, Urinary bladder cancer, Skin cancer, etc.

Theophylline in tea is used to prevent respiratory diseases like wheezing, shortness of breath, and difficulty breathing caused by asthma, chronic bronchitis, emphysema, and other lung diseases (Sharangi, 2009).

Cooper *et al.* (2005) in their study on 1256 women in the United Kingdom aged 65 to 76 (1134 tea drinkers and 122 non-tea drinkers) reported that tea drinkers had significantly greater mean bone mineral density measurements (5%, adjusted for age and body mass index), independent of smoking status, the use of hormone replacement therapy, coffee drinking, and whether milk was added to tea.

The effects of tea on obesity and diabetes have received increasing attention. Tea catechins, especially EGCG, appear to have antiobesity and antidiabetic effects. African black tea extract has been shown to suppress the elevation of blood glucose during food intake and reduce the body weight (Chacko *et al.*, 2010).

CHAPTER III

MATERIALS AND METHODS

The present investigation entitled “Antioxidant properties in green tea as Influenced by processing techniques” was carried out during the year 2016-2017 in the Quality Control and Post-Harvest Technology (PHT) Laboratory of the Department of Horticulture, Assam Agricultural University, Jorhat, Assam.

The techniques employed and materials used therein were described in this chapter.

3.1 MATERIALS

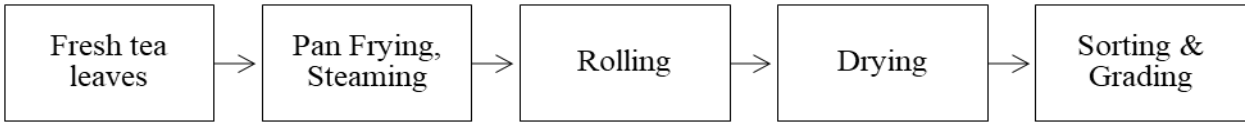
3.1.1 Collection of sample

Tea is primarily processed as black tea or green tea. In the recent past, consumer preference has been shifting towards green tea because of their innumerable health benefits. Small tea growers have started manufacturing green tea from the tea leaves of their own small holdings. However the technique of manufacture varies among those processing units.

Thus for the present study, to study the variation in antioxidant properties of green tea as influenced by the processing techniques, organic green tea were collected from different growers of Assam. The samples manufactured by similar processing techniques were collected for four major tea flushes *viz*., during the months of April, May, August and October. Random samples of standard grades of tea packed by the growers, for marketing, were the samples for the study. Green tea samples manufactured by following a particular processing technique were mixed together thoroughly and sampling was done from this mixture for the present study.

3.1.2 Fermentation inhibition

Fixing or 'kill-green' refers to the process by which enzymatic browning of the wilted leaves is controlled through the application of heat. It is held that the longer it takes to fix the leaves, the more aromatic will be the tea. Fixing is carried out via steaming, pan-frying, etc. Application of steam heats the leaves more quickly than pan-frying, as a result of which teas taste 'green' and vegetal while the pan-fired ones taste toasty. This procedure is carried out for green and yellow teas. Indian green tea produced in Assam is typically heat-treated by steaming immediately after harvest. It is then rolled and dried. Green tea manufacturers in India generally use a conventional roller. Various growers follow different methods to inhibit the fermentation prior to rolling, to process green tea. Some of the noted ones are: roasting, steaming, pan-frying and blanching. Though these methods take the aid of high temperature which is mostly followed in India, there are many methods which take the help of low temperature as well; specifically liquid nitrogen treatment. The basic green tea manufacturing method that is commonly followed as:



Green Tea produced in the month of April (M1), May (M2), August (M3) and October (M4) were collected from different growers of Assam who employed any of the following processing techniques for green tea production and considered as treatments for the present study –

Treatments:

T1	Steaming for 30 sec
T2	Dipping or scalding in water (100 ^{o}C) for 10 sec
T3	Dipping or scalding in water (100 ^{o}C) for 1 min
T4	Steaming for 1 min
T5	Steaming for 3-4 min
T6	Pan roasting

3.2 METHODS

3.2.1 Determination of moisture content

Moisture content is usually shown as a percentage of water contained in a solid, liquid or vapor material. Where sample is vapor or liquid, it can be in percentage of weight of water contained in its sample's volume and it can be called hygroscopic moisture or humidity. In the present study, the moisture content of each of the sample was measured by using AND MX-50 moisture analyzer (AND Co. Ltd., Tokyo, Japan). MX-50 is a heating and drying method analyzer that compares weight before and after heating and drying. 2g of each of the samples were taken directly after opening the packaged tea samples provided by the growers.

3.2.2 Determination of colour (CIELab parameters)

CIE L*a*b* (CIELab) is a color space specified by the International Commission on Illumination (French Commission internationale de l'éclairage, hence its CIE initialism). It describes all the colors visible to the human eye and was created to serve as a device-independent model to be used as a reference.CIELab parameters, i.e. L*, a*, b*, Hue and Chroma values were determined in a HunterLab Colour Quest XE Colorimeter(Hunter Associates Laboratory, Inc., Virginia, USA). 2.8g of tea samples

were infused in 150ml of distilled water and were allowed to rest for 6 minutes. The infused tea samples were then analysed.

The conversion of a* and b* to Chroma and hue is done using the following formulas:

$$\text{Hue } (H^*) = \text{Tan}^{-1}\, b/a$$

$$\text{Chroma } (C^*) = \sqrt{a^2 + b^2}$$

3.2.3 Determination of phytochemicals and antioxidant properties

3.2.3.1 Determination of total phenol

Total phenol was assayed by the method of Malik and Singh (1980).

Phenols react with the phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium to produce a blue-coloured complex (molybdenum blue) which can be estimated spectrophotometrically at 650nm.

Phenol was extracted with 80 per cent ethanol in a ratio of 1:10 (w/v) with several repeats, centrifuged and pooled the supernatants, evaporated the pooled materials to dryness and the residue was dissolved in a known volume of distilled water. Different aliquots were pipetted out and the final volume was made up to 3.0ml with distilled water. Added to it, 0.5ml of Folin-Ciocalteau reagent and after 3 minutes, 2.0ml of 20 per cent sodium carbonate solution and all the tubes were kept in a boiling water bath for 1 minute. After cooling, absorbance was measured in UV-visible spectrophotometer (Varian Cary 50 scan) at 650 nm against a reagent blank. Catechol was used to make the calibration curve. The concentration of the total phenols in the test sample was expressed as mg catechol equivalent/g material.

3.2.3.2 Determination of total flavonoid content

The total flavonoids were determined by the method as described by Aiyegoro and Okoh (2010).

Flavonoids were extracted with 80 per cent ethanol in the ratio 1:10 (w/v) and kept on a rotary shaker for 24 hour. The extracts were filtered through Whatman No. 42 filter paper and the volume was adjusted with 80 per cent ethanol. To 0.5ml of extract, 1.5ml 95 per cent ethanol, 0.1ml of 10 per cent aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water were added in order and after 30 minutes of incubation at room temperature, the absorbance were measured at 415nm in a UV-visible spectrophotometer (Varian Cary 50 scan). Quercetin was used to make the calibration curve. The result was expressed as mg of quercetin equivalents /g of dry weight.

3.2.3.3 Estimation of DPPH radical scavenging activity

Antioxidant activity was measured according to the method Vani *et al.* (1997) by using DPPH.

Samples were extracted with 95 per cent methanol in a ratio of 1:10 (w/v), concentrated over boiling water bath and redissolved in methanol to a concentration of about 10mg/ml and stored in a refrigerator until analysis. A solution of DPPH (0.05mM) was prepared in 99.5 per cent methanol. Methanolic extracts equivalent to 20, 40, 60, 80 and 100 µg dry samples were taken and the volumes were made up to 100 µl. To each of these, 2.9ml of DPPH solutions were added, followed by vortexing and kept at dark for 30 minutes. Absorbance was measured at 517nm along with blank where each of 1ml methanol and DPPH solution was taken, the percentage of inhibition was calculated by following a formula:

$$\text{Percentage of inhibition (\%)} = \frac{Absorbance of\ blank - Absorbance\ of\ test sample}{Absorbance\ of blank} \times 100$$

3.3Statistical analysis

a) Factorial Completely Randomized Design

The data recorded for each parameter were subjected to analysis of variance (ANOVA), for factorial completely randomized design as suggested by Gomez and Gomez (1984). The treatment mean squares were tested for significance by F-test and the difference of treatment means were tested by estimating critical difference.

The standard error of differences (S.Ed.±) was calculated by using the following expression:

$$\text{S.Ed.} \pm = \sqrt{\frac{\text{Error mean square}}{\text{Pooled number of replicatio n}} \times 2}$$

b) Correlation

Correlation is a broad class of statistical relationships involving dependence. It often refers to the extent to which two variables have a linear relationship with each other. This method is useful because they can indicate a predictive relationship that can be exploited in practice. The measure of correlation investigates the nature and extent of relationship between two or more related variables. When correlation is studied between two variables, it is called simple correlation. Correlation may be linear or non-linear. If the rate of change in the two variables is the same, then the correlation is linear, otherwise it is non-linear.

