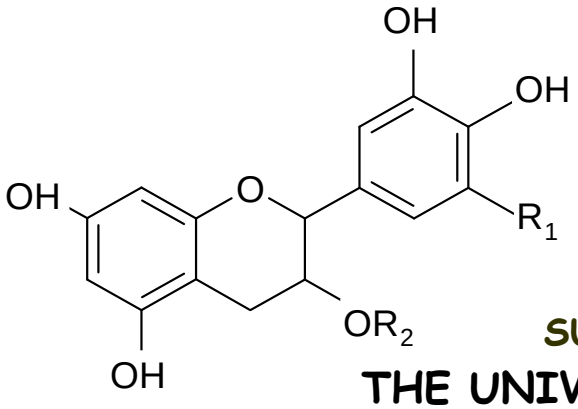
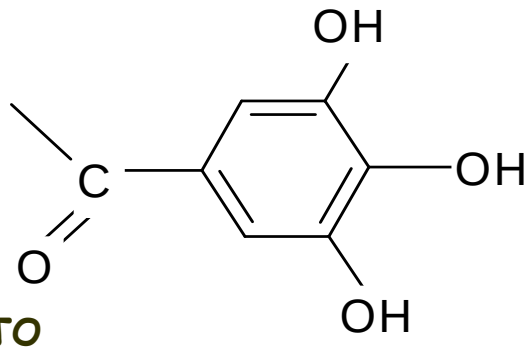
Bio-chemical and Technological Investigations on Tea





THESIS

SUBMITTED TO

THE UNIVERSITY OF MYSORE

FOR THE AWARD OF THE DEGREE

DOCTOR OF PHILOSOPHY (FOOD SCIENCE)

By

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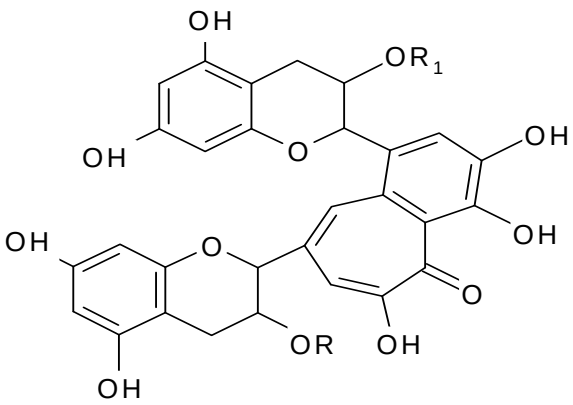

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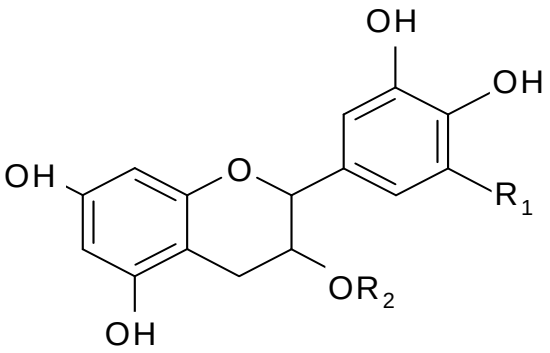
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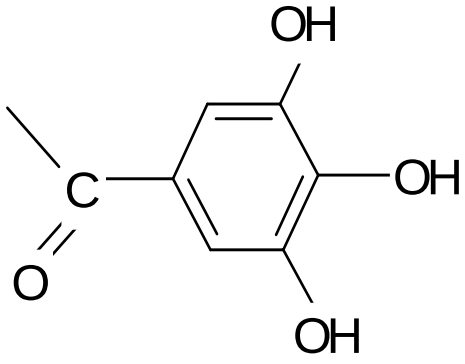
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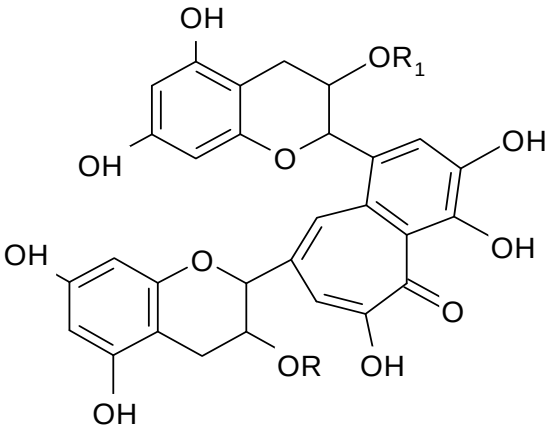
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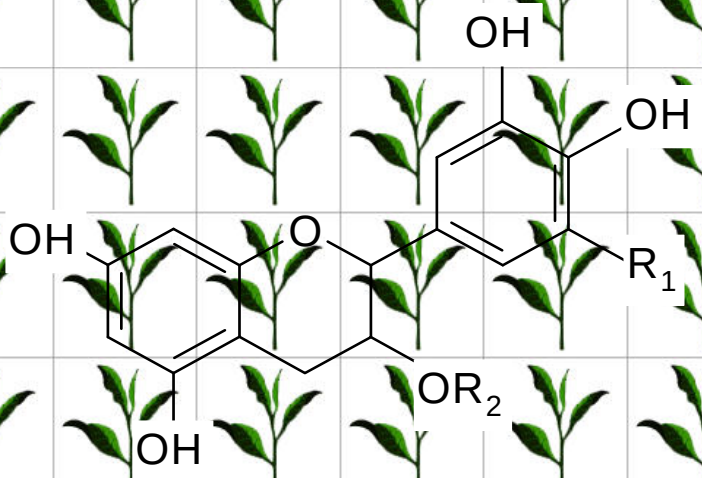
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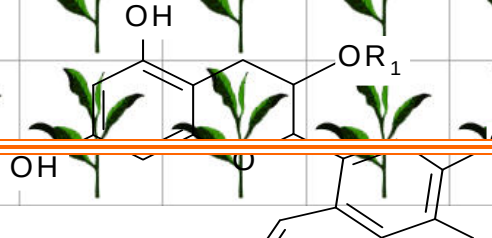
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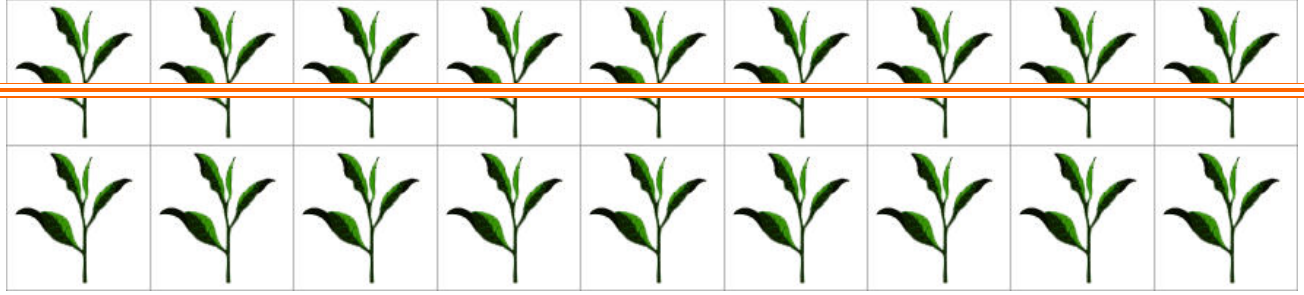

Dr. L. Jagan Mohan Rao
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CERTIFICATE

I certify that this thesis entitled, '***Bio-chemical and Technological investigations on tea***' submitted **by Mr. Borse Babasaheb Bhaskarrao** for the award of the Degree of Doctor of Philosophy in Food Science of the University of Mysore, is an outcome of the work carried out by him under my guidance in the Department of Plantation Products, Spices & Flavour Technology, Central Food Technological Research Institute, Mysore.

Dated: (**Dr. L. Jagan Mohan Rao**)

Dr. L. Jagan Mohan Rao
Sr. Scientist,
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Mysore – 570 020

ATTENDANCE CERTIFICATE

I certify that this thesis entitled, '***Bio-chemical and Technological investigations on tea***' submitted **by Mr. Borse Babasaheb Bhaskarrao** for the award of the Degree of Doctor of Philosophy in Food Science of the University of Mysore, is an outcome of the work carried out by him under my guidance **with full attendance,** in the Department of Plantation Products, Spices & Flavour Technology, Central Food Technological Research Institute, Mysore.

Dated: (**Dr. L. Jagan Mohan Rao**)

FT/PPSFT/BBB/2008 **28.11.2008**

Mr. B.B.Borse, P.P.S.F.T. Dept.
CFTRI, Mysore – 570 020,

The Registrar (Evaluation),
University of Mysore,
Crawford Hall, Mysore.

Through proper channel

Ref: Ex.9.2/Ph.D./ BBB /492/ 01- 02, Dtd. 09.06.2004

Sub: Submission of Ph.D. Thesis

Sir,

I, Mr. B.B.Borse, P.P.S.F.T. Dept. CFTRI, Mysore had registered for Ph.D. in the area of Food Science, under the guidance of Dr. L. Jagan Mohan Rao, Scientist, P.P.S.F.T. Dept. CFTRI, Mysore w.e.f. 10.12.2001. I have completed my research work and the final **thesis** entitled '**BIOCHEMICAL AND TECHNOLOGICAL INVESTIGATIONS ON TEA'** along with the **synopsis** (5 +15 copies enclosed) is **submitted herewith** for further processing at your end. Please find enclosed following challans dt. 27.11.2008 towards the fees paid,

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2. F&R Exam. A/c- challan no.28205 for Rs.11,100/- paid towards the tution fee.
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4. F&R Exam. A/c- challan no. 28203 for Rs.5,500/- paid towards the Exam. fee/ Ph.D. thesis submission fee.

Thanking you,

Yours faithfully,

(B.B.Borse)

Signature of the Guide

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Signature of the Chairperson/ HEAD HRD

Encl. overleaf

1. Completed application
2. Guides certificate
3. Copy of Ph.D. registration
4. Copy of Masters degree certificate
5. Xerox copies of all fees paid challan
6. CD of the Thesis – 2Nos.

06.12.2006

From
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CFTRI, Mysore,
Mysore – 570 013

To
The Registrar (Evaluation),
University of Mysore,
Mysore.

Through proper channel

Ref: Ex.9.2/Ph.D./492/BBB/2001- 02, Dtd. 11.06.2003

Sub: Request for extension

Sir,

I have registered for Ph.D. in the area of Food Science, under the guidance of Dr. L. Jagan Mohan Rao, Scientist, P.P.S.F.T. Dept. CFTRI, Mysore w.e.f. 10.12.2001 and completing five years on 10.12.2006 (copy enclosed). In this regard, I hereby request an extension for enabling me to submit the thesis. Please find enclosed a challan (F&R) for Rs.11000/- paid towards the extension fee (challan no. , dated .12.2006).

Thanking you,

Yours faithfully,

(B.B.Borse)

Signature of the Guide

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Signature of the Chairperson/ HEAD HRD

DECLARATION

I, **Mr. Borse Babasaheb Bhaskarrao**, hereby declare that this thesis entitled, '***Bio-chemical and Technological investigations on tea***' submitted to the University of Mysore for the award of the degree of **Doctor of Philosophy in Food Science** of the University of Mysore, is an embodiment of the results of the research work carried out by me under the guidance of **Dr. L. Jagan Mohan Rao**, Sr. Scientist, Department of Plantation Products, Spices & Flavour Technology, Central Food Technological Research Institute, Mysore during the period 2001-2008. I further declare that the results of the work or part thereof have not been submitted for any fellowship or degree/diploma.

Dated: (**Borse Babasaheb Bhaskarrao**)

Dedicated to

the supreme, the supreme creator, director, principal actor and the supreme father of all souls,

the Brahm (incorporeal sixth element) nivasi-Trimurti Shiva (the rachiyata of the trimurti - Brahma, Vishnu, Shankara and the paradise - the new Deity world order / swarga/ heaven),

the almighty authority, the GOD (the true spiritual - father, teacher, sadguru of all the human souls),

the intellect of the intellectuals, the ocean of divine virtues (Knowledge, peace, bliss, happiness, love, power and purity),

the remover of sorrow and bestower of happiness , the incorporeal-sentient point of self luminous divine light,

the supreme guide, the liberator, for his unlimited mentoring, guidance and the empowerment all through.

His Dictum – ‘‘Always be soul conscious and remember only me”- Ancient Raja Yoga.

ACKNOWLEDGEMENT

My heartfelt thanks to my guide **Dr. L. Jagan Mohan Rao**, Sr. Scientist, PPSFT dept. for his constant encouragement, valuable guidance and unstinted support throughout this study.

My sincere thanks to Padmashree **Dr. V. Prakash**, Director, CFTRI for his kind support, encouragement and keen interest in this study.

