

**ANALYSES OF TANNINS IN
REGIONAL SPECIFIC CEYLON TEAS**

By

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Dedicated to

My ever loving late father,

My most Precious Mother

Who nurtured and groomed me

To be a Valued Citizen.

Also to

My loving wife

&

Little daughter

DECLARATION

I do hereby declare that the work described in this thesis was carried out by me under the supervision of Dr. K.K.D.S. Ranaweera and Mr. Chaminda Munasinghe and a report on this has not been submitted in whole or in part to any University or any other institution for another Degree/diploma.


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TABLE OF CONTENT

	Page No.
Acknowledgement	05
Abstract	06
Chapter 01	
1- Introduction	09
1.1 History of tea	09
1.2 History of tea plant	09
1.3. Agriculture of tea	10
1.4. Manufacturing process of black tea.	11
1.5 Objectives of the study	13
Chapter 02	
2. Literature review	14
2.1 Chemistry and Biochemistry of tea	14
2.2 Polephenols in tea	14
2.3 Composition of a typical tea beverage	16
2.4 Physiological effects of tea	16
2.5 Polephenols as antioxidants	16
2.6 Sri Lankan tea industry	17
2.7 Different regions and sub-districts where teas are grown In Sri Lanka	18
Chapter 03	
3.1 Experimental / Methodology	20
3.2 Methodology used	22
3.3 Measurement of sample	23
3.4 Preparation of standard solution series	24
3.5 Calculation of tea polephenols	25
3.6 Calculation of the dilution of tea extracts	25
3.7 Relating tea extract absorption values with the standard series	26
4.0 Results and Discussion	26
4.1 Analyses and Interpretation of results	27
4.2 Statistical analyses of results	34
4.3 Significance of relationship between variables and predictors	42
5. Conclusion and Recommendations	43
References	44
Appendices	45

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ABSTRACT

Tea in general is considered as a health beverage, although varying claims are still being made concerning the health benefits of tea.

Sri Lanka is the third largest exporter of tea, with the widest variety offered to international customers. Japan is a larger importer of Ceylon teas with a percentage share of 5.94, with a consumption of 130,000 metric tonnes to 2,188,000 metric tonnes of black tea in total in 1993. Majority of its customers focus on the health benefits provided by same, claims that the levels of tannin content in Ceylon teas is having a declining trend with time since of late. They being the customer, are interested in understanding the probable cause(s) for same.

They are also concerned on how the Tannin content varies in regional specific Ceylon black tea and also in understanding the factors contributing to the said variation such as the Agro climatic conditions, soil conditions etc...

Therefore, they proposed an analysis to be carried out in order to verify same. Certain concerns could be noted in this study, such as the differences in the methodologies adopted in analysing the tannin contents, which could most probably be the cause for varying results and also the analysis method below adopted by the Tea Research Institute has analysed for total polyphenols and catechines and the method adopted in this study has focussed only on tannins. However, owing to Unilever safety guidelines, the methanol extraction of tea could not be carried out. Hence, had to carryout only the hot water extraction method.

Method used by the Tea Research Institute :- ISO method for total polyphenols - ISO 14502-1 (2005) is as follows:

Procedure :-

1. Tea sample has to be grinded.
2. Extraction into Methanol/water (70%)
3. Folin-Ciocalteu reagent added.
4. Spectroscopic measurement at 765 nm.
5. Standard graph is prepared using gallic acid as standard.

Analysis results for Total polyphenols and individual catechins according to the said method.

Region	% of Total Polyphenols	% of Total catechins
Nuwara Eliya/ Udapussellawa	18.47	6.41
Western Region	15.06	2.12
Uva	13.82	1.9
Mid Country	18.34	3.68
Low Country	19.69	4.59

Some interesting observations risen from the results could be seen in experiment carried out are as follows:-

- There is no direct co-relation of temperature to the variation in tannin content in any of the agro climatic subdivisions chosen for the study.
- There has been a positive co-relation with the rain fall in the 04 month period in two regions.

viz.:- Uva region and Nuwaraeliya / Udapussellawa region

- There has been a negative co-relation with the rain fall in the 04 month period in the other three regions,
viz.:- Mid grown, Western region and Low grown region.

These are further discussed under '**RESULTS AND DISCUSSION**' below

CHAPTER 01

1. INTRODUCTION

Tea is the most popular beverage in the world, second only to water. Tea (*Camellia sinensis*) (L). O. Kuntze is considered to be the most popular non alcoholic beverage next to water. What are plucked for processing are the tender leaves and the bud of the tea plant, which is referred to as the flush.

What are Tea Tannins?

Tannins are a group of very astringent compounds which bind themselves to protein. Tannins also combine with enzymes because these are proteins. The tanning of enzymes leads to inactivation or reduction of enzyme activity .