The amount of relationship between two variables is measured by a quantity, called the correlation coefficient. Professor Karl Pearson, the statistician, presented a coefficient to measure the degree of correlation or product moment correlation. It is denoted by r and formula of correlation coefficient of x and y variables is given by

$$r(x,y)=\frac{Cov(xy)}{\sigma \times \sigma y}$$

$$=\frac{1/n\sum(xi-\bar{x})(yi-\bar{y})}{\sqrt{1/n\sum(xi-\bar{x})^2 1/n(yi-\bar{y})^2}}$$

- The correlation coefficient has certain properties:
- Correlation coefficient (r) is a dimensionless quantity.
- It is independent of change in origin and scales.
- The value of r is such that $-1 \le r \le +1$ The + and – signs are used for positive linear correlation and negative linear correlations, respectively.
- Positive correlation: A value of exactly +1 indicates a perfect positive correlation. Positive values indicate a relationship between x and y variables such that as values for x increase, values for y also increase.
- Negative correlation: A value of exactly -1 indicates a perfect negative correlation. Negative values indicate a relationship between x and y such that as values for x increase, values for y decrease.
- No correlation: A value close to 0 indicates that there is no linear correlation or a weak linear correlation. A value near zero means that there is a random, non-linear relationship between two variables.
- A perfect correlation of ±1 occurs only when the data points all lie exactly on straight line. If r = +1, the slope of this line is positive. If r = -1, the slope of this line is negative.

A correlation coefficient greater than 0.8 is generally described as strong whereas a correlation coefficient less than 0.5 is described as weak.

c) Standard deviation

The standard deviation is a more accurate and detailed estimate of dispersion because an outlier can greatly exaggerate the range. The standard deviation shows the relation that set of scores has to the mean of the sample. It is the square root of

the sum of the squared deviation from the mean divided by the number of scores minus one.

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(xi-\mu)^2}$$

$$\mu = \frac{1}{N}\sum_{i=1}^{N} xi$$

N = number of scores

CHAPTER IV

EXPERIMENTAL FINDINGS

The results of the present investigation on "Antioxidant properties in green tea as influenced by processing techniques" are presented in this chapter.

4.1 Moisture Content

Moisture content varies greatly according to the environmental factors, manufacturing methods, etc. During withering, certain amount of leaf moisture is reduced however most of it is reduced in the process of drying where the leaf moisture content is brought down to 3 to 4 per cent. Data pertaining to the moisture content in the green tea samples manufactured by different processing techniques and collected from different flushes are given in Table 4.1.

Table 4.1 Moisture Content (%) in green tea samples

	M_1	**M_2**	**M_3**	**M_4**	**MEAN**
T_1	4.75	5.39	6.56	4.63	5.33
T_2	4.79	5.14	6.34	5.25	5.38
T_3	4.09	5.27	6.60	3.30	4.81
T_4	4.10	4.57	6.18	4.57	4.86
T_5	3.87	5.35	6.60	4.96	5.20
T_6	5.21	6.04	6.76	5.16	5.79
MEAN	4.47	5.29	6.51	4.65	
	S.Ed. (±)		**C.D. (5%)**		
T	0.06		0.12		
M	0.05		0.09		
T X M	0.12		0.23		

The result revealed a significant difference in the moisture content of green tea samples. The highest average moisture content (6.51%) was recorded in the samples of August flush (M_3). In the samples of August flush (M_3), all the treatments had the least effect on the moisture content which ranged from 6.18 per cent to 6.76 per cent. Samples of April flush (M_1) had the lowest average moisture content (4.47%). In general, there was an increasing moisture content in green tea from April to August flush which then decreased in the October flush. Amongst the different processing techniques employed in the manufacture of green tea, the lowest average moisture content was recorded for T_3 (4.81%) and the highest for T_6 (5.79%).

The analysis of variance showed that the treatment followed by the growers, month of manufacturing and their interaction, all had significance effects on the moisture content in the green tea samples.

4.2 CIE Lab parameters

Changes in CIE Lab colour values i.e.lightness (L*), redness (a*) and yellowness (b*) green tea are presented in the Tables 4.2, 4.3 and 4.4. Hue and Chroma were derived from L*, a* and b* values using standard formula and are presented in the Tables 4.5 and 4.6.

4.2.1 CIE L* value of infused green tea samples

L* depicts the lightness of the food material. The brightest and lightest colour i.e. white, gives L* = 100. Green tea infusion being bright and light in colour gives high L* value. The lightness of the green tea samples under study are presented in the Table 4.2. It revealed that there were significant effects of both the manufacturing method as well as the flushes on the L* value of infused green tea samples.

Table 4.2 Variation in CIE L* value in infused green tea samples

	M_1	M_2	M_3	M_4	MEAN
T_1	86.59	91.22	88.61	91.06	89.37
T_2	82.79	88.16	85.27	84.65	85.22
T_3	88.09	86.85	86.45	87.45	87.21
T_4	87.10	88.44	88.57	88.36	88.12
T_5	84.40	85.71	89.12	86.97	86.55
T_6	84.73	86.89	83.40	89.94	86.24
MEAN	85.62	87.88	86.90	88.07	
	S.Ed. (±)		C.D. (5%)		
T	0.17		0.34		
M	0.14		0.28		
T X M	0.35		0.68		

The average lightest tea was observed for the October flush (highest L* value of 88.07) while the least average light tea was found in April flush with L* value of 85.62. Steaming for 30 sec (T_1) resulted in the lightest tea with average L* value of 89.37 while the average lowest was found in T_2 (85.22) where the green tea leaves were dipped in boiling water for 10 seconds. M_2T_1 gave the lightest tea while the darkest tea was observed in case of M_1T_2.

The interaction between the treatments followed by the growers and the month of manufacture had a significant effect on the lightness of the infused tea as shown by the analysis of variance.

4.2.2 CIE a* value of infused green tea samples

Infused green tea has a greenish colour because of non- degraded enzymes and chlorophyll and other chemical constituents. Thus an infused green tea will have a negative value of a* which denotes a green colour. The a* of the green tea

samples along the various processing methods for the four tea flushes are given in the Table 4.3.

Table 4.3 Variation in CIE a* value in infused green tea samples

	M_1	**M_2**	**M_3**	**M_4**	**MEAN**
T_1	1.82	-2.34	-1.36	-2.34	-1.05
T_2	-0.41	-1.58	-0.5	-1.59	-1.02
T_3	-0.04	0.21	0.41	0.45	0.26
T_4	-0.91	-1.76	-0.68	-0.56	-0.98
T_5	0.82	-0.92	-0.49	-0.47	-0.26
T_6	2.57	0.14	0.03	0.02	0.69
MEAN	0.64	-1.04	-0.43	-0.75	
	S.Ed. (±)		**C.D. (5%)**		
T	0.14		0.28		
M	0.12		0.23		
T X M	0.29		0.56		

The data showed a significant effect of processing methods and harvesting period on a* value of the infused green tea. The average redness was lowest (-1.04) in the May product which had the most greenish tinge and average redness was highest (0.64) in that of April product. Processing techniques had significant effects on a* value, T_3 and T_6 had the positive a* values indicating redness of tea while the others had the negative a* values with T_1 being the most negative (-1.05). T_6 showed the highest average a* value (0.69). The results revealed that pan roasting (T_6) had a negative impact on a* value while T_1 (steaming for 30 sec) was found best.

The analysis of variance showed a significant effect on the redness of the infused green tea among the various treatments, their month of manufacturing and their interaction.

4.2.3 CIE b* value of infused green tea samples

CIE b* is the yellowness of a sample. Higher the yellowness of the sample, higher is the b* value. A properly infused green tea has a b* value in the range of 25 to 35. The data on yellowness of the green tea samples under study are given in the Table 4.4.

Table 4.4 Variation in CIE b* value in infused green tea samples

	M_1	**M_2**	**M_3**	**M_4**	**MEAN**
T_1	31.35	28.99	33.34	33.22	31.73
T_2	33.05	34.62	27.38	34.62	32.42
T_3	29.85	32.12	32.43	32.23	31.66
T_4	28.8	32.35	31.13	31.26	30.88
T_5	27.65	33.02	30.92	34.4	31.5
T_6	29.77	32.73	30.66	32.17	31.33
MEAN	30.08	32.31	30.98	32.98	
	S.Ed. (±)		**C.D. (5%)**		
T	0.2		0.39		
M	0.16		0.32		
T X M	0.4		0.77		

The data showed a significant change along all the methods of manufacturing and the time of manufacturing. The average yellowness was highest (32.98) in the October product and average lowest value (30.08) was found in the products of April. Dipping in 100 ^{o}C water for 10 sec (T_2) resulted in the highest average yellowness while T_4 (Steaming for 1 min) gave the lowest (30.88) average yellowness of green tea.

The analysis of variance showed that the treatment followed by the growers and the month of manufacturing and their interaction, all have a significance effect on the yellowness of the infused green tea samples.

4.2.4 CIE Hue (H*) value of infused green tea samples

Hue is one of the main properties of a colour, defined technically as "the degree to which a stimulus can be described as similar to or different from stimuli that are described as red, green, blue, and yellow". Hue of the green tea samples along the various manufacturing methods across the four flushes are given in the Table 4.5.