My sincere thanks to the former head(s) and Present head **Dr. P. Srinivas**, scientists, technical staff and colleagues from PPSFT, CIFS, FED, S.S., FSAQCL, B&N, HRD, TTBD, GST and all other departments for their support.

My heartfelt thanks to **my seniors and colleagues (former and present) from** team CFTRI/CSIR for kind support and help in this endeavour.

My sincere thanks to the one and all who have helped, encouraged, nurtured me directly/indirectly to realize the goal.

My sincere thanks to the CSIR, New Delhi and M/s HLL (now HUL), Bangalore for funding the research project and also to the Director, CFTRI, nodal scientists, P. Is., members, and regular/temporary staff of the projects for kind support.

Last but not the least my wholehearted thanks to the all who have helped, supervised, assisted (directly/indirectly) in data acquisition, analysis, typesetting, design-layout, proof reading, printing, binding and submission of this thesis.

My heartfelt thanks to my better half, Mrs. Surekha & son, Aniket for their unstinted support, time, sacrifice and strength throughout this study and career.

My special thanks to the *laukik, alaukik and parlaukik* parents, sisters, brothers and families for their unlimited support to achieve the goal.

My sincere thanks to the Supreme, the almighty authority, the intellect of all the intellectuals, the ocean of knowledge, divine attributes, power and purity - the **GOD** (the true - *father, teacher, satguru* of all the souls) for his unlimited mentoring and empowerment all through to achieve the goals successfully and for guiding me to the higher heights of learning.

Finally, I place on record my thanks to one and all by their name and capacity, for having helped me directly or indirectly in researching, realizing this wonderful dream and the S&T contribution. In return I sincerely wish the best of luck, health, wealth and happiness to the one and all and hope that this work would be of help to the society at large.

(Borse, B. B.)

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ABBREVIATIONS

AAPH	2,2-azobis (2-amidino propane) hydrochloride
ABTS	2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid radical cation)
AOAC	Association of official Analytical chemists
ASTM	American Society for Testing and Materials
BD	Bulk density
BHA	Butilated hydroxy Anisole
BOP	Broken orange peko
C	Catechin
CFD	Cross flow dryer/dried
CG	Catechingallate
CIS	Commonwealth of Independent states
CTC	Cursh-Tear-Curl
DB	Dry basis
DM	Dislike Moderately
DMPO	5,5-dimethyl-1-pyroline-1-oxide
DNA	Deoxyribonucleic Acid
DPPH	1,1-diphenyl-2-picrylhydrazyl radical
DS	Dislike Slightly
DVM	Dislike Very Much
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
ESR	Electron Spin Resonance
FI	Flavour Index
GA	Gallic acid
GC	Gallocatechin
GCG	Gallocatechingallate
GC-MS	Gas Chromatograph - Mass Spectrometer
GMS	Glycerol mono stearate
HDG	Hydroxydeoxyguanosine
HG	High grown
HPLC	High Performance Liquid Chromatography
HPS	Higly Polymerized Substances
iNOS	Inducible nitric oxide synthase
IRD	Infra red dryer/dried
ISO	International standards organisation

KI	Kovat's Index
LDL	Low density lipoprotein
LM	Like Moderately
LPS	Lipopoly-saccharide
LS	Like Slightly
LVM	Like Very Much
m Pa	mlli Pascal
MDA	Malondialdyhyde
MMC	Mitomycin C
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
NIST	National Institute of standards and Technology
NLND	Neither Like Nor Dislike
NO	Nitric oxide
NVFC	Non volatile flavour compounds
OD	Optical density
OD	Outer diameter
ODC	Ornithine decarboxylase
ORAC	Oxygen Radical Absorbing Capacity
Q	Quercetin
QP	Quadrupole
ROS	Reactive Oxygen Species
RPM	Rotation per minute
RSA	Radical scavenging activity
RT	Retention Time
SCE	Sister chromatid exchange
SD	Standard deviation
SDE	Simultaneous distillation and solvent extraction
TBARS	Thiobarbutyric acid reactive substances
TEAC	Trolox equivalent antioxidant capacity
TF	Theaflavin
TFDG	Theaflavin digallate
TFMG	Theaflavin monogalate
TFs	Theaflavins
TI	Terpene Index
TIC	Total ion chromatogram
TR	Thearubigin
TRs	Thearubigins
TSS	Total soluble solids
VFC	Volatile Flavour Compounds

CONTENTS

SYNOPSIS OF THE THESIS

BIO-CHEMICAL AND TECHNOLOGICAL INVESTIGATIONS ON TEA

Submitted to the

UNIVERSITY OF MYSORE

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Doctor of Philosophy

In

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By

Borse B. B., M.Tech.(Food Science),

Plantation Products Spices and Flavour Technology Department

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE,

Mysore – 570 020, India.

November 2008

SYNOPSIS

BIOCHEMICAL AND TECHNOLOGICAL INVESTIGATIONS ON TEA

The synopsis of the completed Doctoral research work entitled '**BIOCHEMICAL AND TECHNOLOGICAL INVESTIGATIONS ON TEA'** is presented in the following paragraphs:

Ch. 1. INTRODUCTION AND REVIEW OF LITERATURE

In this Chapter a brief introduction is presented followed by the status of Indian tea industry, processing, tea quality, determination, isolation and identification of quality indicators and health benefits. The review of literature covers aspects relating to the chemical composition, quality co-relation and bioactivities in health benefits of teas as well as objectives and scope of the research work. Besides, the general pathway of biogenesis leading to the volatile aromatic compounds present in the tea have also been discussed. The brief account is given below.

Sheng Nung the chinese emperor (2737 B.C.) was the first to recognize the stimulant effect of tea. Tea is one of the important agro-industrial plantation crops of India. Tea is the beverage with which most of the Indians start their day. The recent research findings indicative of several health benefits have further popularized tea as a beverage. During the year 2007, India produced 945 million kg of tea from 38,705 gardens spread over an area of 4, 35, 057 ha. Out of this, domestic consumption accounts for 76 per cent and exports accounts for 24 per cent.

Tea plant belongs to the *Camellia* species of *Theaceae* family. The two basic varieties are recognised namely chinese variety *Sinensis* and Assamese variety - *Assamica*. The commercially grown tea plant is highly heterogenous. Tea flush contains polyphenols, amino acids, organic acids, polysaccharides, lipids, carotenoids, caffeine, chlorophylls, minerals and volatiles. The polyphenols which includes catechins constitute 25-30% of the fresh flush on dry

weight basis. These are converted to theaflavins, thearubigins, theaflavic acids and bisflavanols during the manufacture of black teas and these are responsible for colour, briskness, brightness and astringency. Theaflavins are determined qualitatively and quantitatively whereas quantitative determination of thearubigins has been possible tentatively but their structures are yet to be explored exhaustively. Caffeine is the major alkaloid present in tea and it is responsible for stimulating action. Highly efficient HPLC method to determine soluble caffeine is reported from this laboratory. Carbohydrates play an important role in the formation of tea aroma. Lipid concentration increases with the maturity of the leaves and is responsible for the formation of C_6 volatiles during the manufacture of black tea.

Three types of organic acids are present in tea viz., dicarboxylic acids, fatty acids and monocyclic acids. Monocyclic acids (eg. Quinic and Shikimic) are the precurors of polyphenols. Chlorophyll a and b are reported to be present in the tea and are converted to phaeophytins which are responsible for black colour of commercial tea. ß-carotene is the major compound among the carotenoids and degrades to character impact volaitle compounds such as theaspirone, ß-ionone and related compounds. Although K (Potassium) is the major mineral found the Cu (Copper) and Al (Aluminium), are important for the colour and taste of brewed teas.

Theanine is the most abundant amino acid and accounts for 50% of the total amino acids and 1% of the dry weight of tea. Theanine is a constituent of the "thearabigin" fraction while glutamic acid and ethylamine are its precursors. Amino acids and glucose interact with tea polyphenols during thermal processing and yield coloured moieties and Amadori products, which improve the flavour of tea.

Catechins, theaflavins and thearubigins contribute to the bitterness, astringency, brightness and total colour of black tea infusion. Further thearubigins are responsible for body and richness of the tea brew. Theaflavin digallate is having lowest threshold value for the astringency. Caffeine contributes towards the bitter taste in tea. Characteristic umami or brothy taste of

black teas is due to the presence of amino acids. The ionone related aroma compounds such as theaspiranes are formed from carotenoids and they are found to have different odour properties. The aroma quality of tea with respect to theaspiranes is yet to be exploited.

Volatile flavour compounds (VFC) play a major role in detemining the unique flavor of tea. Although >600 compounds are reported but the unique composition for character impact aroma of black tea is not yet established. The aroma quality of black teas with respect to the VFCs is measured by different ratios/indices viz., Terpene index, Wickremasinghe-Yamanishi ratio, Mahanta ratio, Yamanishi-Botheju ratio. Wickremasinghe-Yamanishi ratio is the ratio of sum of the peak areas of compounds eluting before linalool to the sum of the peak areas linalool plus all compound that elute after linalool. Smaller the ratio better is the quality. Mahanta ratio is the sum of the peak areas of terpenoids to non-terpenoids. Yamanishi-Botheju ratio is the ratio of peak area of linalool to E-2-hexenal. All the three ratios mentioned above have limitations for their applicability.

Another aroma quality indicator called flavour index (F.I.), the ratio of VFC II to VFC I is reported for kenyan clonal black teas and F.I. is positively correlated to tasters evaluations. This confirms that F.I. is a good aroma quality indicator for Kenyan black teas. However, it should only be used qualitatively since the olfactory perception limits of individual VFC are different. A suitable ratio for Indian black teas with reference to aroma and quality is yet to be explored and the limitation is vide variation in weather. A new approach in terms of novel quality index for tea through the present work has been innovated (chapter 2).

Tea is a good source of flavanoid antioxidants which has a role in prevention of cancer and coronary heart diseases. Tea is known to improve blood flow, eliminate alcoholic toxins, relieve joint pains and acts as a diuretic and improves resistance to diseases.

Flavonoids present in tea can effectively stabilize free electrons through several mechanisms viz., delocalisation of electrons, formation of intramolecular hydrogen bonds and rearrangement of their molecular structure. This may be

the reason for their antioxidant property. The catechins ranked depending on their antioxidant potential as ECG > EGCG > EC > GC > EGC > C. Theaflavins and thearubigens inhibited the formation of TBARS and these are more effective than vitamin E, glutathione, vitamin C and synthetic phenolic antioxidants. Catechins were also found to be the scavengers of peroxynitrites which are capable of oxidising LDL.

Theaflavins and catechin gallates are more effective scavengers of aqueous and lypophilic stable radicals than many other flavonoids and many antioxidant vitamins. The inhibition mechanism of tea flavonoids is independent of metal ion chelation properties.

Tea flavonoids were found to reduce oxidative damage in animals from radiation, chemical oxidants, diet stress. Drinking of tea beverage was shown to reduce oxidative biomarkers in chronic smokers. Tea was found to reduce the metabolism of compounds to known carcinogens and enhance their detoxification. Thus it is claimed to inhibit variety of cancers such as oesophagal, gastrointestinal, lung and skin cancers.

A cup of black tea is reported to be three times and two times more effective than one serving of common vegetables and one serving of common fruits respectively.

Ch. 2. PROFILING OF INDIAN BLACK TEAS

This chapter describes analytical determination of volatiles and non-volatiles by different methods for profiling Indian black teas. Chromatographic techniques for separation and their determination using spectroscopic techniques besides flavour indices have also been discussed and the quality co-relation on the scientific basis is discussed.

Indian teas especially Darjeeling, Assam and Nilgiris are valued world over for their superior aroma and taste.

In order to improve our scientific understanding on objective tea quality and thereby to help retain supremacy in the world tea trade, it was proposed to

make an in depth study to generate fingerprint profile of teas grown in different regions of India which ultimately may result in a database with respect to volatile flavour compounds (VFC) as well as non volatile flavour compounds (NVFC) which are responsible for aroma, taste and quality of tea.

Novel approach for overall quality based on Seasonal, regional variations and bio-chemical quality fingerprint

A study was carried on tea samples collected from nine regions spread over four seasons. Profiling of the black tea samples from four seasons (S_1=April-June), (S_2=July-Sept.), (S_3=Oct.-Dec.) and (S_4=Jan.-March) based on bio-chemical fingerprint was accomplished.