1.1. HISTORY OF TEA

Tea has been discovered by the Chinese emperor Shen Nung where he claimed that it was able to detoxify 72 kinds of poison (Teranishi et al., 1995)

Tea became very popular in both east and west and the present tradition of the afternoon tea established particularly as a British habit. Cultivation of tea was introduced to several other countries, with the growing popularity of tea.

1.2. HISTORY OF TEA PLANT

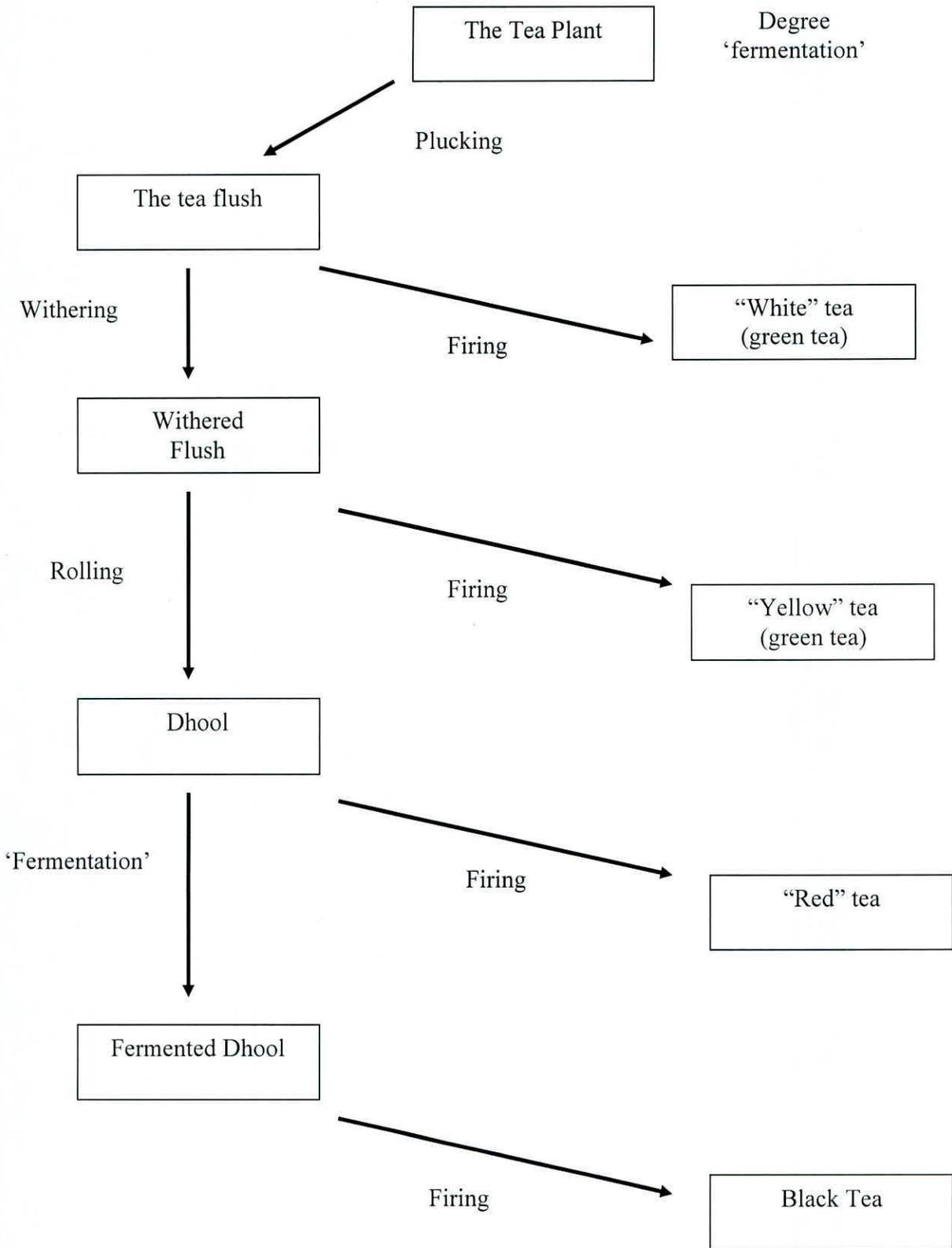
Tea plant was classified as *Thea sinensis* in 1753, in the binomial system originated by Linnaeus. Many synonyms have been given, but now it is generally accepted that the tea plant is classified in the family Theaceae and in *Camellia* species.

Commercially cultivated tea consist of natural hybrids of 03 main types. Viz- *Camellia sinensis var sinensis* for small leaves China plants.

1.3. AGRICULTURE OF TEA

Tea grows best in tropical and sub-tropical areas where adequate rain fall of approximately 2,000 mm, good drainage and slightly acidic soils prevail. In hot tropical areas quality is improved by planting at higher altitudes as practiced in Sri Lanka, where tea cultivation is done at elevations upto 2,000 meters.