Table 4.5 Variation in CIE H* value in infused green tea samples

	M_1	**M_2**	**M_3**	**M_4**	**MEAN**
T_1	2.1	5.21	1.82	0.06	2.3
T_2	-2.73	0.93	-1.23	6.88	0.96
T_3	1.21	-0.51	0.99	0.31	0.5
T_4	0.09	0.98	-1.23	-1.44	-0.4
T_5	0.47	-1.66	0.23	-0.11	-0.27
T_6	-0.95	1.74	5.91	1.05	1.94
MEAN	0.03	1.11	1.08	1.12	
	S.Ed. (±)		**C.D. (5%)**		
T	1.02		1.99		
M	0.83		1.63		
T X M	2.03		3.99		

A significant change along both the manufacturing methods and the flushes was observed for the H* value of infused green tea samples. The average maximum hue in the tea sample (1.12) was recorded for the October flush while the average minimum hue (0.03) was recorded in April. On the other hand, samples steamed for 30 sec (T_1) showed the average maximum hue (2.3) while the average lowest hue value (-0.4) was observed in samples steamed for 1 min (T_4). The highest hue (5.91) was observed in pan roasted samples obtained during August flush while the lowest (-2.73) was recorded in samples obtained during April flush and treated by dipping in 100 ^{o}C water for 10 sec.

The treatments followed by the growers, the month of manufacturing and their interaction, all showed a significant effect on the hue value.

4.2.5 CIE Chroma (C*) value of infused green tea samples

Chroma is the quality of a colour's purity, intensity or saturation. The data pertaining to the chroma of the green tea samples manufactured by various processing methods from the four flushes are shown in the Table 4.6.

Table 4.6 Variation in CIE C* value in infused green tea samples

	M_1	**M_2**	**M_3**	**M_4**	**MEAN**
T_1	31.42	33.37	29.09	33.3	31.79
T_2	33.06	27.39	34.66	34.65	32.44
T_3	29.85	32.43	32.12	32.24	31.66
T_4	28.81	31.13	32.4	31.26	30.9
T_5	27.67	30.92	33.05	34.4	31.51
T_6	29.88	30.66	32.73	32.17	31.36
MEAN	30.11	30.98	32.34	33	
	S.Ed. (±)		**C.D. (5%)**		
T	0.2		0.38		
M	0.16		0.31		
T X M	0.39		0.77		

A significant change was observed with the processing methods and the flushes from which the samples were collected. The average maximum chroma value (33.00) was recorded for samples from October flush while the average minimum chroma value (30.11) was observed for April flush. Samples processed with dipping in 100 ^{o}C water for 10 sec (T_2) showed the average maximum chroma (32.44) with highest saturation (34.66) for the August flush.

The interaction between the treatments followed by the growers and the month of manufacture had a significant change on the chroma value of the infused tea as shown by the analysis of variance.

4.3 Total polyphenol content

Polyphenols are a structural class of natural, synthetic or semisynthetic, organic chemicals characterized by the presence of large multiples of phenol structural units. The total polyphenol contents of the green tea samples (mg catechol equivalents/g) in green tea samples as affected by the processing methods and the flushes are presented in the Table 4.7.

Table 4.7 Total polyphenol content (mg CE/g) in green tea samples

	M_1	**M_2**	**M_3**	**M_4**	**MEAN**
T_1	15.19	14.04	20.38	17.74	16.84
T_2	14.35	16.98	13.04	11.48	13.96
T_3	11.36	11.68	15.55	14.34	13.23
T_4	9.39	11.3	15.68	12.47	12.21
T_5	8.71	10.58	12.46	10.73	10.62
T_6	10.66	15.1	15.41	19.46	15.16
MEAN	11.61	13.28	15.42	14.37	
	S.Ed. (±)		**C.D. (5%)**		
T	0.1		0.21		
M	0.09		0.17		
T X M	0.21		0.41		

The average highest total polyphenol content (15.42 mg CE/g) was recorded for the August flush with highest total polyphenol content (19.46 mg CE/g) in pan roasted samples for the October flush. The lowest average total polyphenol content (11.61 mg CE/g) was observed for the April flush with lowest (8.71 mg CE/g) in samples prepared by steaming for 3-4 min (T_5). Amongst the processing methods,

samples treated with steaming for 30 sec showed the maximum average total polyphenol content of 16.84 mg CE/g while the lowest average total polyphenol content (10.62 mg CE/g) was observed in T_5.

The interaction between the treatments followed by the growers and the month of manufacture had a significant effect on the total polyphenol content of the green tea sample as shown by the analysis of variance.

4.4 Total flavonoid content

Tea is rich in flavonoids or bioflavonoids which are a class of plant secondary metabolites. Table 4.8 shows the data on total flavonoid content (mg quercetin equivalents /g) of the green tea samples as affected by different manufacturing methods and the flushes.

Table 4.8 Total flavonoids content (mg QE/g) in green tea samples

	M_1	**M_2**	**M_3**	**M_4**	**MEAN**
T_1	19.86	23.44	23.91	23.26	22.62
T_2	13.66	19.7	20.81	20.86	18.76
T_3	22.02	22.47	20.82	15.74	20.26
T_4	17.6	20.78	20.13	19.94	19.61
T_5	16.17	19.05	20.55	18.94	18.68
T_6	15.65	18.55	19.03	19.69	18.23
MEAN	16.45	20.39	21.15	20.78	
	S.Ed. (±)		**C.D. (5%)**		
T	0.12		0.24		
M	0.1		0.19		
T X M	0.24		0.47		

The highest average total flavonoid content (21.15 mg QE/g) was recorded in August flush while the lowest average total flavonoid content (16.45 mg QE/g) was observed for the April flush. On the other hand, samples steamed (T_1) for

30 sec showed the average maximum total flavonoid content (22.62 mg QE/g) while the average lowest total flavonoid content (18.23mg QE/g) was recorded for treatment T_6 (pan roasting). The flavonoid contents in green tea were found to vary from 13.66 to 23.91 mg QE/g depending on the processing methods and time of harvest.

The analysis of variance showed a significant effect on the total flavonoid content of the infused green tea among the various treatments, their month of manufacturing and their interaction.

4.5 DPPH free radical scavenging activity

Radical scavenging activity of the crude methanolic sample extracts were measured by colorimetric assay using DPPH as a source of free radical. The results are presented in Table 4.9.

Table 4.9 DPPH radical scavenging activity (% inhibition) of green tea samples

	M_1	**M_2**	**M_3**	**M_4**	**MEAN**
T_1	72.33	74.71	85.03	82.98	78.76
T_2	70.69	76.87	82.30	80.16	77.51
T_3	67.22	67.32	84.58	74.37	73.37
T_4	67.63	74.89	87.83	80.33	77.67
T_5	66.57	76.04	88.12	77.13	76.96
T_6	61.33	75.07	87.15	79.07	75.66
MEAN	67.63	74.15	85.84	79.00	
	S.Ed. (±)		**C.D. (5%)**		
T	0.50		0.97		
M	0.40		0.79		
T X M	0.99		1.94		

The highest average activity (85.84% inhibition) was recorded in case of August flush while the lowest activity (67.63% inhibition) was observed for April

flush. On the other hand, steaming for 30 sec (T_1) resulted in the higher average free radical scavenging activity (78.76% inhibition) while the average lowest (73.37% inhibition) was observed for samples dipped in 100 ^{o}C water for 1 min (T_3). Maximum inhibition (88.12%) was recorded for August flush steamed for 3-4 min.

The month of manufacture, treatment followed by the growers and their interaction showed a significant change as shown by the analysis of variance.

4.6 Correlation Data

From the above studies on different parameters, all the treatments were selected for further correlation studies. The data pertaining to correlation among all the above factors are given in Table 4.10, 4.11, 4.12 and 4.13 for the month of April, May, August and October respectively.

The significance of correlation coefficient was tested by comparing the observed value of correlation coefficient with the table value at 5 per cent and 1 per cent probability level. To find out the relationship between the different parameters, simple correlation coefficients among the studied parameters have been analysed for the month April, May, August and October.

Table 4.10. Correlation among the different manufacturing methods in the month of April

	Moisture	L*	a*	b*	Hue	Chroma	TPC	TFC	TAC
Moisture	1								
L*	-0.40	1							
a*	0.61	-0.13	1						
b*	0.62	-0.34	-0.04	1					
Hue	-0.40	0.78	0.23	-0.41	1				
Chroma	0.64	-0.34	-0.02	1.00**	-0.41	1			
TPC	-0.06	0.74	0.52	-0.41	0.90*	-0.40	1		
TFC	-0.36	0.93**	-0.01	-0.20	0.86*	-0.20	0.75	1	
TAC	-0.16	0.05	-0.38	0.54	0.20	0.53	-0.10	0.18	1

** Correlation is significant at 0.01 level

* Correlation is significant at 0.05 level

The analysis illustrates that the lightness of the green tea samples had a positive correlation with the total flavonoid content (0.93) at 1 per cent probability level in the month of April. Again the yellowness had a positive correlation at 1 per cent probability level with the chroma of the green tea samples in the month of April. However the hue angle of the green tea samples showed a positive correlation at 5 per cent level, both with the total polyphenol content (0.90) and total flavonoid content (0.86).