The brief of the research findings is given below:

Codes for Region / Grade / Garden

Region / Grade / Garden	Code
Tamilnadu, Parajulie	A
Tamilnadu, Pandiar	B
Darjeeling Medium	C
Darjeeling Premium	D
Assam AFTL	E
Assam Magor	F
Nilgiris HG	G
Dooars, Aibheel	H
Dooars, Chinchula	I
Palampur G1	J
Nilgiris HG-CTC	K
Dibrugarh, Rose kandy	L
Palampu G2	M
Assam, Cachar best	N
Assam, Cacher Med.	O
Darjeeling, Kurti	P
Assam BOP	Q
Nilgiris Waynad	R
Annamalai	S
Assam OP	T

Seasonal variation of TF/TR ratio over tea producing region/grade and quality

The TF content of a tea or the ratio TF/TR is considered to be a good quality indicator of tea. Accordingly seasonal variations of TF/TR ratios over the coded tea producing regions / grades in all the four seasons (s_1, s_2, s_3, s_4) were studied. The teas having TF/TR ratios up to 0.04, >0.04-0.08 and >0.08 can be considered to be a good, better and best quality indicator of tea quality respectively. Teas from the region/grade A-I are the better (TF/TR ratios >0.04-0.08) to best (TF/TR ratios >0.08) quality teas over all the four seasons except for the teas from region A, C, D (s_1), which fall under good quality category considering their TF/TR ratios (upto 0.04).

Also the teas from region/grade K-L (s_2), N,O (s_1), PQ (s_3) and RS (s_4) are the better (TF/TR ratios >0.04-0.08) quality teas except for the teas from region J (s_2), M(s_3) and T(s_4) teas, which fall under good quality category considering their TF/TR ratios (upto 0.04).

The teas from region/grade JKL (s_1, s_3), M –T (s_2), ORST (s_3), MP (s_1), Q (s_4) are also falling under good quality category teas, considering their TF/TR ratios (upto 0.04).

Seasonal variation of sum of Yamanishi-Botheju and Mahantha ratio over tea producing region/grade and tea quality

The VFC (Volatile Flavour Compounds) content of a tea or the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) is considered to be a good quality indicator of tea. Accordingly seasonal variations of or the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) over the coded tea producing regions / grades in all the four seasons (s_1, s_2, s_3, s_4) were studied. Accordngly based on the sum of the VFC ratios (i.e.Yamanishi-Botheju ratio and Mahanta ratio) the teas can be categorized as a good (upto 1), better (>1-4) and best (>4) quality indicator of tea respectively.

The teas from regions/grade A-J (all seasons) have better (>1-4) to best (>4) quality as indicated by sum of the VFC ratios (i.e.Yamanishi-Botheju ratio and Mahanta ratio) except for teas from regions/grade AEFHIJ(s_2),K-T (s_2),

BDFHIJ(s_1), BIJ(s_3) which are good (upto 1) quality teas, as indicated by sum of the two VFC ratios. Also the teas from regions/grade M (s_3), O (s_1), P (s_3), RST (s_4) are good (upto 1) quality teas as indicated by sum of the two VFC ratios.

Seasonal variation of Borse-Rao quality index over tea producing region/grade and tea quality

A new approach in terms of novel quality index for tea has been innovated through present work and the results are presented. The sum of TF/TR ratios of tea and the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) added together is proposed for the first time as a new and novel quality index, hence forth referred to as Borse-Rao quality index, considered to be an overall quality indicator of tea as both the non-volatiles/volatiles are given due consideration in this quality index. Accordingly seasonal variations of the Borse-Rao quality index over the coded tea producing regions / grades in all the four seasons (s_1, s_2, s_3, s_4) were studied. Based on the the Borse-Rao quality index teas can be categorized as a good (upto 1), better (>1-4) and best (>4) quality tea respectively.

The teas from regions/grade having Borse-Rao quality index more than four are C (s_2, s_4), D (s_3, s_4), G (s_1, s_2, s_3), H (s_3) and I (s_4) are the best (>4) quality teas.

The teas from regions/grade having Borse-Rao quality index ranging from one to four are A (s_1, s_2, s_3, s_4), B (s_2, s_3, s_4), C (s_1, s_3), E(s_3, s_4), F(s_3, s_4), H (s_2, s_4), I (s_3, s_4), J(s_2), M (s_3) and RS (s_4) and indicate that these are better (>1-4) quality teas.

The rest of the teas from regions/grade having Borse-Rao quality index upto one are good quality teas which are BDFH (s_1), EF (s_2), I (s_1, s_2), JKL (s_3), K-T (s_2) and P-T (s_3).

The profile of Indian black teas in terms of a bio-chemical fingerprint is carried out in present study which will not only help in understanding the intrinsic

quality objectively but also help in tracing the origin of the teas based on the markers identified.

For the first time a novel approach has been evolved to mark teas on the basis of TF/TR ratio, VFC ratios and a novel tea quality index (Borse-Rao quality index) is proposed which takes both volatiles and non-volatiles into account.

Ch. 3. FUNCTIONAL INGREDIENTS FROM UNUSED GREEN TEA LEAVES: ACTIVITY AND APPLICATIONS

Despite several reports on the radical scavenging activity of green tea from two leaves and a bud, the radical scavenging activity of green tea from coarse and pruned leaves in particular is not studied. The pruned and coarse tea leaves are tea plantation waste, India is one of the largest producers of tea. Therefore, testing of its radical scavenging properties is of interest primarily in order to find new promising sources for natural antioxidants. In this chapter novel approach for preparation of the green teas from the pruned or coarse tea leaves and optimization of extraction conditions to obtain catechin rich radical scavenging conserve and its application with the following two objectives is presented.

1. To isolate active conserves from coarse and pruned green tea leaves.
2. To use the active conserves for food applications

Processes for utilization of pruned/coarse green tea leaves (a plantation waste) have been worked out. A novel process for green tea preparation has been standardized and patented.

Processes for isolation, fractionation and enrichment (50-70 % catechin) and separation of radical scavenging conserve (90-94% RSA @ 10-15 ppm) from pruned/coarse green tea leaves have been standardized and patented.

Application of this catechin-rich radical scavenging conserve in nutraceutical ice-cream and cookies was worked out and patented.

The details of the study are presented in the following paragraphs:

Normal, coarse and pruned fresh tea leaves (low grade) were procured and subjected to enzyme inactivation using cross flow dryer (80-120^{o}C, 4 – 8h), and continuous infra red dryer (70-120^{o}C, 0.5-1.5h) at different temperature and time intervals. The chemical parameters are found to be in the following range, Caffeine (1.30-3.20%); Total polyphenols (11.5-15.5%). Sensory characteristics were found to be similar to that of the commercial green tea samples.

Forty four compounds have been identified from the volatiles of green teas by comparing the mass spectra as well as retention indices reported in literature, followed by retention times of the GC peaks with those of reference compounds run under identical conditions where ever possible. The broad classification of the compounds identified includes ten terpenoids, three aromatic compounds, eight alcohols, seven aldehydes, four acids, eight esters and four compounds derived from carotenoids. Ethyl hex-(2*E*)-enoate and dihydroactinidiolide are exclusively present in the green teas derived from coarse leaves.

In general, coarse green teas irrespective of the method of processing contained more number of the volatile constituents, whereas the normal green teas irrespective of the method of processing contained less number of volatile constituents eluting before linalool. This can be attributed to the normal leaf quality, which contributes less volatiles from the group of constituents (Gr. I) which are undesirable, which is the prevalent practice in the industry as well. It is also evident from the results that normal as well as a commercial sample of green tea contained almost half the number of identified volatile constituents in the present investigation as compared to the coarse green teas contained. Dihydroactinidolide and ethyl hexenoate were found to be important markers and both were present in coarse green teas, whereas both were not found in the normal green teas including the commercial one. It can be concluded that the commercial green tea (Nilgiris green tea) is also prepared from the normal tea leaves. This can be very well used in spotting the normal / coarse green tea or the admixture of the coarse green tea leaves with the normal green tea leaves. Other important marker volatiles found were the both heptadienals [(E,Z)-2, 4 /

(E,E)-2,4]. Coarse green tea contained both the heptadienals, whereas normal green teas did not contain any of the identified heptadienals in the present investigation. *cis*-3-hexenyl-n-hexanoate, ∝-ionone, *cis*-geranylacetone and β-ionone-5,6-epoxide were not found in a normal green tea. This can be attributed to the method of processing (CFD) and higher temperature (110°C) used.

Green tea extract

The extraction of green tea samples at lab scale, using different solvents (viz., Ethyl acetate, acetone, ethyl alcohol, methyl alcohol and their aqueous mixtures) was carried out.

The radical scavenging activity (RSA) of these extractives at 50 and 100 ppm concentrations were evaluated using the DPPH model system. The order of activity and extractability are as follows: Methanol > Ethanol> Acetone > Ethyl acetate The aqueous alcoholic mixtures showed higher activity and polyphenol extractability than the respective single solvents.

It was found that the yields of the extractives from green teas of coarse leaves are relatively low on the expected lines and the radical scavenging activities of the extractives of green teas from coarse leaves are marginally low at different concentrations. This observation indicated that the green teas from coarse leaves could be used for the preparation of radical scavenging conserves, by separating / enriching the active components using suitable technique.

Fractionation of the green tea extract

The extractives were subjected to liquid-liquid extraction using water and low molecular weight ester to fractionate the catechins into the solvent fraction. These extracts were analyzed for total polyphenol content and evaluated for radical scavenging activity. The polyphenol content of the solvent extracts found to be 30±2.3% as gallic acid equivalents for coarse leaves, while, polyphenol content of the solvent extract of normal leaves is found to be 31±2.4% as gallic acid equivalents. The total polyphenol content in the aqueous portion of these extracts is 23±2.1% as gallic acid equivalents for normal leaves, while that for coarse leaves extracts is found to be 18±3.0% as gallic acid equivalents. The

yields of the solvent extracts are found to be 15±0.8% for coarse leaves and for normal leaves the yield of solvent extract is found to be 17±0.8%. The yield of the aqueous extract is 17±0.9 % for coarse leaves and for normal leaves the yield of solvent extract is found to be 19 ±1.0 %. However, the radical scavenging activity of the solvent extracts from both normal and coarse leaves is found to be same (92±1% at 15 ppm). The RSA of the aqueous extracts is found to be lower. Hence, it may be concluded that the solvent used separated the compounds responsible for the radical scavenging activity.

The data obtained reveal that the green tea extracts / conserve is free radical inhibitor and primary antioxidant that react with DPPH radical, which may be attributed to its hydrogen donating ability.

HPLC profiling of green tea extractives, chemical composition and quantification

The total catechin content in the green tea extract based on the comparison of peak areas of each peak with that of authentic samples and from calibration curves was found to be in the range of 20–30 %. After fractionation the solvent extract is enriched with catechin and the total catechin content is found to be in the range of 55-85 %, while the HPLC profile of aqueous extracts showed only the presence of gallic acid and caffeine.

Extracts from unused fresh green tea leaves have the potential for large-scale application as natural antioxidants. Extracts of the green tea are becoming increasingly important as functional ingredients in the diet and are being added to a range of foods and beverages.

Improved method for the active conserve

To minimize the processing cost and to control the epimerisation of catechins during processing, alternate methods were explored. Green tea sample from fresh batch was subjected to aqueous alcoholic extraction. The extract was concentrated to remove the alcohol to the extent possible. The

obtained miscella was cooled to 10^{o}C and kept over night at that temperature. The separated solids were filtered and dissolved in low molecular weight ester and the filtrate was also treated with the same ester. Solvent was removed from the combined portion and the yield was found to be 12±2%. Both aqueous and ester portions were subjected to HPLC analyses. Ester soluble portion was found to contain most of the catechins. The aqueous portion (filtrate) was freeze dried and the solid yield was ~12±2%. Total polyphenol content and Radical scavenging activity (RSA) of the aqueous portion and ester portions were evaluated. RSA of the aqueous portion was found to be in the range of 70-85% at 40-50 ppm concentration, while that of ester portion was in the range of 85-90% at 10-15 ppm concentrations. The total polyphenol content in the aqueous portion was found to be 23-25%, while that of in the ester portion are in the range of 27-32%. The project economics of the process for catechin conserve is also presented.

Catechin-rich nutraceutical ice-cream

Ice-cream was prepared using the food ingredients along with the polyphenol conserve. The concentration of tea catechin conserve was tried in the range of 5-200 ppm. The optimum range of tea catechin concentration was found to be in the range of 20-30 ppm.

The results of the sensory analysis for ice-cream with tea antioxidant extract show that , 15% of the respondents rated under like very much (LVM), 45% of the respondents under like moderately (LM) and another 40% under Like Slightly (LS). However the ice-cream samples are acceptable as the scores are falling on ‘Like’ category.

Catechin-rich nutraceutical cookies

The cookies were prepared using the required food ingredients along with green tea catechin conserve. The concentration of green tea catechin conserve was tried in the range of 10-400 ppm. The optimum concentration was found to be in the range of 20-50 ppm by sensory evaluation.

The results of the sensory analysis indicated that 53% of the respondents rated cookies as LVM, 32% of the respondents rated it as LM and 15% of the respondents rated it as LS indicating the product is acceptable. As the results are falling on the ‘Like’ category, the product is acceptable.

Ch. 4. SUMMARY AND CONCLUSION

The significant findings of the complete study have been presented in a comprehensive way under Summary and Conclusions.