1.4. MANUFACTURING PROCESS OF BLACK TEA



When oolong and black teas are to be produced, the fresh leaves are allowed to be produced, the fresh leaves are allowed to wither until the moisture content of the leaves is reduced to 55% to 72% of the leaf weight.

1.4.1 Withering

Harvested teas are handled in a manner to prevent bruising and to promote dissipation of heat generated during continued respiration. The moisture content of tea is reduced from 75% to 55% approximately, by a flow of cool air.

1.1.2 Rolling

Rolling helps to establish proper conditions for enzymatic oxidation of the flavanols, by atmospheric oxygen. Rolling is accomplished by disruption of the cell structure which facilitate enzyme – substrate contact. The leaf mass is also maintained in a physical state

1.4.3 Fermentation (Oxidation)

During this process the green leaf is converted to black tea. Although this is referred to as fermentation, it became recognised around 1901 as an oxidation process initiated by the tea enzymes. This process actually starts at the onset of maceration and it allowed continuing under ambient conditions. Temperature of the leaf is maintained around 25 - 30 C.

1.4.4 Firing

At this stage of processing, fermenting enzymes are deactivated by passing hot forced air over the tea leaves. Many organochemical processes are accelerated during this period, as are the enzymatic reactions before thermal inactivation.

Firing also reduces the moisture level to 2% - 3%. This is critical as incomplete inactivation can cause accelerated deterioration during storage.

1.4.5 Sorting

The dried teas are sorted into different grades, by passing it over a series of vibrating screens of different mesh sizes. Electro statically charged rollers preferentially attract and remove fibre and stalks in the processed black tea.

1.5 OBJECTIVES OF THE STUDY:

- To study the variation of Tannin content in regional specific Ceylon black tea.
- To understand the factors contributing to the said variation such as the Agro climatic conditions, soil conditions etc...
- To recommend tea drinking consumer segments, the means of extracting the maximum tannin content to the brew, in order to obtain maximum possible health benefits.

CHAPTER 02

2. LITERATURE REVIEW

2.1 CHEMISTRY & BIOCHEMISTR OF TEA

Tea is the subject of many biochemical investigations as it produces a number of unique natural products. in contrast to the isolation and quantification of the Tea flush, consisting of the terminal bud and two adjacent leaves of the tea plant

Tea is rich in high amounts of anti oxidant flavonoids, which help clean harmful compounds from the blood.

2.2 POLYPHENOLS IN TEA:

The term polyphenol is an inclusive descriptor referring to the millions of natural and synthetic aromatic molecules that are substituted with multiple hydroxyl groups. Polyphenols of Black tea are poorly understood but seemingly well defined group of compounds. These are principally responsible for the colour and astringency and partially responsible for flavour. The most abundant class of chemical compounds in fresh tea flush is the polephenols. (made up of many phenol molecules) The phenol molecule has a single hydroxyl group attached to an aromatic ring; the polyphenols have a number of these – OH groprs. Of the polyphenolic categories, the flavonoids are the most abundant in tea flush. Flavonoids are 2- phenyl benzopyran based compounds, classified into six groups:

Flavones.

Flavanones.

Isoflavones.

Flavanols (including catechines or flavan – 3- ols)

Flavonols

Anthocyanidins

Flavanols and Flavonols are the main components in fresh leaf. Catechines are the major flavanols in tea. EGCG, EGC, ECG, EC,GC and C and they undergo oxidative dimerisation or polymerisation during black tea manufacture.

Flavonols (such as quercetin, kaempferol, myricetin and their glycosides) and anthocyanidins are also found in the flush in appreciable amounts, but they do not undergo much change during black tea manufacture. The tea leaves contain two enzymes, polyphenol oxidase and peroxidase, which are involved in the oxidation of polyphenols.

These may be subdivided by several chemical backbone structures comprising of:-

Simple tea polyphenols

Gallic Acid and its quinic acid ester (or depside, as quinic acid esters are commonly referred), theogallin, have been identified in tea.

Other polyphenols Flavones and their glycosides such as Apigenin have been detected in tea but represent a very small fraction of the polyphenols present.

2.2.1 Tannins:-

Although it is commonly stated that there are no tannins (meaning hydrolyzable tannins such as pentagalloylglucose) in tea, this statement is not strictly true. In addition to the gallic acid esters of the catechins and their oxidation products (which can be hydrolyzed to produce gallic acid readily and precipitate proteins), there is also a small quantity of hydrolysable tannin (Nonaka et al., 1984; Yoshida et al. 1990; Hatano et al., 1994.) The unique hydrolysable tannins in tea are typically "hybrid" tannins such as camelliatannin A, which is a galloylglucose derivative with pendant catechines. The tannic acid derivatives common to gall-nuts and tree bark are not present in significant quantities in tea infusions.

2.2.2 Catechins:

Of the total catechines consumed by humans, a large percentage passes out unchanged in the faeces (Hara, 1997) and of that absorbed into the body, approximately 60% are excreted in the urine and the rest in the bile (Brown