Table 4.11. Correlation among the different manufacturing methods in the month of May

	Moisture	L*	a*	b*	Hue	Chroma	TPC	TFC	TAC
Moisture	1								
L*	-0.21	1							
a*	0.54	-0.76	1						
b*	-0.08	-0.69	0.35	1					
Hue	0.16	0.92**	-0.55	-0.71	1				
Chroma	0.08	0.31	0.01	-0.88*	0.34	1			
TPC	0.38	0.07	0.26	-0.69	0.27	0.84*	1		
TFC	-0.30	0.69	-0.35	-0.77	0.51	0.68	0.23	1	
TAC	0.01	0.15	-0.55	0.23	0.20	-0.53	-0.32	-0.55	1

** Correlation is significant at 0.01 level

* Correlation is significant at 0.05 level

The correlation coefficient data in the month of May showed that the yellowness of the green tea samples was negatively significant to chroma (-0.88) however chroma was positively significant to the total polyphenol content (0.84), both at 5 per cent probability level. Moreover the lightness of the green tea sample had a positive correlation with the hue angle of the green tea sample at 1 per cent probability level (0.92).

Table 4.12. Correlation among the different manufacturing methods in the month of August

	Moisture	L*	a*	b*	Hue	Chroma	TPC	TFC	TAC
Moisture	1								
L*	-0.38	1							
a*	0.37	-0.58	1						
b*	0.34	0.45	-0.15	1					
Hue	0.83*	-0.58	0.28	0.30	1				
Chroma	-0.23	-0.43	0.50	-0.89*	-0.27	1			
TPC	0.34	0.35	-0.16	0.95**	0.44	-0.85*	1		
TFC	-0.05	0.53	-0.71	0.47	-0.21	-0.74	0.30	1	
TAC	0.14	0.34	-0.01	0.38	0.25	-0.15	0.56	-0.37	1

** Correlation is significant at 0.01 level

* Correlation is significant at 0.05 level

Moisture showed a positive correlation with the hue angle (0.83), the redness showed a negative correlation with chroma (-0.89) and chroma showed a negative correlation with the total polyphenol content (-0.85) at 5 per cent significant level in the month of August. However, the yellowness showed positive correlation with the total polyphenol content (0.95) at 1 per cent significant level.

In the month of October, the correlation coefficient data showed that the lightness and yellowness was positively correlated with the total polyphenol content (0.92) and the chroma (1.00) respectively, both at 1 per cent probability level. The total flavonoid content was also significant at 1 per cent probability level with the total antioxidant activity (0.98). The redness of the green tea sample was negatively significant with the total flavonoid content (-0.91) and total antioxidant activity (-0.85) both at 5 per cent significant level.

Table 4.13. Correlation among the different manufacturing methods in the month of October

	Moisture	L*	a*	b*	Hue	Chroma	TPC	TFC	TAC
Moisture	1								
L*	-0.08	1							
a*	-0.44	-0.12	1						
b*	0.45	-0.53	-0.46	1					
Hue	0.42	-0.66	-0.33	0.64	1				
Chroma	0.45	-0.51	-0.48	1.00**	0.64	1			
TPC	-0.29	0.92**	0.26	-0.62	-0.76	-0.62	1		
TFC	0.65	0.36	-0.91*	0.26	0.18	0.27	-0.02	1	
TAC	0.59	0.40	-0.85*	0.07	0.12	0.08	0.02	0.98**	1

** Correlation is significant at 0.01 level

* Correlation is significant at 0.05 level

CHAPTER V

DISCUSSION

Green tea has nowadays become a very popular drink because of both the surging interest in tea in general as well as its proven healthful properties.

Green tea is processed by having the leaves steamed or pan fried soon after being harvested from the tea plant. This prevents oxidation, keeping the leaves soft, pliable and green in color. There are numerous types of green tea available. These differing types of green tea, while they originate from the same plant, all ends up differing in tastes and look due to the way the plant is grown and where the tea plant is located.

Green tea was used in traditional Chinese and Indian medicine to control bleeding and heal wounds, aid digestion, improve heart and mental health, and regulate body temperature. Recent studies have shown green tea can potentially have positive effects on everything from weight loss to liver disorders, type II diabetes, and Alzheimer's disease.

According to Annon (2007), the polyphenols in tea have been shown to decrease tumor growth in laboratory and animal studies and may protect against damage caused by ultraviolet UV-B radiation. In countries where green tea consumption is high, cancer rates tend to be lower, but it is impossible to know for sure whether it is the green tea that prevents cancer in these particular populations or other lifestyle factors.

Researchers believe that it is the high level of polyphenols in tea that helps kill cancerous cells and stop them from growing. However, the exact mechanism by which tea interacts with cancerous cells is unknown. However, other

studies have not found that tea can reduce cancer risk. The amount of tea required for cancer-preventive effects also varies widely in studies - from 2-10 cups per day.

According to Annon (2005), “there is no credible evidence to support qualified health claims for green tea consumption and a reduced risk of gastric, lung, colon/rectal, esophageal, pancreatic, ovarian, and combined cancers”.

A 2006 study published by Kuriyama *et al.* (2006) concluded that green tea consumption is associated with reduced mortality due to all causes, including cardiovascular disease. The study followed over 40,000 Japanese participants between the ages of 40 and 79 for 11 years, starting in 1994. The participants who drank at least 5 cups of green tea per day had a significantly lower risk of dying (especially from cardiovascular disease) than those who drank less than one cup of tea per day. Green tea contains catechins, polyphenolic compounds that are thought to exert numerous protective effects, particularly on the cardiovascular system. An analysis of published studies in 2011 found that consuming green tea, either as a beverage or in capsule form, was linked to significant but modest reductions in total and LDL or "bad" cholesterol.

Studies concerning the relationship between green tea and diabetes have been inconsistent. Some have shown a lower risk of developing type 2 diabetes for green tea drinkers than for those who consumed no tea, while other studies have found no association between tea consumption and diabetes at all.

Green tea may promote a small, non-significant weight loss in overweight and obese adults; however, since weight loss in the studies was so minimal, it is unlikely that green tea is clinically important for weight loss.

According to Annon (2011), researchers tested the effect of a component of green tea, CAGTE (or "colon available" green tea extract), after it had been digested, to see how it affected a key protein in Alzheimer's disease.

According to Annon (2011), “this study adds to previous research that suggests green tea might help to reduce the risk of Alzheimer's disease. However, the researchers used a far higher dose of the active green tea chemical than would ever be found in the human body. More research is needed to see whether green tea is protective at a much lower dose, and to understand the mechanism involved”.

Other studies have found that green tea might be helpful in preventing dental cavities, stress, chronic fatigue, treating skin conditions, and improving arthritis by reducing inflammation. Further research is needed to firm up these theories.

The present study was undertaken to study the physiochemical, phytochemical and antioxidant properties of green tea, collected from different growers of Assam, India; with respect to different manufacturing techniques along four tea flushes. The results of the present study are discussed in this chapter.

5.1 Physiochemical properties of green tea

5.1.1 Moisture Content

Since moisture content may be classified into various statuses with names to them, evaluation of and dealing with measured data requires a special care. Moisture adhered to a material’s surface is called water of adhesion, free water or hygroscopic moisture and moisture adhered to a material with a certain condition such as pressure, temperature, volume etc. is called absorbed water or equilibrium moisture content. Water chemically bonded to a material itself or inside of the material is crystal water or hydrated water while its moisture is bond water or combined water.

The measurement of tea moisture content is important for processing and storing tea. The moisture content of tea affects the quality and durability of the product. Tea should be packed at maximum moisture content of 4 per cent. To pack with moisture content of less than 3 per cent would not be economically viable. Packaging with more than 4 per cent moisture constant will affect the keeping

properties of the tea and eventually resulting liquor becomes tired, flat, mouldy, out of condition, etc.

The present study showed a significant difference between the moisture content of different green tea samples along the different manufacturing methods and the four tea flushes. The data ranged from 6.51 per cent in the month of August which was an autumn flush with moderate precipitation in context to Indian tea cultivation, to 4.47 per cent in the month of April which is considered as the summer flush and thus having low moisture content. While along the treatment, the range was obtained from 4.81 per cent in the made tea where the green leaves has undergone a treatment of dipping in boiling water for 1 minute which makes the green leaves flaccid in short duration, to 5.79 per cent where the leaves were pan roasted. Pan roasting is a difficult method as the leaves become brittle and subjected to get burned very quickly. Thus most of the growers, in fear of damaging the leaves, tend to roast the leaves till halfway, which eventually did not dry up all the moisture content in the green leaves which hamper the overall quality. Moreover there are other manufacturing faults which cannot be omitted from the processing unit especially during drying which affects the moisture content of the made tea.

The present study was almost similar to the data found by Li *et al.* (2012), who studied on the moisture content of 738 representative samples, including fresh tea leaves, manufactured tea and partially processed tea.