[Borse, B.B.]
Student

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Guide

CHAPTER-1

CHAPTER-2

CHAPTER-3

CHAPTER-4

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1. INTRODUCTION

Tea is one of the most popular commonly consumed non-alcoholic beverages on this planet and enjoyed by larger section of population due to its refreshing properties (Weisburger, 1999; Liao *et al*, 2001). Three major varieties of teas (viz., green tea, oolong tea, black tea) are produced, with various degrees of fermentation. The principal tea produced and consumed in the world is black tea. Tea industry is one the chief foreign exchange earners in India. The role played by Indian tea industry in employment generation is very significant. It has not only provided employment but also created a sense of entrepreneurship among certain sections of population. There are nearly one million workers employed directly and another ten million who are indirectly dependent on the Indian tea industry. The tea trade not only plays important role in Indian economy but also in world economy. With the recent research findings on the biological activities of different varieties of teas, the health savvy consumer is switching over to tea and it is gaining more importance as a beverage due to its antioxidant potential / health attributes.

The origin of tea dates back to the year 2737 BC discovered by Chinese emperor Sheng Nung while boiling water under a tree. Use of tea was increased by the spread of Buddhism in China and by the edict of the imperial court that tea should replace the use of wine. Tea in China became so popular after Ming

Dynasty (1368 – 1644 AD) that it became the national drink and even today it holds the status quo.

China enjoyed flourishing barter trade and communication with Rome, Iran, India, Afghanistan, Korea and Japan in middle of sixth century. Opening of sea-lanes led further expansion of tea trade. Tea became very popular in the West. The Tea Act 1773 gave British East India Company (BEIC) monopoly in tea trading to America and caused the “Boston Tea Party” and sparked the American War of Independence.

1.2. CULTIVATION

In 805 AD, tea seeds and cultivation techniques were imported to Japan from China. Tea seeds brought from China were planted in Korea (828 AD). Cultivation of tea was introduced to several countries like Indonesia (1684), India (1780), USSR (1833), Sri Lanka (1839), Malawi (1875), Iran (1900), Kenya (1903) and Turkey and Argentina (1924). Now tea is grown in very wide range of latitudes from 45ºN (Russia) to 30ºS (South Africa) and from 150ºE (New Guinea) to 60ºW (Argentina).

Of late due to advancements in agricultural science, horticultural practices, engineering and technological innovations, elite varieties and clones have been developed. Recently tissue culture, biotechnology and computer technology have added value to the cultivation techniques. But the other areas of concern are unscientific use of chemical fertilizers and pesticides that have caused damage

to the soil, productivity and most importantly to the environment. These concerns have given rise to organic farming and to some extent, it is now practiced in tea plantations. Pruning and harvesting is mechanized to cope up with manpower problems.

1.3. PRODUCTION

World tea production for year 2000 was 2936 million kgs as compared to 3645 million kgs in 2007 indicating an increase of 709 million kgs which is close to the Indian tea consumption (Table 1.1).

Table 1.1.: World tea production (million kilograms)

Year	World Production
2000	2936
2001	3060
2002	3081
2003	3197
2004	3326
2005	3372
2007	3645

Not only the share of India in global tea production has declined over the years, but has also passed on the long retained status as the largest tea producer with 26.0% of the world (Table 1.2) output to China (28.7%). India is the only country which can boast of producing so many varieties of tea.

Table 1.2.: Share of India in world tea production (%)

Year	Production
1953	41.0
1963	39.0
1973	38.0
1983	28.0
1993	29.0
1998	30.0
2005	27.5
2007	26.0

Teas from Darjeeling, Assam and the Nilgiris are world famous for their taste and flavour. Of the total tea produced in India in 2007, 90.0% was CTC, 8.0% was orthodox and 1.0% each of Darjeeling and green tea. And for south India (Table 1.3) in 2007 it was, 83.0% CTC, 16% orthodox and 1.0% of green tea.

Table 1.3.: Tea production in India (million kilograms)

Region / year	2004	2005	2006	2007
India	893	945	981	945
South India	231	227	228	220
North India	662	718	753	725

Being located in backward and rural areas, the tea plantations supplement the economy of the different regions and provide a higher standard of living at the grass root level.

There has been a significant increase in production and productivity in the last five decades (Table 1.4). The number of gardens has gone up manifold registering an increase of ~500 percent over 1951. Bulk of this increase had taken place in the Ninties, particularly in the small growers segment in Assam and North Bengal. When compared to 1951, the production in 2006-07 has trebled from 285 to 981 and 945 million kg. Much of the increase could be attributed to improvement in productivity as the land under cultivation had shown a meagre, increase of 38%. The production and productivity have shown a significant increase of ~325 % and ~210 % respectively.

Table 1.4.: Growth of Indian tea industry (1951 – 2007)

Year	No. of gardens	Area (ha)	Production (m. kg)	Yield (kg/ha)
1951	06214	3,16,870	285	901
2007	38705	5,40,050	945	1900
Increase over 1951	32491	2,23,180	660	999

1.4. CONSUMPTION

India is not only the largest producer of tea but also the largest consumer in the world. Nearly 24% of the global production and 75% of India's total production is consumed in the country (Table 1.5). The most significant growth is in domestic consumption. From a mere 73 million kg in 1951, domestic consumption has gone up to as much as 760 million kg.

Table 1.5.: Share of India in world tea consumption (%)

Year	Consumption
1953	12
1963	16
1973	21
1983	19
1993	22
1998	23
2005	25
2007	21

More interestingly even with the consumption of nearly 75% of the total production, the per capita consumption in India is one of the lowest in the world with 660 gram per head. With the increasing awareness about health attributes of tea and improved living standards, demand for domestic consumption continues to grow steadily. It is this demand which is expanding forward the industry and compelling it to grow as fast as possible. More importantly the rise in domestic consumption is the industry's greatest strength. From 1951 to 1958, the domestic consumption on an average registered a compound growth rate of 4.74% per annum, whereas the production growth rate was only 2.4%. This gap needs to be bridged in order to retain India's pre-eminent position as the largest producer of tea.

It was estimated that India will require about 1000 million kg of tea by the end of the Ninth Plan in 2001-02, to meet the growing internal consumption plus the demand for export calculated on the basis of retaining India's share of the world tea market. It has been achieved on her own production (981 million kilograms in 2005-06) by the end of the tenth plan.

1.5. EXPORTS

The one area of Indian tea industry which has shown steady decline since 1950 is the export front (Table 1.6). In 1951, exports were around 206 million kg and in 1988, it remained at the same level. It will be of interest to note that tea export exceeded 97 % of the total production in 1900. This pattern of tea export had continued for next five decades. It was only after 1950s, that internal consumption of tea began to rise rapidly and the share of export from India started declining. In 2007, India accounted for 14% of the world exports in comparison to 45 % in the late forties.

Table 1.6.: Share of India in world tea exports (%)

Year	Production
1953	48
1963	39
1973	27
1983	24
1993	15
1998	17
2005	13
2007	14

The information on production and export share of major tea producing countries is presented in table 1.7. The overall scenario in India is quite different from that of other major producing countries such as Kenya and Sri Lanka. While these countries export as much as 95% of their produce, it is the reverse in the case of India.

Table 1.7.: Production and export share of major tea producing countries (%) over last decade (1997- 2007)

Country	Production	Export
India	30	13
China	22	18
Sri Lanka	10	22
Kenya	10	17
Indonesia	05	06
Total	77	76

In 2004, the value of Indian tea exports was Rs.1841 Crores, and in 2005, it was Rs.1738 Crores. However, in terms of volume, the exports of tea have come down to 192 million Kg. (2005) from 198 million Kg (2004) and later increased to 219 million Kg during 2007. Our markets also need to be diversified. There are several global markets like Syria, Tunisia and Pakistan, which are yet to be explored by India.

1.6. BOTANY OF TEA

Botanical Name :*Camellia sinensis* (L) O.Kuntze
Family :*Theaceae*
Genus :*Camellia*
Species :*Sinensis*

Varieties: 1. *Camellia sinensis* var. *sinensis* (Chinese jat) and

2. *Camellia sinensis* var. *assamica* (Assam jat).

According to binomial system of Linnaeus, the tea plant was classified as *Theasinensis* in 1753. Many synonyms were given, but in general it is now accepted that the tea plant be classified in the family *Theaceae* and in the

camellia genus. There are many varieties evolved from different cultivation practises and number of other variables.

Camellia sinensis, (L) O.Kuntze, is grown in a very wide range almost world over from CIS (Formerly USSR) to South Africa, and from New Gueinea to Argentina, i.e. from 45°N to 30°S and from 150° E to 60°W of latitudes.

Sealy, (1958) reported, *Camellia sinensis* variety *sinensis* (Chinese jat) and *Camellia sinensis* variety *assamica* (Assam jat), are two main varieties or jats of tea. Since propagation of tea plants was originally by seed, it added to the heterogeneity. It was necessary to adopt vegetative propagation to evolve true to type plants. Hence vegetative propagation methods have been introduced (Visser and Kehl, 1958) and are practiced for new plantations and for filling in vacancies in existing plantations of tea and also for replanting. But the commercially grown tea plant is generally a highly heterogeneous mixture with contributions from other varieties of Camellia such as *C. irawadiensis* and *C. cambodiensis* (Wight and Barua, 1954). Barua (1989) and Banerjee (1992) have discussed comprehensive accounts of botany, botanical classification and physiology of tea.

1.7. PROCESSING

Tea can be processed in a number of ways, but usually it is made into green or black tea.

1.7.1. Green tea

Greeen tea is the unfermented form of tea or the unoxidised form of tea prepared by keeping the polyphenols in native state. The processing of green tea involves withering, rolling, steaming / pan firing / radiation fixing, drying, sorting, grading and packing. The major producers and consumers are China, Japan and Sri Lanka.

1.7.2. Black tea

Black tea processing as a whole consists of a number of mechanical operations combined with or alternated by chemical and enzymatic reactions followed by drying. The fundamental process in making black tea, incorrectly called fermentation, consists of a series of oxidations and condensations of certain substances in the withered leaf initiated by the rolling process. One may feel that tea manufacture is a simple process, but it is not so and numerous important factors affect the final product. The five steps involved in the black tea processing are:

1. Withering
2. Rolling / leaf distortion
3. Fermentation
4. Drying
5. Sorting and grading

The freshly plucked young shoots of the tea bush, which is the basic material for tea processing, are withered by moisture evaporation during initial 16 to 20 h to prepare the leaf for further processing. Withering continues to a

stage in which the material physically can be rolled without breaking up excessively and has undergone certain chemical changes, in which the concentrated juice can be wrung out by a twisting action. During the rolling of the withered leaf, the cell contents of the bruised material are mixed and aerated. The polyphenolic bodies in the leaf belonging to the catechin group are more or less oxidised by an enzyme taking up atmospheric oxygen, initially to yellow theaflavins and subsequently to red and brown thearubigins. After rolling, the material is subjected to further fermentation by spreading it under adequate conditions of temperature and humidity for such a period that the best possible quality of made tea is obtained from the given basic material. After fermentation many other changes take place including the development of the characteristic aroma of tea. At a specified time fermentation is arrested almost completely by removing the moisture from the fermenting material by drying. Drying not only arrests or stops the fermentation but also provides a dry finished tea. Dried tea can be easily handled and can withstand prolonged storage without deterioration. Sorting and grading is done to achieve clean grades and uniform size which is useful to the users. Grading is an important operation for marketing of tea, ensuring the correct particle size, shape, and cleanliness required by the buyer. Major grades of black tea incase of orthodox tea are whole leaf, broken, fannings and dust and incase of CTC tea are broken, fannings and dust, which are separated from each other by sifting the made tea through standard wire meshes. The blending is an operation wherein a blend of teas from different growing regions and grades of tea is prepared in order to keep the quality of tea

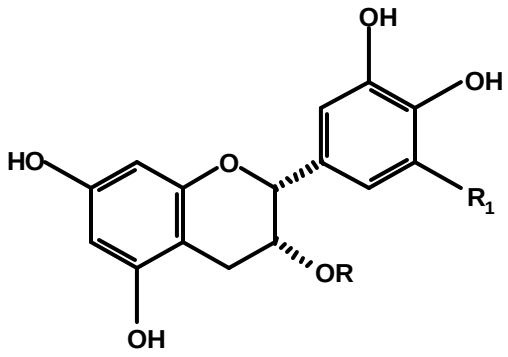
uniform as per the market requirement. The professional tea taster is an integral part of blending operation and plays a vital role in blend formulation.

1.8. BIO-CHEMICAL MOIETIES IN TEA

The tea contains different classes of chemical moieties. These classes of compounds in tea have been studied (Vuataz *et al*, 1959; Millin and Rustidge, 1967). These include polyphenols, amino acids, caffeine, nucleotides, carbohydrates, lipids, organic acids, chlorophyll, carotenoids, unsaponifiable compounds, saponins, minerals and the very important volatile compounds.