5.1.2 CIE Lab Parameters

The perfect colour of infused leaf is that of bright copper or red for black tea and bright greenish yellow for green tea. Colour should be of an even shade throughout and all the leaves of approximately the same size. Although there are some perfect colours, they are by no means a common feature but are generally reserved for tea produced during the quality period on estates in districts renowned for producing quality tea. It is, therefore, reasonably safe to assume that bright infused leaf denotes quality liquor and with it, flavours may also be present. The following terms are used by tasters to distinguish between degrees of variation:

- Bright greenish
- Mixed
- Dull
- Dark

Bright greenish: This usually implies that a tea has been under oxidized. Greenness is caused by leaf, which has failed to oxidize fully. Liquors obtained from a bright greenish infused leaf are generally quite brisk but to the point of possessing an unpleasant astringency or greenness. Colour and strength in cup may be lacking and quality will not be at its maximum; flavour on the other hand may not have been suffered.

Mixed: - This term is used when variation in colour between individual pieces of leaf is marked. The term is usually coupled with another such as dull, and more especially green. Pieces of green leaf in infused leaf are simply portions, which have failed to oxidize and are usually caused by coarse plucking, low withers or inadequate rolling. Both coarse plucking and low withering affect rolling; instead of the leaves being twisted they are merely cracked and broken. In this way leaf cells are not ruptured and consequently oxidation does not begin. Daily variations in manufacture will also produce mixed infused leaf in the bulk. Liquor characters will depend on the adjectives accompanying the description “mixed” and may vary between extremes.

Dull: – Common plains teas manufactured during the monsoon period are often dull due to extremes of heat and the ideal conditions for bacterial development. Over oxidation is also responsible for this dull appearance. Liquors are generally coloured but are plain to the palate and completely devoid of quality. A little briskness may be present but never pungency.

Dark: – Dark infused leaf is brought about by badly burning the tea in the firing machines or by severe bacterial infection. In both cases the effect on the liquor is most unpleasant.

Some tasters prefer to use the term “dull” to cover both the meaning of “dull” and “dark”. Depth of colour will vary between all grades from the same estate. Generally the smaller the grade the more coloured the liquor; in this way leaf grades are expected to have a lighter liquor than the Brokens, Brokens a lighter liquor than the Fannings, etc. Geographical location also plays an important part. Brightness is a very important factor. Tea liquors should never be dull to the eye and degree of brightness often corresponds closely to degree of quality. Common teas although possessing good depth of colour, are sometimes dull in appearance.

The present investigation showed that CIE L* values were quiet high indicating lightness of the infused tea leaves and for green tea to be perfect, producing a bright green liquor. The study revealed a significant change along both the manufacturing method as well as the tea flushes. The average lightest tea sample was recorded for the October flush, steamed for 30 seconds. The average darkest tea sample was observed for April flush processed by dipping in 100^{o}C water for 10 seconds. Decrease in L* value suggests darkening of the product and can be attributed to the occurrence of non-enzymatic browning (Muzzaffar *et al.*, 2016). But in green tea manufacturing, the enzyme is inhibited and hence there is less degradation of enzyme and polyphenols resulted in bright coloured made tea.

Chlorophyll is proven to be the influential compounds for the colour of dry tea leaves. Water soluble chlorophyll releases when the tea leaves are infused and thus increases both the greenness and turbidity of the made tea. Among the flavonoids, catechins and flavonols detected in green tea infusions, quercetin shows to be the most important phenolic compound contributing to the greenness of the tea infusion (Wang *et al.*, 2006).

The CIE a* value indicates the redness of the sample tested. Negative data reveals greenness of the tea infusion. The average values ranged from -1.04 in the month of October to 0.64 in the month of April. Among the treatment, the average a* was in the range of -1.05 (T_1) to 0.69 (T_6). Treatment T_6 was pan roasted which might be the reason of showing the least green colour as the roasting methods

generally initiates burning of the green leaves if not done by expertise personnel. The overall data justified the natural greenness of the made tea as green tea is highly rich in flavonoid and there is less degradation of chlorophyll during manufacturing. The a* value of the present data is quite similar to the findings of Laddi *et al.* (2011). They worked on the influence on color attributes of freshly brewed tea with time due to variations in temperature conditions.

The CIE b* value of the present study was found to be high towards the yellow axis which is a desirable cup characteristic of a green tea infusion. The average lowest yellowness was found in the month of April (30.08) while in the month of October the average yellowness was the highest (32.98). Amongst all the treatment, T_2 showed the average highest yellowness (32.42) where the green leaves were dipped in boiling water for 10 sec. However, the average lowest yellowness was found in T_4 (30.88) where the green leaves were steamed for 1 min. Treatment T_5 had the average mean yellowness to be 31.50 which was also steamed but for 3 to 4 min. This showed that steaming of green leaves had a negative impact on the yellowness of the infused liquor.

Hue simply describes the wavelength of the color. It is the actual "color" that follows a natural order of red (R), yellow (Y), green (G), blue (B) and purple (P); designated as principle hues. Between each were intermediate hues yellow-red (YR), green-yellow (GY), bluegreen (BG), purple-blue (PB) and red-purple (RP). The infused green tea samples in the present study gave a wide range of H* (-2.73 in the month of April in treatment T_2 to 5.91 in the month of August in treatment T_6).

Chroma (saturation) may be defined as the strength or dominance of the hue. Thus it is the quality of a colour's purity or intensity. For an instant, a gray colour is a neutral having an extreme low chroma, fire engine red may be a high chroma while brick red may be a middle chroma. Light yellow colours have potentially high chroma value, as much as in the range of 30 to 40. In the present study too, the data ranged from 27.39 in the month of May in Treatment T_2 to 34.65 in

T_2 as well in the month of October. In treatment T_2, the green leaves were manufactured by dipping in boiling water for 10 seconds. Thus it justified that for good tea liquor, specifically for green tea liquor, this particular treatment is suitable.

5.2 Phytochemical and Antioxidant Properties

5.2.1 Total Phenol Content

Phenolic compound of plant act as primary antioxidants or free radical scavenger (Ayoola *et al.*, 2008). Tea is rich in phenols and phenols have got potent medicinal properties. Phenols sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule (Khoddami *et al.*, 2013). Polyphenols are secondary metabolites of plants and are generally involved in the defense against UV radiation or aggression by pathogens. Plant polyphenols offered some protection against development of cancers, cardiovascular diseases (Graf *et al.*, 2005). Phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes (Dai and Mumper, 2010).

The present study reveals the total phenolic content to be in the range of 8.71 to 20.38 mg CE/g in the April and August flushes respectively. Shonisani (2010) reported phenolic content in 1st tea flush to be 7.68 mg CE/g. (Anesini *et al.*, 2008) estimated the total polyphenol concentration in green tea. They reported total phenol content in the range of 21.02 ± 1.54 to 14.32 ± 0.45 per cent of Catechol equivalent. Balentine *et al.* (1997) reported that the green tea contained almost 30 to 42 per cent polyphenols on the dry weight which was very high as compared to the present study. This shows that due to geographical diversity, the biochemical composition of tea shows a wide difference. From the present study, it was observed that T_1, i.e., steaming green leaves for 30 seconds was the best amongst the treatments. August flush, i.e. the autumn flush resulted best in the polyphenolic compounds.

5.2.2 Total Flavonoid Content

Tea is rich in flavonoids which have got immense medicinal properties. Flavonoids comprise the most studied group of polyphenol. This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocyclic (Pandey and Rizvi, 2009). Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as anti-tumor activities but the best described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Tapas *et al.*, 2008).

The present study revealed a high amount of total flavonoid content in the August flush followed by the October flush. The flavonoid content ranged from 13.66 mg QE/g in the month of April with leaves dipped in 100 ^{o}C water for 10 sec to 23.91 mg QE/g in the month of August with treatment T_1, steaming for 30 sec. Like total polyphenol content, T_1 showed the maximum total flavonoid content indicating a better treatment in the preservation of antioxidant activities. Balentine *et al.* (1997) worked on flavanols and flavonols, the two main sub-division of Flavonoid. It was found to be 30 per cent of the dry weight of fresh leaf. According to (Punyasiri *et al.*, 2004) the enormous content of flavan-3-ols is the most striking feature of tea leaf chemistry, it is up to 20 per cent (w/w, dry) and depends mostly on tea variety.

5.2.3 DPPH free radical scavenging activity

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from substance to an oxidizing agent. Oxidation reaction can produce free radicals can start chain reaction that damage cells. Antioxidant terminates these reactions by removing the free radical intermediates, and inhibits other oxidation reaction. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997).

The present study reveals DPPH (%) inhibition to be in the range of 61.33 per cent for April flush in pan roasted samples to 88.12 per cent for the August flush with leaves steaming for 3-4 minutes. The data showed a higher inhibition for August flush over and above the other flushes. For the August flush, total phenol and flavonoid contents were also high and this might probably was the reason for the higher free radical scavenging activity. There was a gradual increase in the total phenol and flavonoid contents in the April, May and August flushes and hence the antioxidant activities of green tea and then a decline in the antioxidant activity in the October flush.

Scavenging effects of tea catechins and their epimerized, acylated, and glucostylated derivatives on 1,l -diphenyl-2-picrylhydrazyl (DPPH) radical were evaluated by electron spin resonance spectrometry by Nanjo *et al.* (1996). Tea catechins and their epimers were shown to have 50 per cent radical scavenging ability in the concentration range of 1 to 3 µM, which was found similar with the present study.