1.8.1. Polyphenols

The total content of polyphenols in tea flush is 25-30%, on a dry weight basis. These compounds are mainly flavanols, together with flavonols and flavonol glycosides, flavones, acids and depsides. During processing of black tea about 90-95% of the flavanols undergo enzymatic oxidation to products which are directly responsible for the characteristic colour of tea brews, their astringency and unique taste. Roberts named these oxidation products as theaflavins and thearubigins. The structures of important polyphenolic moieties from tea are presented in Fig. 1.1 and 1.2 (Sanderson, 1972a).

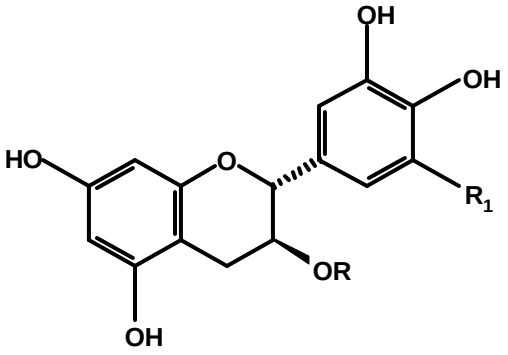


epi-CATECHIN R = R_1= H

epi-GALLOCATECHIN R = H R_1= OH

epi-CATECHIN GALLATE R = GALLOYL R_1= H

epi-GALLOCATECHIN GALLATE R = GALLOYL R_1= OH



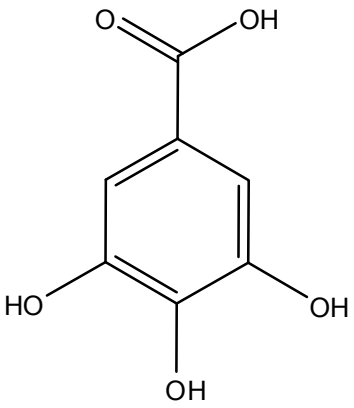
CATECHIN R = R_1= H

GALLOCATECHIN R = H R_1= OH

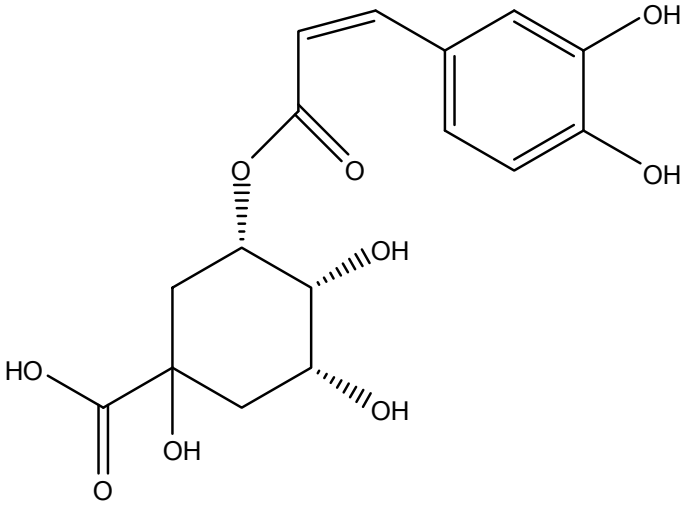
CATECHIN GALLATE R = GALLOYL R_1= H

GALLOCATECHIN GALLATE R = GALLOYL R_1= OH

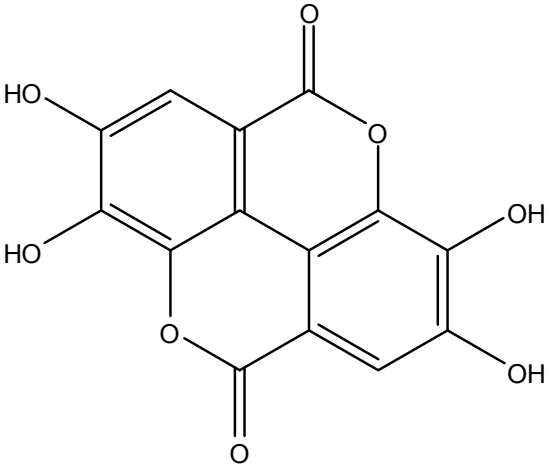
Figure 1.1. Structures of phenolic moieties from tea – catechins



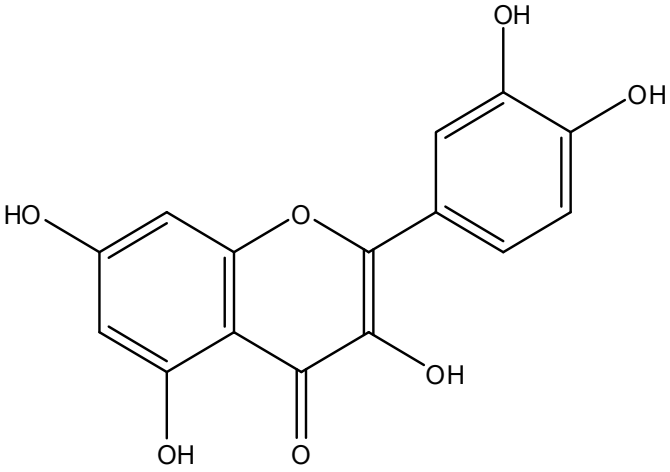
Gallic acid



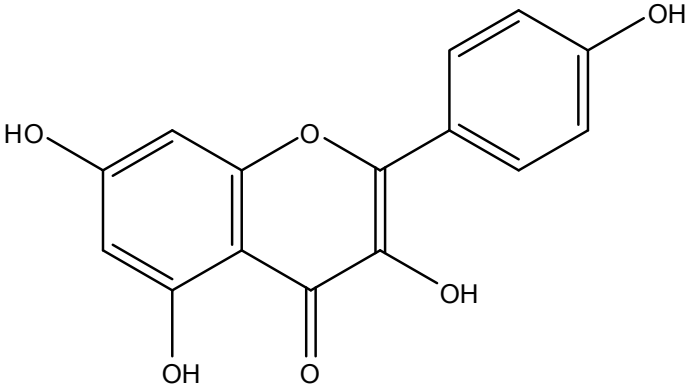
Chlorogenic acid



Ellagic acid



Quercetin



Kaempherol

Figure1. 2. Structures of phenolic moieties in tea

The reaction of prime importance in black tea processing is the oxidation of pairs of catechins by polyphenol oxidase, leading to the formation of the black tea pigments theaflavins and then compounds undergo further condensation giving rise to thearubigins. Theaflavins consists of four major components; theaflavin, theaflavin monogallates A&B, and theaflavin digallate which are formed by the paired oxidation of catechins as follows:

Epicatechin + Epigallocatechin + $3/2O_2$ ⟶ Theaflavin +CO_2+ $2H_2O$

Epicatechin + Epigallocatechingallate + $3/2O_2$ ⟶ Theaflavin-3-gallate + CO_2 + $2H_2O$

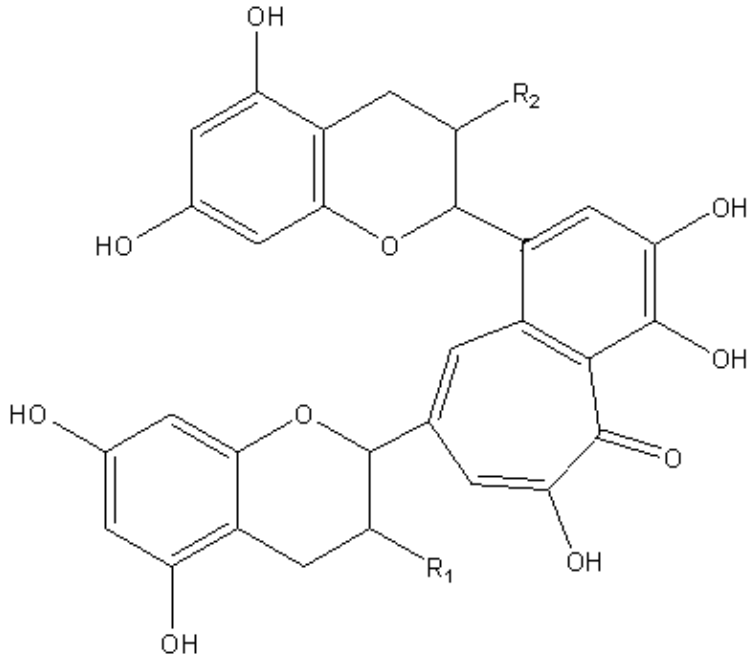
Epicatechin gallate + Epigallocatechin + $3/2O_2$ ⟶ Theaflavin-3'-gallate + CO_2 + $2H_2O$

Epicatechin gallate + Epigallocatechin gallate + $3/2O_2$ ⟶ Theaflavin-3,3'-digallate + CO_2 + $2H_2O$

Model tea fermentation systems were used to establish these reactions (Co and Sanderson 1970 and Sanderson *et al* 1972b). The chemical structure and configuration of theaflavin as a benzotropolone derivative was established (Roberts, 1962; Takino *et al*, 1965; Brown *et al* 1966) as shown in Fig. 1.3. Also isolation and characterization of the theaflavin gallates have been accomplished (Bryce *et al*, 1970 and Coxon *et al* 1970).

Photometric methods have been developed for quantitative determination of total theaflavin content (Roberts and Smith, 1961; Roberts and Smith, 1963; Hilton, 1974) included. The approximate relative proportions were reported (Coxon *et al*, 1970) as theaflavin-3-gallate (8%), theaflavin-3′-gallate (20%), theaflavin-3-3′-digallate (40%), isotheaflavin (4%), and epitheaflavic acids (Coxon

et al, 1970). Near-infrared (NIR) spectroscopy (Ikegaya *et al*, 1989), ref. included Gel filtration (Lea and Crispin, 1971), gas-liquid chromatography of trimethylsilyl esters (Collier and Mallows, 1971) and high-performance liquid chromatography (Robertson and Bendall, 1983) were used for estimation of theaflavin andindividual gallate esters.



	R1	R2
Theaflavin	OH	OH
Theaflavin-3-gallate	Gallate	OH
Theaflavin-3'-gallate	OH	Gallate
Theafavin-3-3'-digallate	Gallate	Gallate

Figure1.3. Structures of Theaflavins from black tea

Studies on the chemistry of thearubigins (Brown *et al*, 1969 and Brown *et al*, 1969a) showed the presence of five fractions, which were degraded to flavan-3-ols, flavan-3-ol gallates, anthocyanin, delphinidin and gallic acid. It was suggested that the thearubigin fractions investigated were mixtures of polymeric proanthocyanidins and flavanoid residues. Thearubigins constitute as much as 10-20% of the dry weight of black tea (Sanderson, 1972a), which is 10-20 times greater than the dry weight of the theaflavins (1-2%). These are the major oxidation products of catechins during fermentation and make a highly significant contribution to the depth of colour and strength of tea brews. One of the moieties of thearubigins is the theaflavins, and it is known that during fermentation the theaflavins reach peak (Roberts, 1958 and Owuor *et al*, 1986), after which they are believed to undergo further oxidation to produce thearubigins (Cloughley and Ellis, 1980; Cloughley 1980). The chemistry underlying the changes which occur during tea leaf fermentation is reviewed and used as a basis for proposals for the structure of thearubigins, the major pigments of black teas (Haslam, 2003).

Black tea beverage typically contains approximately 31% (w/w) flavonoids as 9% catechins, 4% theaflavins, 3% flavonols and 15% undefined catechin condensation products. A typical cup of tea (prepared from 1-2 g of black tea powder) contains approximately 600 mg of total solids and 200 mg of flavonoids.

1.8.2. Alkaloids

The alkaloid caffeine and to a lesser extent, theobromine and theophylline (Fig. 1. 4) are well known components of tea.

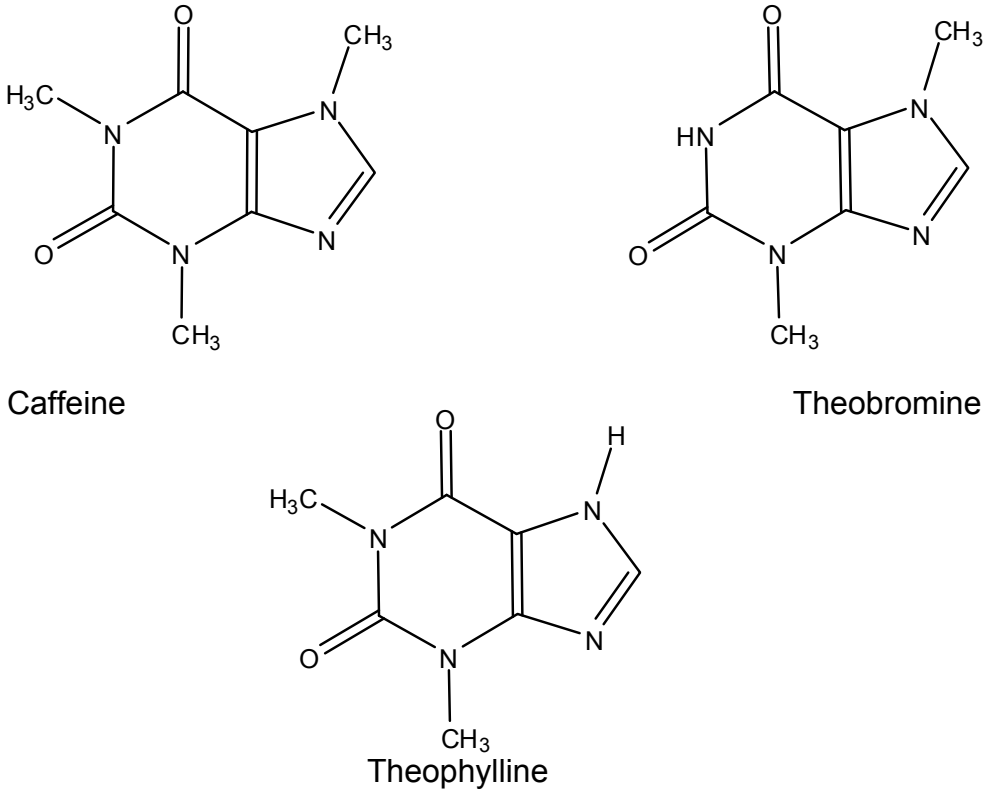


Figure1.4. Structures of Alkaloids from tea

Caffeine makes a significant contribution to the briskness and creaming properties of tea. Briskness is a taste sensation, which makes an important contribution to the evaluation of tea whereas the creaming property of tea brews, the turbidity that developed when a tea brew is cooled, is an indication of tea quality. A cream, which is bright and golden, is preferred to one which is dull and muddy. A study of cream revealed that it is an insoluble complex of caffeine, theaflavin gallates, polysaccharides, protein, and other compounds (Wickremasinghe and Perera, 1966).

Several methods have been described for the quantitative estimation of caffeine. These include spectrophotometry (Newton, 1969), gas chromatography (Weerasinghe *et al*, 1982), high performance liquid chromatography (Herath and

Roberts, 1981; Ikegaya, 1985; Pura Naik and Nagalakshmi, 1997), and infrared spectroscopy (Ikegaya *et al*, 1987).

The stimulant effect of tea is due to caffeine. It activates the central nervous system and gives a feeling of freshness and alertness, which is a positive effect of tea drinking if consumed in reasonable quantity.

1.8.3. Carbohydrates

It has been postulated that simple sugars play a part in the formation of tea aroma. Starch is a major polysaccharide and the enzymatic breakdown products of pectin have been implicated in the development of tea quality during processing (Lamb and Ramaswamy, 1958).

Investigation on the polysaccharide fraction of the ethanol insoluble material of tea flush (Selvendran and Perera, 1971) showed the presence of hot water soluble polysaccharides, proteins, ammonium oxalate soluble pectin acids, sodium hypochlorite soluble compounds, hemicelluloses A and B, and ∝-cellulose. More recently (Sakata *et al*, 1989), a new tea constituent 2-O-(β-L-arabinopyranosyl)-myoinositol has been characterised as a major (ca.0.8%) constituent of black teas. The data on carbohydrates of black tea infusion is presented in Table 1.8., (Fig. 1.5)

Table 1.8.: Carbohydrates in Black tea infusion

Carbohydrates	Amount of constituent (% x10^2)
Pectin	0.05
Fructose	0.60
Glucose	0.57
meso-Inositol	0.15
Sucrose	0.48
Maltose	0.03
Raffinose	0.10
Total sugars	2.2
Total Polysaccharides (by difference)	1.3

Source: Sanderson *et al*, 1976.

1.8.4. Lipids and fatty acids

The total lipid content of black tea varies from 4% to 9%, and the transformation of lipids to volatile compounds during processing of tea was believed to be associated with the development of flavour (Selvendran *et al*, 1978; Wright and Fishwick, 1979; Mahanta *et al*, 1985). The amounts of lipids in different shoot components were presented in Table 1.9. It is evident that lipid content increases as the leaves mature, and is related to the maturity of the chloroplast (Bhuyan *et al*, 1991).

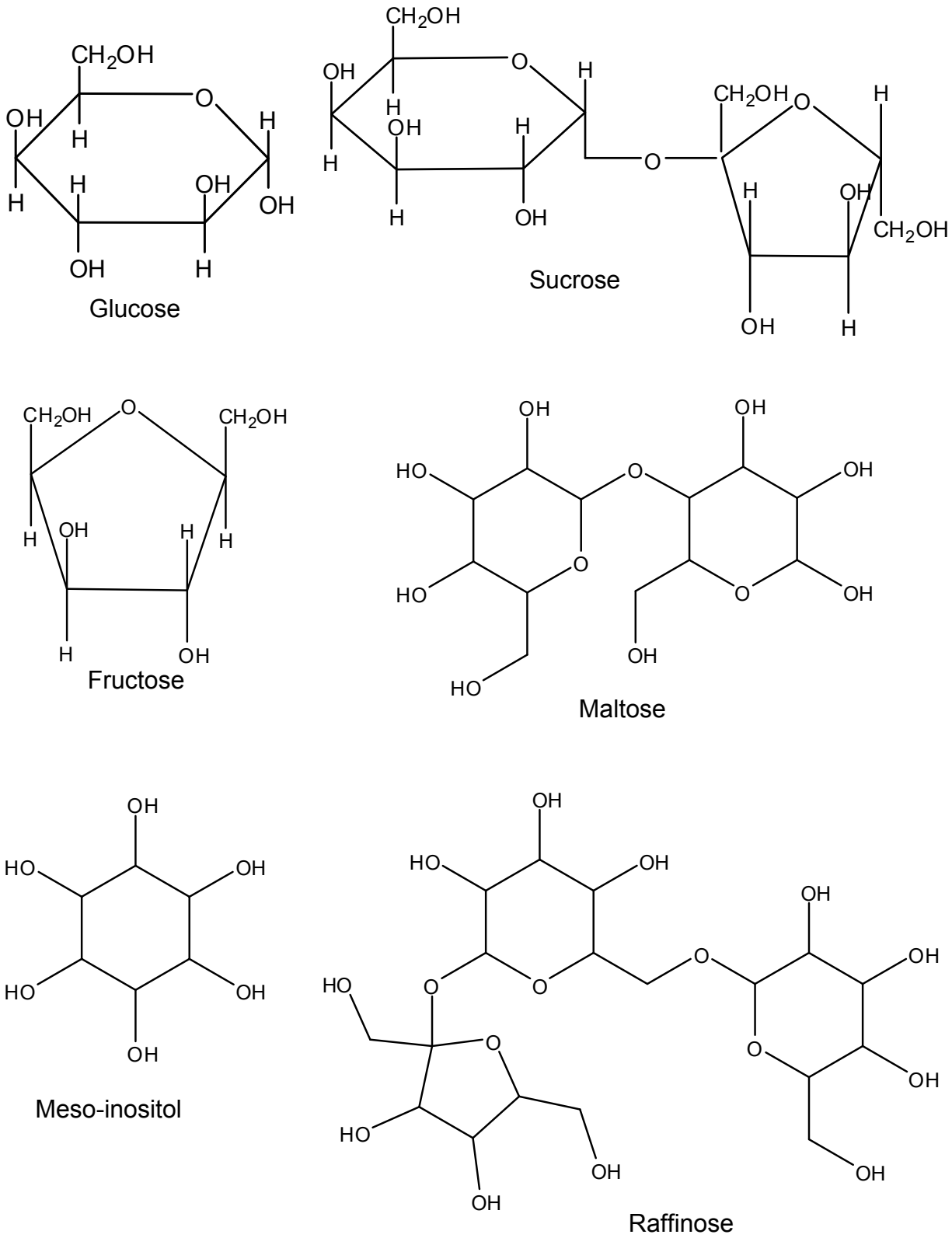


Figure1.5. Structures of carbohydrates from tea

The lipid content and fatty acid composition of tea shoot and manufactured tea were studied by Bhuyan *et al*, 1991. Glycolipids accounted for about 60% of the total lipids and contained a high level of linolenic acid (Fig. 1.6).

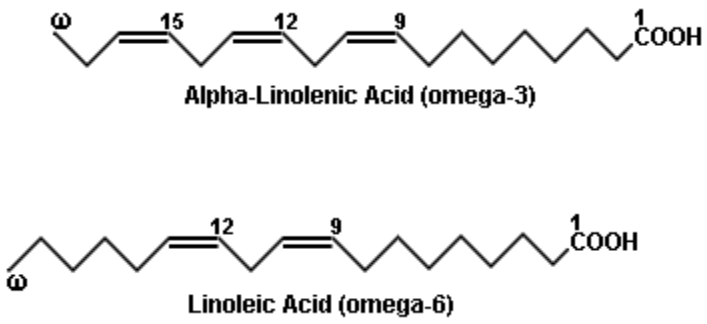


Figure 1.6. Structure of Linoleic and linolenic acid

Palmitic acid occurred in higher concentration in phospholipids, but myristic and lauric acids were abundant in neutral lipids.

Table 1.9.: Amounts of lipids in shoot components

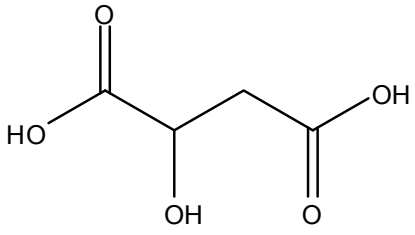
Components	Amounts of lipid (mg/g, dry weight)
Bud	36
First leaf	40
Second leaf	60
Third leaf	67
Fourth leaf	72
Stem	30
Whole shoot	52

Source: Bhuyan *et al*, 1991.

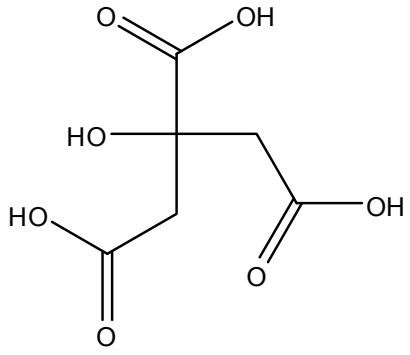
Identification of the fatty acids of tea leaves (Zaprometov, 1961; Saijo, 1973; Owuor, 1990) showed that linolenic acid was the most abundant, followed by linoleic, palmitic, stearic, oleic, and palmitoleic. The relatively high levels linolenic and linoleic acids are of interest in view of their oxidative degradation to C_6 aldehydes, alcohols and esters. These C_6 compounds play a significant role in tea flavour (Wickremasinghe *et al*, 1973).

1.8.5. Organic Acids

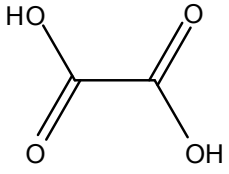
Malic acid was identified (Bokuchava, 1936 and; Sakato *et al*, 1955) in fresh tea and fermented tea leaves, and this was followed by detection of oxalic, citric, and succinic acids (Fig 1.7).



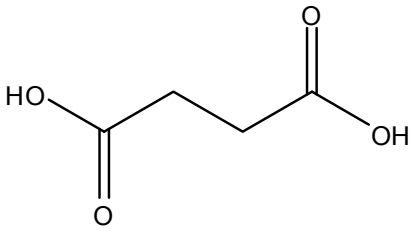
Malic acid



Citric acid



Oxalic acid



Succinic acid

Figure1.7. Organic acids identified from tea leaves and fermented tea

Later investigations (Sanderson and Selvendran, 1965) showed ten organic acids in extracts of tea flush, of which five were identified as oxalic, malic, citric, isocitric and succinic acids. Oxalic acid, present as crystals in the vacuoles of tea leaf cells (Green, 1971), is the predominant organic acid. The occurrence of quinic and shikimic acids in tea flush was also demonstrated, in keeping up their role as precursors of polyphenols (Neish, 1966).

1.8.6. Chlorophylls

Chlorophylls a and b (Fig. 8) were reported to be present in tea flush (Wickremasinghe and Perera, 1966; Co and Sanderson, 1970; Kawamura *et al*, 1985;), which contains 1.4 mg of chlorophylls per gram on dry weight basis. A relationship between chlorophyll content of various tea samples and grassy taste was evaluated (Lelyveld and Smith, 1989), and concluded that high chlorophyll content was associated with grassy taste.