CHAPTER VI
SUMMARY AND CONCLUSION

The present investigation was on "Antioxidant Properties in Green Tea as influenced by Processing Techniques", with the objective to identify the basic phytochemical and antioxidant properties of the green tea manufactured by the Small Tea Growers Assam, India, with a vision on their manufacturing variance for inhibiting the enzymes. The result of the present investigation may be comprehensively summarizer as follows:

1. Moisture Content was found to be in the optimum amount, with higher value being in the month of August. It is probably due to the increased humidity as August had a moderate precipitation as compared to the other months. The highest moisture content (6.76%) was found in pan roasted samples of August flush and lowest (3.30%) in the October flush dipped in water (100 ^{o}C) for 1 min.
2. The tea samples were very bright, green and yellow in colour with optimum Hue angle and Chroma (saturation). The average highest L* value (lightness) was found to be 88.07 present in October flush and steaming for 30 sec resulted in the highest value for L* (89.37).The average lowest a* value (greenness) was reported for the May flush (-1.04). In this case also steaming for 30 sec resulted best. The average highest b* value (yellowness) was reported to be highest in the samples from October (32.98) and in this case samples dipped in water (100 ^{o}C) for 10 sec was the best.
3. The average total polyphenolic content was found to be highest in the samples of August (15.42 mg CE/g) and steaming for 30 sec resulted in the highest total polyphenol (16 mg CE/g).
4. Green tea samples from August had a highest amount of total flavonoid content (21.15 mg QE/g). The average highest total flavonoid content (22.62 mg/g) was found in samples treated by steaming for 30 sec.

5. Free radical scavenging activity was highest in samples from August with 85.84% inhibition. The lowest (67.63%) was recorded for samples of April flush. Amongst all the treatments, highest inhibition was recorded for samples steamed for 30 sec.

CONCLUSION

It has been found from the present investigation that green tea has high amount of compounds with antioxidant properties. The values could not be standardised as green tea samples were collected from different gardens based only on their manufacturing methods. There were wide geographical diversity as well as variation in the genotypes used in the study. Keeping in mind, all the variations that have good effect on the made tea, the growers may give emphasis on certain enzyme inhibiting methods like steaming for 10 sec and dipping in boiling water for 1 min and the products from August flush has a good cup characteristics along with higher quantitative wellness in context to green tea and for manufacturing green tea. Low temperature treatment is also beneficial for inactivating the enzymes. Researches are being conducted on liquid nitrogen in this regard. Colour of the infused green tea has always been a strong benchmark for its quality. Moreover a hypothetical fact that low temperature always restores the inherent properties of the food. Thus a field of study on this context can be extended. A tester's score can be used to compare the organoleptic parameters with the consumer acceptance. This would provide a defined effect of low temperature treatments over the high temperature treatments on the green leaves to inactivate the enzymes.

FUTURE PROSPECTS

Though the health benefits of green tea have been known for centuries, recent research is providing concrete evidence of these benefits.

- Studies have shown that green tea can prevent cancer since it contains catechin, the major component of tea. A study in Japan showed that residents in areas devoted to green tea production in the central and western regions of Shizuoka Prefecture, who

drink the tea daily, have a significantly lower death rate for all types of cancer compared to other regions.These findings were supported by animal experiments that showed green tea reduced the growth of tumors.

- Green tea catechin has also been shown to limit the excessive rise in blood cholesterol in both animals and humans, as well as prevent high blood pressure.
- Other benefits of catechin include killing bacteria and influenza viruses, preventing halitosis, inhibiting increase of blood sugar, and fighting cariogenic bacteria. Green tea (especially matcha) also contains important vitamins (C, B complex, and E), fluoride (for preventing cavities), amino acids (for lowering blood pressure), and polysaccharides (lowers blood sugar). Green tea is a strong antioxidant as well and is even more powerful than vitamin E or vitamin C due to the presence of polyphenols, such as epigallocatechin gallate (EGCG).
- Extracts of green tea may also make strains of drug-resistant bacteria that cause skin infections more sensitive to penicillin, British researchers report. The investigators also found that diluted tea extract acted synergistically with antibiotics, making them more potent against particular strains of this type of bacteria.
- In addition to preventing or curing these more common diseases, preliminary research indicates the antiviral capability of green tea catechin may have some beneficial effect in fighting AIDS. Laboratory tests have verified that catechin can inhibit the activity of the AIDS virus. Instead of simply being known as a popular Japanese beverage, green tea may thus become an important "new" medicine of the twenty-first century for the entire world.

BIBLIOGAPHY

Aiyegoro, O. A. and Okoh, A. I. (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complement. Alt. Med.,* **10**(21).

Ali, J. (2015). Role of processing conditions in determining tea quality. *Res. Gate*.

Ames, B. N. and Gold, L. S. (1991). Endogenous mutagens and the cause of aging and cancer. *Mutant Res. Fund. Mol. Mech. Mut.,* **250**, 3-16.

Anand, J.; Rai, N.; Kumar, N. and Gautam, P. (2012). Green tea: a magical herb with miraculous outcomes. *Int. Res. J. Pharm.,* **3**(5), 139-148.

Andjelkovic´, M.; Camp, J. V.; Meulenaer, B. D.; Depaemelaere, G.; Socaciu, C.; Verloo, M. and Verhe, R. (2006). Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.,* **98**, 23–31.

Anesini, C.; Ferraro, G. E. and Filip, R. (2008). Total polyphenol content and antioxidant capacity of commercially available tea (*Camellia sinensis*) in Argentina. *J. Agric. Food Chem.,* **56**, 9225–9229.

Anonnymous, (2005). https:///www.fda.gov.

Anonnymous, (2007). https:///www.cancer.gov.

Anonnymous, (2011). https:///www.alzheimers.org.uk.

Anonnymous, (2011). https:///www.detechter.com

Ayoola, G. A.; Ipav, S. S.; Sofidiya, M. O.; A, A.; Adepoju-Bello; Coker, H. A. and Odugbemi, T. O. (2008). Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda* Oliv (Guttiferae). *Int. J. Health Res.,* **1**(2): 87-93.

Balentine, D. A.; Wiseman, S. A. and Bouwens, L. C. (1997). The chemistry of tea flavonoids. *Critic. Rev. Food Sci. Nutr.,* **37**: 693-704.

Barrett, D. M.; Beaulieu, J. C. and Shewfelt, R. (2010). Color, flavor, texture and ntritional qality of fesh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. *Crit. Rev. Food Sci. Nutr.,* **50**(5), 369-389.

Barua (2008a). Romancing the *Camellia assamica* (Assam and the story of tea). *Assam Rev. Tea News*, pp. 18-27.

Barua, D. N. (2008b). *Science and practice in tea culture*. Tea Research Association, Kolkata, Kolkata.

Bharadwaz, A. and Bhattacharjee, C. (2012). Extraction of polyphenols from dried tea leaves. *Int. J. Sci. Eng. Res.*, **3**(5).

Botheju, W. S.; Amarathunge, K. S. P. and Abeysinghe, I. S. B. (2011). Simulation of trough withering of tea using one dimensional heat and mass transfer finite difference model. *Trop. Agric. Res.*, **22**(3): 282 - 295.

Bradfield, A. E.; Penny, M. and Wright, W. B. (1947). The catechins of green tea Part I. *J. Chem. Soc.*, **32**.

Cabrera, C.; Nez, R. G. and Pez, M. C. L. (2003). Determination of tea components with antioxidant activity. *J. Agric. Food Chem.*, **51**: 4427-4435.

Cakir, A.; Yildirim, M. A. A.; Duru, M. E.; Harmandar, M. and Kazaz, C. (2003). Isolation and characterisation of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium L.* by activity-guided fractionation. *J. Ethnopharmacol.*, **87**: 73-83.

Chacko, S. M.; Thambi, P. T.; Kuttan, R. and Nishigaki, I. (2010). Beneficial effects of green tea: A literature review. *Chin. Med.*, **5**(13).

Chen, A.; Chen, H.-Y. and Chen, C. (2014). Use of temperature and humidity sensors determine moisture content of oolong tea. *Sensors*, **14**, 15593-15609.

Cheng, H. Y. and Fan, H. J. (2007). Effects of withering time and blanching condition on catechins content and quality of green tea made from. **8**.

Choudhury, D. N. M. and Goswami, M. R. (1983). A rapid method for determination of total polyphenolic matters in tea (*Camellia sinensis* L.). *Two and a Bud*, **30**, 59-61.

Cooper, R.; Morré, D. J. and Morré, D. M. (2005). Medicinal benefits of green tea. *J. Altern. Complement. Med.*, **11**(3).

Dai, J. and Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, **15**(10), 7313-7352.

Das, S. C. (2011). Tea cultivars in North East India. *Tea Res. Assoc.*, 146-279.

Eguchi, S. and Eguchi, A. (2001). Methods of manufacture of Japanese tea and the newly developed automatic leaf roasting device. **2**, 260-263.

Erturk, Y.; Ercisli, S.; Sengul, M.; Eser, Z.; Haznedar, A. and Turan, M. (2010). Seasonal variation of total phenolic, antioxidant activity and minerals in fresh tea shoots (*Camellia sinensis* var. *sinensis*). *Pak. J. Pharm. Sci.*, **23**(1), 69-74.