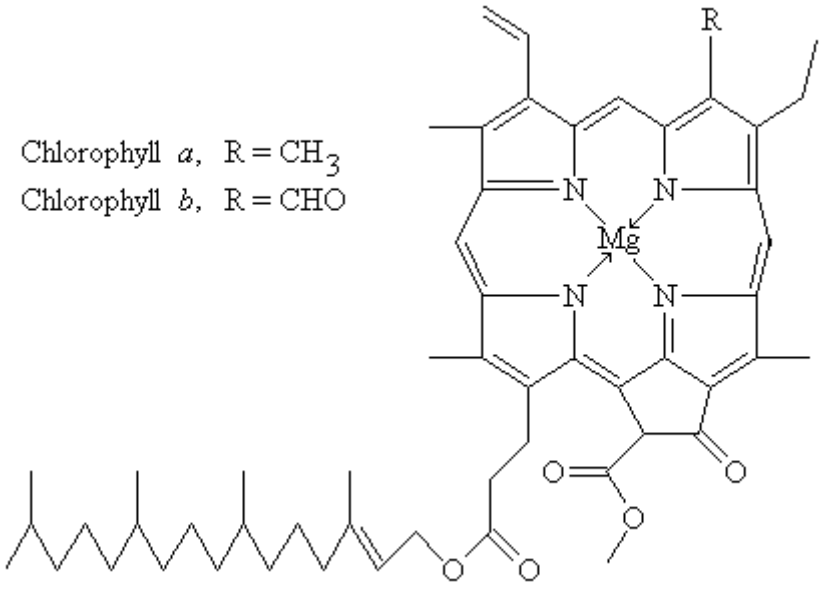


Figure1.8. Structure of chlorophyll a and b

There is large decrease of chlorophylls during the processing of black tea, and this decrease can be accounted for by their transformation to phaeophytins and phaeophorbides (Wickremasinghe and Perera, 1966; Co and Sanderson, 1970). This breakdown of chlorophylls during processing determines whether the appearance of the tea will be black (phaeophytin) or brown (phaeophorbide). The blackness of tea is also equally valued in trade since it is an important property of the product.

1.8.7. Carotenoids and Xanthophylls

In earlier studies on the extracts of tea, fourteen compounds derived from carotenoids (Fig. 9) were identified (Tirimanna and Wickremasinghe, 1965), which decreased during the processing to black tea. It was speculated that the degradation of carotenoid compounds would yield volatile compounds, which contribute to the aroma of tea. Quantitative studies showed that the carotenoid compounds steadily decreased from about 0.53% (dry weight basis) in fresh leaf to about 0.026% in the final processed product and that β-ionone and several other volatile compounds were formed from β-carotene in a model tea fermentation system (Sanderson *et al*, 1971). In studies of the thermal degradation products of β-carotene (Yamanishi *et al*, 1989), several ionone related compounds were identified; among them were dihydroactinidiolide and theaspirone, both important flavour components of black tea. The structures of ionone-related compounds found in made teas are presented in figure 9. (Kawashima and Yamanishi, 1973; Kawakami, 1982; Hazarika and Mahanta, 1983; Kanasawud and Crouzet, 1990). Total carotenoid content increases with

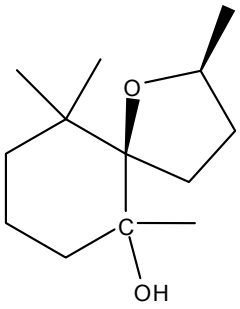
increasing leaf maturity as do the carotenoid and xanthophyll contents. About sixteen carotenoids were identified, and it was found that β-carotene constitutes about 90% of the total carotenes. Lutein constitutes the bulk of the xanthophylls, about 82% of the total xanthophylls in the first leaf (Venkatakrishna *et al*, 1977).

A simple, rapid and reproducible method using reverse-phase high performance liquid chromatography for the separation and classification of carotenoids and chlorophylls present in fresh tea leaves revealed the presence of twenty eight pigments (Taylor and McDowell, 1991).

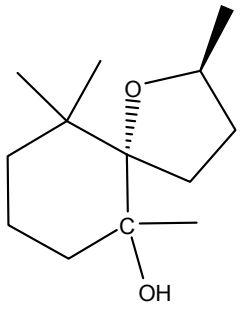
1.8.8. Minerals

In the initial reports, there is a wide variation in the aluminium content in tea leaves, ranging from 1000 to 10,000 ppm (Winton and Winton, 1935). Chang and Gudnason (1982) reported that the amount of K in the infusion (100 ml) was more than 5 mg per gm of brewed tea, whereas the amount of the last group of minerals was only about 0.01 μg per gram. Cu and Al are important because Cu is a component of polyphenol oxidase, and Al is a factor that improves the colour and taste of brewed tea. In another investigation on brewed infusion, the element in highest concentration was found to be K, followed by P, Mg, Ca, Al, Mn, Cu, Na, Si, Zn, B, Ba, Fe, Ni, Pb, Sr, Co and Cd (Natesan and Ranganathan, 1990).

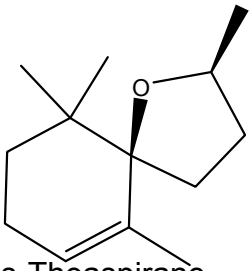
Tea also contains an unusually high level of fluoride, ~150 ppm in black teas (Singer *et al*, 1967). This amount of fluoride in tea may be one of the reasons as to why drinking tea lowers the incidence of dental caries.



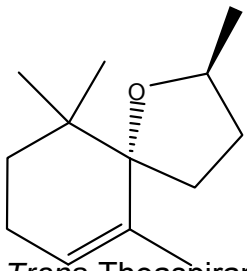
Cis-6-Hydroxydihydro theaspirane



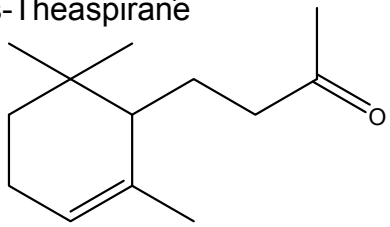
trans-6-Hydroxydihydro theaspirane



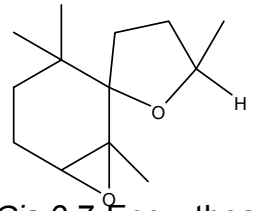
Cis-Theaspirane



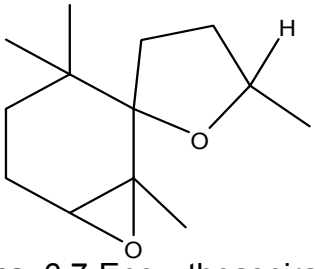
Trans-Theaspirane



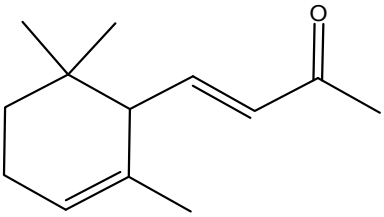
7,8 –Dihydro-α-ionone



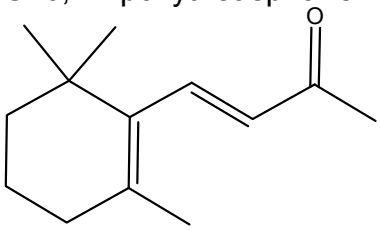
Cis-6,7-Epoxytheaspirane



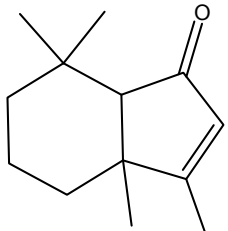
trans -6,7-Epoxytheaspirane



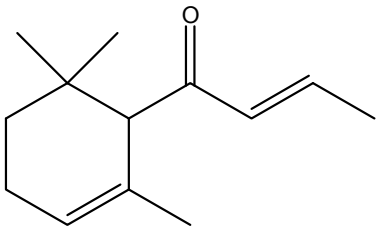
α-Ionone



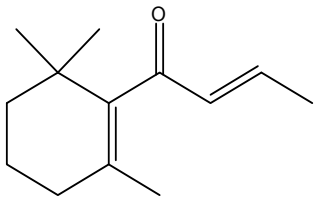
*β -Ionone



5,5,9-Tetramethylbicyclo[4,3,0]-non-8-en-7-one



α- Damascone



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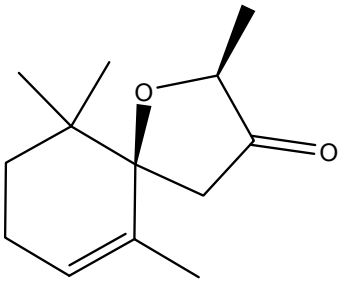
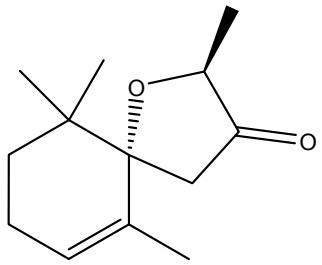
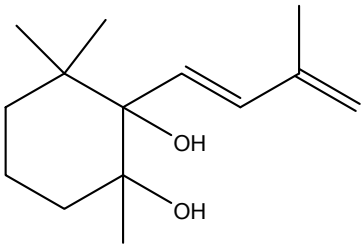


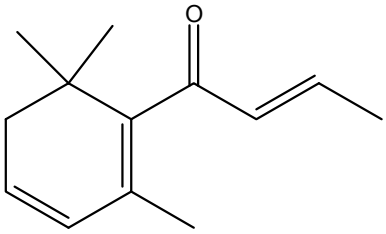
Cis –Theaspirone



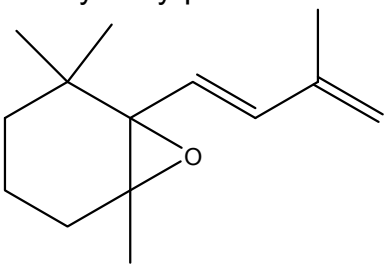
Trans -Theaspirone



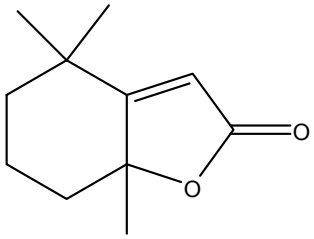
5,6-Dihydroxy-β-ionone



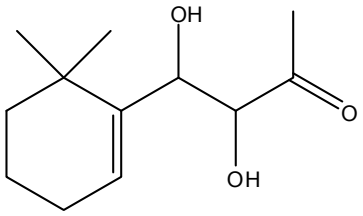
β -Damascenone



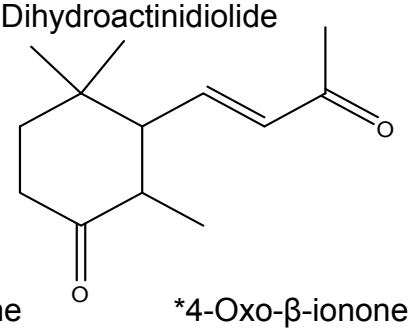
*5-6-Epoxy-β-inonone



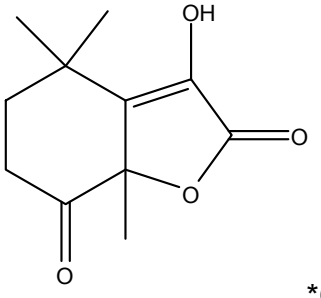
*Dihydroactinidiolide



1’, 2’-Threo-1’, 2’-dihydroxy-β-ionone



*4-Oxo-β-ionone



Loliolide

*Compounds produced from β-carotene by heating

Figure1.9. Ionone-related compounds in made tea

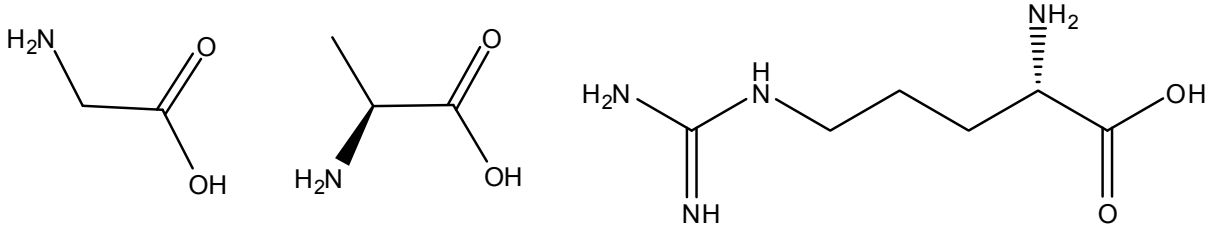
1.8.9. Amino Acids

Theanine was reported in tea for first time by Sakato (1950) and identified as N-ethyl glutamine in the same year (Sakato *et al*, 1950). Theanine, which is particularly abundant in high-quality green teas, has been reported as the prime factor in the taste of green tea (Sakato, 1957). The presence of theanine, an Angiosperm, is unique to tea, although its occurrence was reported later in the mushroom *Xerocomus badius* (Casimir *et al*, 1960). Studies of the biosynthesis of theanine, in tea indicate that the site of biosynthesis is the root (Sasaoka and Kito, 1964; Konishi,1969; Wickremasinghe and Perera, 1952) and that the precursors are glutamic acid and ethylamine. The occurrence of L-glutamic acid-N-methylamide in tea, biosynthesized from L-glutamic acid and methylamine was reported (Konishi and Takahashi, 1966). Theanine is the most abundant and accounts for 50% of the total amino acids and 1% of the dry weight of tea. It is particularly abundant in the roots of the tea plant and occurs in all parts of the tea plant with the exception of the seed, where pipecolic acid is present in the early stages of seed development (Ozawa *et al*, 1969). The exact role of theanine in the tea plant is not firmly established, but it was suggested (Wickremasinghe *et al*, 1973) that theanine may function as an hydrogen bond breaker, in keeping with the properties of N-ethylamindes. Theanine is a constituent of the thearubigin fraction, which is responsible for much of the colour of tea brews. It has been proposed that ethylamine is formed from alanine by alanine decarboxylase, and that this amine reacts with glutamic acid in the presence of L-glutamate ethylamine ligase to produce theanine (Takeo, 1974). Studies of

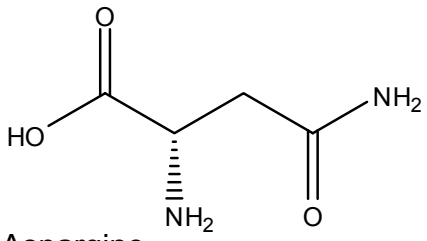
theanine formation in suspension of tea cells (Matsuura and Kakuda, 1990 and Matsuura *et al*, 1992) have confirmed the role of ethylamine as precursor of theanine.