Esmaeili, M. A. and Sonboli, A. (2010). Antioxidant, free radical scavenging activities of *Salvia brachyantha* and its protective effect against oxidative cardiac cell injury. *Food Chem. Toxicol.*, **48**, 846–853.

Fatima, M. and Rizvi, S. I. (2011). Health beneficial effects of black tea. *Biomed.*, **31**(1), 3-8.

Gadow, A. V.; Joubert, E. and Hansmam, C. F. (1997). Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chem.*, **60**(1), 73-77.

Gomez, K.A. and Gomez, A.A. (1984). Statistical procedures for agricultural research 2nd ed. John wiley and sons New York, p. 680.

Graf, B. A.; Milbury, P. E. and Blumberg, J. B. (2005). Flavonols, flavones, flavanones, and human health: epidemiological evidence. *J. Med. Food*, **8**(3), 281-290.

Gramza, A.; Lemañska, K. P.; Korczak, J.; Sowicz, E. W. and Rudzinsk, M. (2005). Tea extracts as free radical scavengers. *Pol. J. Environ. Stud.*, **14**(6), 861-867.

Grubesic, R. J.; Vukovic, J.; Kremer, D. and Knezevic, S. V. (2007). Flavonoid content assay: Prevalidation and application on *Plantago* species. *Act Chim. Slov.*, **54**, 397-406.

Gulati, A.; Rajkumar, S.; Karthigeyana, S.; Suda, R. K.; Vijayan, D.; Thomas, J.; Rajkumar, R.; Das, S. C.; Tamuly, P.; Hazarika, M. and Ahuja, P. S. (2009). Catechin and catechin fractions as biochemical markers to study the diversity of Indian tea [*Camellia sinensis* (L.) O. Kuntze] Germplasm. *Chem. Biodivers.*, **6**, 1042-1052.

Guo, Q.; Zhao, B.; Shen, S.; Hou, J.; Hu, J. and Xin, W. (1999). ESR study on the structure antioxidant activity relationship of tea catechins and their epimers. *Biochim. Biophys. Act 1427*, 13-23.

Harborne, J. B. and Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochem.*, **55**, 481-504.

Harbowy, M. E. and Balentine, D. A. (1997). Tea Chemistry. *Critic. Rev. Plant Sci.*, **16**(5), 415-480.

Hazarika, M. and Muraleedharan, N. (2011). Tea in India: An overview. *Two and a Bud*, **58**, 3-9.

Hilton, P. J. and Jones, P. (1973). Relationship between the flavanol composition of fresh tea shoots and the theaflavin content of manufactured tea. *J. Sci. Food Agric.,* **24**, 813-818.

Huafu, W.; Provan, G. J. and Helliwell, K. (2000). Tea flavonoids: their functions, utilisation and analysis. *Trends Food Sc. Technol.,* **11**, 152-160.

Inoue, Y.; Trevanich, S.; Tsujimoto, Y.; Miki, T.; Miyabe, S.; Sugiyama, K.-i.; Izawa, S. and Kimura, A. (1996). Evaluation of catechin and its derivatives as antioxidant : Recovery of growth arrest of *Escherichia coli* under oxidative conditions. *J. Agric. Food Sci.,* **71**, 297-300.

Kalaivani, T. and Mathew, L. (2010). Free radical scavenging activity from leaves of *Acacia nilotica* (L.) wild : an Indian medicinal tree. *Food Chem. Toxicol.,* **48**, 298–305.

Kanatt, S. R.; Chander, R. and Sharma, A. (2007). Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. *Food Chem.,* **100**, 451-458.

Kato, C.; Uritani, I.; Saijo, R. and Takeo, T. (1976). Cellular localization of particulate-bound polyphenol oxidase in tea leaves. *Plant and Cell Physiol.*, **17**, 1045-1052.

Khoddami, A.; Wilkes, M. A. and Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules,* **18**(2), 2328-2375.

Kondo, K.; Kurihara, M.; Miyata, N.; Suzuki, T. and Toyoda, M. (1999). Mechanistic studies of catechins as antioxidants against radical oxidation. *Arc. Biochem. Biophys.,* **362**(1), 79–86.

Kramer, P. J. and Kozlowski, T. T. (1979). Physiology of woody plants. *Nature*, 811.

Kuriyama, S.; Shimazu, T.; Ohmori, K.; Kikuchi, N.; Nakaya, N.; Nishino, Y.; Tsubono, Y. and Tsuji, I. (2006). Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *J. Am. Med. Assoc.*, **296**(10), 1255-1265.

Kursanov, A. L. and Brovchenko, M. I. (1950). Tannins of various organs of the tea plant. *Biokhim. Chain. Proizvod.,* **6**, 53-69.

Laddi, A.; Sharma, S.; Kumar, A. and Rup, N. (2011). Influence on color attributes of freshly brewed tea with time due to variations in temperature conditions. *Int. J. Computer Appl.,* **34**(7), 0975 – 8887.

Lee, L. S.; Kim, S. H.; Kim, Y. B. and Kim, Y. C. (2014). Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity *Molecules,* **19**, 9173-9186.

Li, X.; Xie, C.; He, Y.; Qiu, Z. and Zhang, Y. (2012). Characterizing the moisture content of tea with diffuse reflectance spectroscopy using wavelet transform and multivariate analysis. *Sensors,* **12**, 9847-9861.

Liang, Y.; Lu, J.; Zhang, L.; Wu, S. and Wu, Y. (2005). Estimation of tea quality by infusion colour difference analysis. *J. Sci. Food Agric.,* **85**, 286–292.

Liang, Y.; Wu, Y.; Lu, J. and Zhang, L. (2007). Application of chemical composition and infusion colour difference analysis to quality estimation of jasmine-scented tea. *Int. J. Food Sci. Technol.,* **42**, 459–468.

Liebert, M.; Licht, U.; Böhm, V. and Bitsch, R. (1999). Antioxidant properties and total phenolics content of green and black tea under different brewing conditions. *Z Lebensm. Unters. Forsch. A,* **208**, 217–220.

Lin, X.; Zhang, L.; Lei, H.; Zhang, H.; Cheng, Y.; Zhu, R. and Ruan, R. (2010). Effect of drying technologies on quality of green tea. *Int. Agric. Eng. J.,* **19**(3).

Liu, X. W.; Gao, X. Y.; He, Y. Q.; Gao, X.; Xiao, C.; Wu, G. X.; Zhou, H. G. and Yuan, W. X. (2010). Effect of several trace elements on the tea plant physiological and tea quality. *Guangdong Agric. Sci.,* **6**, 162-165.

Longo, G.; Karan, M.; Sharma, P. D.; Rakesh, D. D.; Vyas, S. and Vasisht, K. (2005). Quantitative analysis of green tea polyphenols in indian cultivars.

Lou, L. (2002). Applications of microwave technology in the tea processing. *Fujian Tea,* **1**, 23-25.

Mahanta, P. K. and Baruah, H. K. (1992). Theaflavin pigment formation and polyphenol oxidase activity as criteria of fermentation in orthodox and CTC teas. *J. Agric. Food Chem.,* **40**, 860-863.

Mahanta, P. K. and Baruah, H. K. (1998). Chemical basis of liquor characteristics and made tea appearance : A brief review. *Two and a Bud,* **35**, 66-70.

Malik, C. P. and Singh, M. B. (1980). Plant enzymology and histo-enzmology. In (pp. 286). New Delhi: Kalyani Publisher.

Mou, E. J. and Xu, Z. R. (2006). Preliminary study on several biochemical components in the different tea resources. *J. Anhui Agric. Sci.,* **34**(4), 685-687.

Muzzaffar, S.; Jan, R.; Wani, I. A.; Masoodi, F. A.; Munaff, M. B.; Wani, T. A. and Wani, G. R. (2016). Effect of preservation methods and storage period on the chemical composition and sensory properties of strawberry crush. *Cogent Food.Agric.,* **2**, 1178691.

Nagalaksmi, S. (2003). Tea: an appraisal of processing methods and products.

Naheed, Z.; Barech, A. R.; Sajid, M.; Khan, N. A. and Hussain, R. (2007). Effect of rolling, fermentation and drying on the quality of black tea. *Sarhad J. Agric.,* **23**(3).

Naik, G. H.; Priyadarshini, K. I. and Mohan, H. (2005). Evaluating the antioxidant activity of different plant extracts and herbal formulations. *Res. Chem. Intermed.,* **31**, 323-329.

Nakao, M.; Takio, S. and Ono, K. (1998). Alkyl peroxyl radical-scavenging activity of catechins. *Phytochem.,* **49**(8), 2379-2382.

Nanjo, F.; Goto, K.; Seto, R.; Suzuki, M.; Sakai, M. and Hara, Y. (1996). Scavenging effects of tea catechins and their derivatives on l,l-diphenyl-2-picrylhydrazyl radical. *Free Radic. Biol. Med.,* **21**(6), 895-902.

Obanda, M.; Owuor, P. O. and Taylor, S. J. (1997). Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. *J. Sci. Food Agric.,* **74**, 209-215.

Pandey, K. B. and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell Longev.,* **2**(5).

Pekal, A.; Biesaga, M. and Pyrzynska, K. (2013). Trace metals and flavonoids in different types of tea. *Food Sci. Biotechnol.,* **22**(4), 925-930.

Punyasiri, P. A. N.; Abeysinghe, I. S. B.; Kumar, V.; Treutter, D.; Duy, D.; Gosch, C.; Martens, S.; Forkmann, G. and Fischer, T. C. (2004). Flavonoid biosynthesis in the tea plant *Camellia sinensis*: properties of enzymes of the prominent epicatechin and catechin pathways. *Arc. Biochem. Biophys.,* **431**, 22-30.

Punyasiri, P. A. N.; Abeysinghe, I. S. B.; Kumar, V.; Treutter, D.; Duy, D.; Gosch, C.; Martens, S.; Forkmann, G. and Fischer, T. C. (2004). Flavonoid biosynthesis in the tea plant *Camellia sinensis*: properties of enzymes of the prominent epicatechin and catechin pathways. *Arc. Biochem. Biophys.,* **431**, 22-30.

Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L.; Bolwell, G. P. and Evans, C. R. (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidant. *Archiv. Biochem. Biophys.,* **322**(2), 339-346.

Samarasingham, S. (1990). A method for processing of green tea. *S. L. J. Tea Sci.,* **59**(1), 16-23.

Sanderson, G. W. and Grahamm, H. N. (1973). Formation of black tea aroma. *J. Agric. Food Chem.,* **21**(4), 576-585.

Sato, D.; Ikeda, N. and Kinoshita, T. (2007). Home processing black and green tea (*Camellia sinensis*). *Food Saf. Technol.*

Seifu, T. (2012). *Ethnobotanical and Ethnopharmaceutical Studies on Medicinal Plants of Chifra District, Afar Region. North Eastern Ethiopia.* M.Sc. thesis submitted to Addis Ababa University, Ethipia.

Shahidi, F. (2004). Functional foods: their role in health promotion and disease prevention. *J. Food Sci.,* **69**, 146-149.

Sharangi, A. B. (2009). Medicinal and therapeutic potentialities of tea (*Camellia sinensis L*.) – a review. *Food Res. Int.,* **42**, 529–535.

Sharma, M. and Thomas, E. V. (2013). Electronic vision study of tea grains color during Infusion. *Int. J. Eng. Sci. Invent.,* **2**(4), 52-58.

Shonisani, N. (2010). *Effects of Brewing Temperature and Duration on Quality of Black Tea (Camellia sinensis) and Equal (50:50) Combination of Bush Tea (Athrixia phylicoides DC.) and Black Tea.* Master of Science in Agriculture (Horticulture) thesis submitted to University Of Limpopo, Limpopo, Africa.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experiment. Physiol.,* **82**, 291-295.

Singh, V.; Verma, D. K. and Singh, G. (2014). Processing technology and health benefits of green tea. *Popular Kheti,* **2**(1), 23-30.

Sinija, V. R. and Mishra, H. N. (2008). Green tea: Health benefits. *Green tea: Health benefits,* **17**(4), 232–242.

Sreejayan, N. and Rao, M. N. (1996). Free radical scavenging activity of curcuminoids. *Arzneim. Forsch.,* **46**(2), 169-171.

Steinmetz, K. A. and Potter, J. D. (1991). Vegetables, fuit, and cancer II mechanisms. *Cancer Cause Cont.,* **2**(4), 27-42.

Su, X.; Duan, J.; Jiang, Y. and Chen, X. D. (2007). Polyphenolic profile and antioxidant activities of oolong tea infusion under various steeping conditions. *Int. J. Mol. Sci.,* **8**, 1196-1205.

Tachebele, B.; Abebe, M.; Birhan, W. and Addis, Z. (2014). Health benefits of green tea with emphasis on diabetes mellitus. *Am. J. Food Nutr.,* **2**(5), 85-91.

Takeo, T. (1996). Tea leaf polyphenol oxidase. *Agric. Biol. Chem.,* **30**(6).

Tapas, A. R.; Sakarkar, D. M. and Kakde, R. B. (2008). Flavonoids as nutraceuticals: a review. *Tropic. J. Pharmac. Res.,* **7**(3), 1089-1099.

Tomlins, K. I. and Mashingaidze, A. (1997). Influence of withering including leaf handling on the manufacturing and quality of black teas - a review. *Food Chem.,* **60**(4), 573-580.

Topuz, A.; Dincer, C.; Torun, M.; Tontul, I.; Nadeem, H. S.; Haznedar, A. and Ozdemir, F. (2014). Physicochemical properties of Turkish green tea powder: effects of shooting period, shading, and clone. *Turk. J. Agric. For.,* **38**, 233-241.

Tram, N. N.; Hien, P. P. and Oanh, H. N. (2015). Optimizing the extraction conditions of phenolic compounds from fresh tea shoot. *J. Food Nutr. Sci.,* **3**(1-2), 106-110.

Vani, T.; Rajani, M.; Sarkar, S. and Shishoo, J. C. (1997). Antioxidant properties of the ayurvedic formulation, triphala and its constituents. *Int. J. Pharmacogn.,* **35**, 313-317.

Vinson, J. A. and Dabbagh, Y. A. (1998). Tea Phenols: Antioxidant effectiveness of tesa, tea components, tea fractions and their binding with lipoproteins. *Nutr. Res.,* **18**(6), 1067-1075.

Wang, H. and Helliwell, K. (2000). Epimerisation of Catechins in Green tea infusions. *Food Chem.,* **70**, 337-344.

Wang, L. F.; Park, S. C.; Chung, J. O.; Baik, J. H. and Park, S. K. (2006). The compounds contributing to the greenness of green tea. *J. Food Sci.*, **68**(8).

Wickremasinghe, R. L. (1978). Monographs on tea production in Sri Lanka. *Facet. Tea Res. Pract.,* **7**.

Xie, Y.; Song, L. and Yang, X. (2006). Application of heat pump drying technology and its developmental trend. *J. Agric. Mechan. Res.,* **4**, 12-16.

Xua, B. and Chang, S. K. C. (2008). Effect of soaking, boiling, and steaming on total phenolic content. *Food Chem.,* **110**, 1-13.

Yen, G. C. and Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimut agenicity. *J. Agric. Food Chem.,* **43**, 27-32.

Yoshioka, H.; Sugiura, K.; Kawahara, R.; Fujita, T.; Makino, M.; Kamiya, M. and Tsuyumu, S. (1991). Formation of radicals and chemiluminescence during the autoxidation of tea catechins. *Agric. Biological Chem.,* **55**(11), 2717-2723.

Zhang, M. H.; Luypaert, J.; Pierna, J. A. F.; Xu, Q. S. and Massart, D. L. (2004). Determination of total antioxidant capacity in green tea by near-infrared spectroscopy and multivariate calibration. *Talanta,* **62**, 25-35.

APPENDIX I

Analysis of variance for the moisture content (%) in green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	7.96	5	1.59	75.94
Month	45.98	3	15.33	731.25
Interaction	7.79	15	0.52	24.78
Error	1.01	48	0.02	

Analysis of variance for the variation in CIE L* value in infused green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	129.43	5	25.89	141.87
Month	68.09	3	22.70	124.40
Interaction	138.71	15	9.25	50.68
Error	8.76	48	0.18	

Analysis of variance for the variation in CIE a* value in infused green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	33.42	5	6.68	53.89
Month	29.18	3	9.73	78.42
Interaction	32.06	15	2.14	17.23
Error	5.95	48	0.12	

APPENDIX II

Analysis of variance for the variation in CIE b* value in infused green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	15.41	5	3.08	13.14
Month	92.06	3	30.69	130.85
Interaction	179.20	15	11.95	50.94
Error	11.26	48	0.23	

Analysis of variance for the variation in CIE H* value in infused green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	74.77	5	14.95	2.41
Month	15.55	3	5.18	0.84
Interaction	286.29	15	19.09	3.08
Error	297.71	48	6.20	

Analysis of variance for the variation in CIE C* value in infused green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	15.55	5	3.101	13.55
Month	92.06	3	30.69	133.73
Interaction	178.66	15	11.91	51.91
Error	11.01	48	0.23	

APPENDIX III

Analysis of variance for the total polyphenol content (mg CE/g) in green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	286.27	5	57.25	866.44
Month	214.66	3	71.55	1082.83
Interaction	118.39	15	7.89	119.45
Error	3.17	48	0.066	

Analysis of variance for the total flavonoids content (mg QE/g) in green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	155.15	5	31.03	356.37
Month	257.98	3	85.99	987.64
Interaction	41.65	15	2.78	31.89
Error	4.18	48	0.09	

Analysis of variance for the DPPH radical scavenging activity (% inhibition) of in green tea samples

Source of Variation	*SS*	*df*	*MS*	*F*
Treatment	217.02	5	43.40	29.48
Month	3195.98	3	1065.33	723.667
Interaction	387.10	15	25.81	17.53
Error	70.66	48	1.47	

T_1

T_2

T_3

T_4

T_5

T_6

Fig. 3.2.Tea samples collected from different growers based on six different treatments

Fig. 3.1. A -Tea samples, B - Infused tea leaves, C - Infused tea