The several other amino acids (Fig.10) are present in tea and were identifed as aspartic acid, threonie, glutamic acid, glycine, ∝-alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, glutamine, asparagine and tryptophan along with theanine (Bhatia and Deb,1965; Roberts and Senderson, 1966).

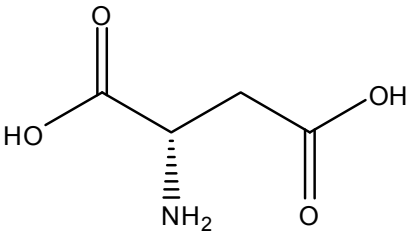
Studies on the interaction of catechins with proteins (Sekiya *et al*, 1984) showed that the major catechin gallates formed precipitates with soybean lipoxygenase, which led to a loss of 10-30% of enzyme activity and altered the nature of the enzyme protein. Tea polyphenols also interacted with amino acids and glucose during heating (Anan and Kato, 1984; Anan *et al*, 1987 and Anan, 1988) to yield coloured compounds and the formation of Amadori porducts (Anan, 1988), which improved the flavour of tea.



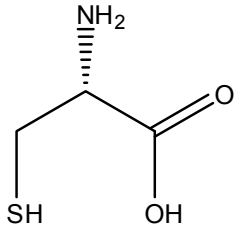
Glycine Alanine Arginine



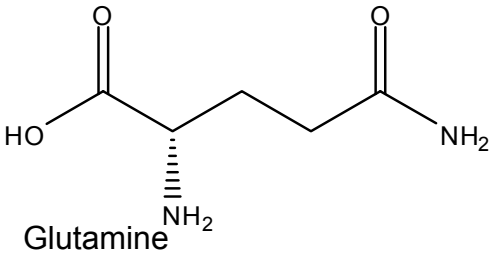
Aspargine



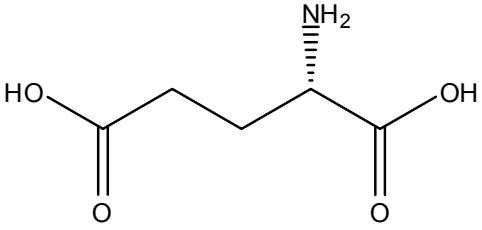
Aspartic acid



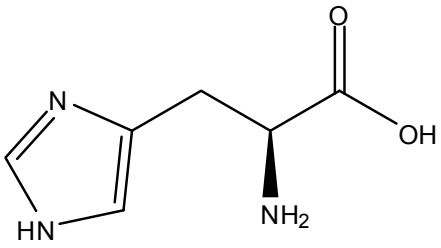
Cysteine



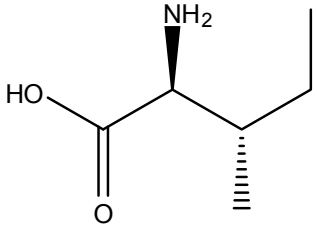
Glutamine



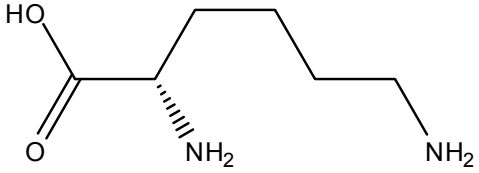
Glutamic acid



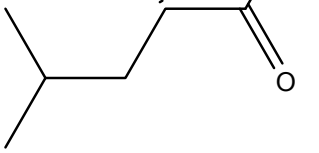
Histidine



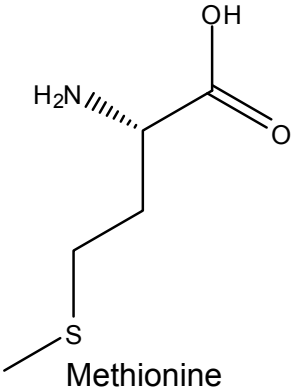
Isoleucine



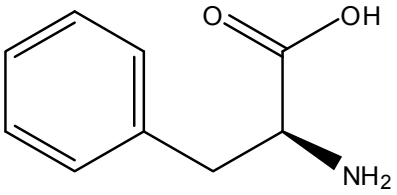
Lysine



Leucine



Methionine



Phenylalanine

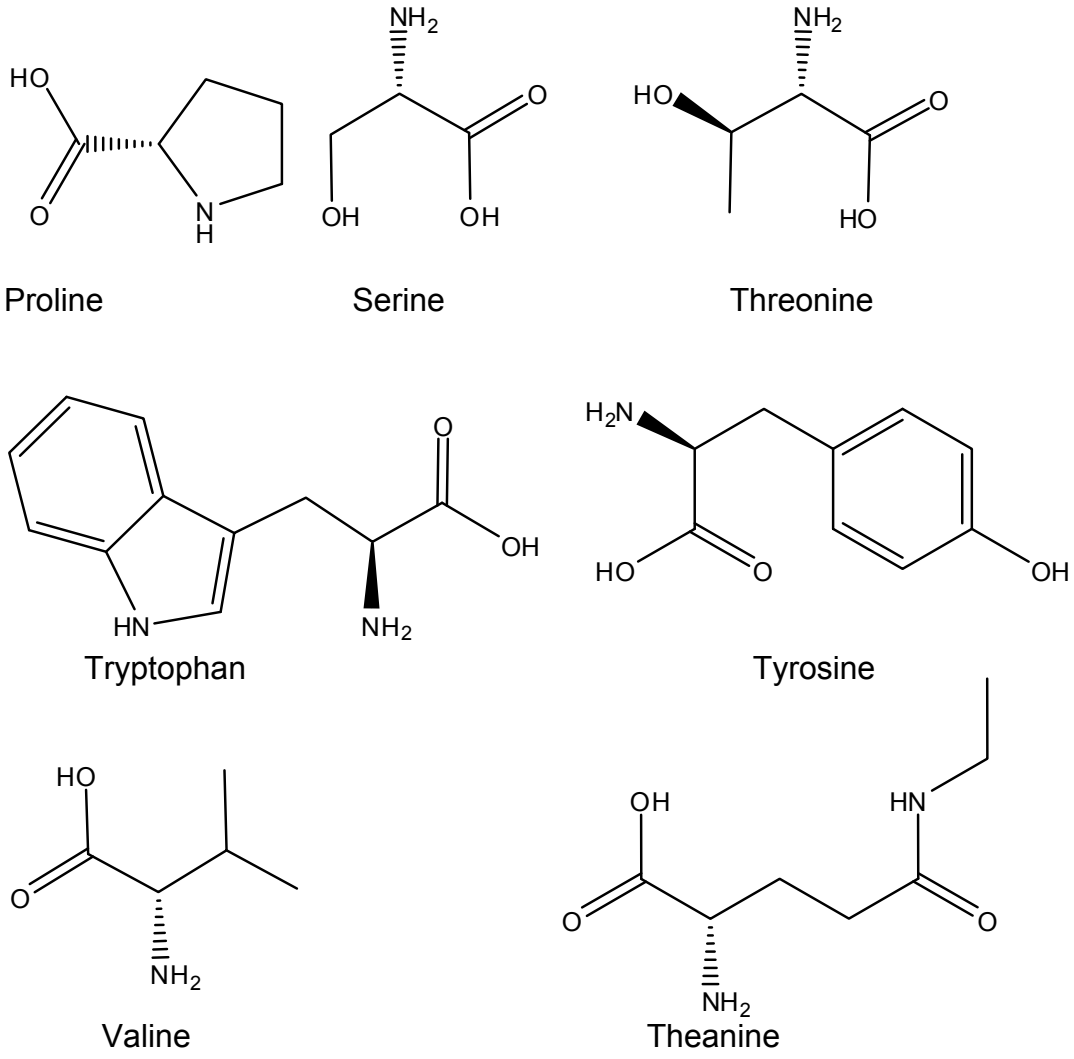


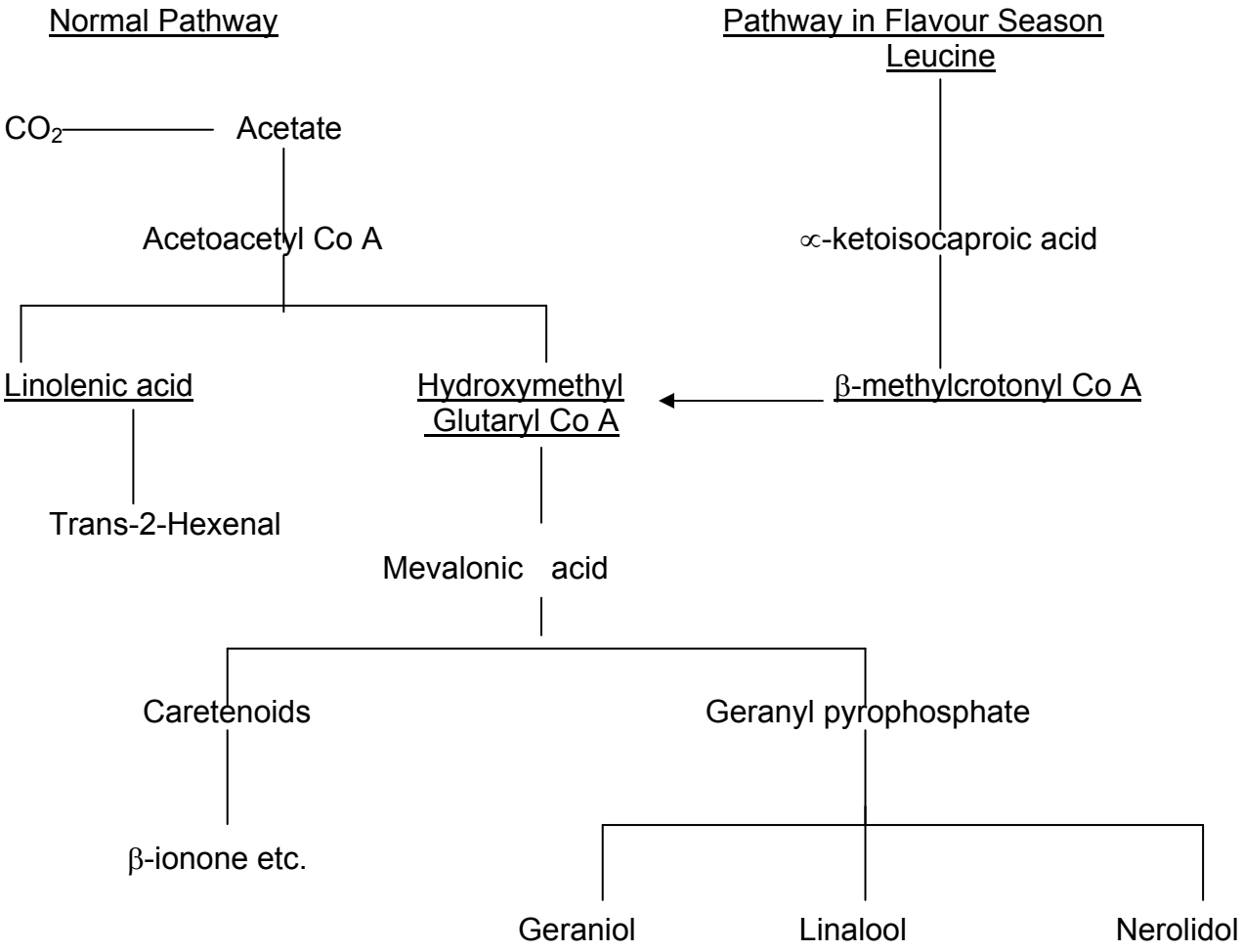
Figure1.10. Structures of amino acids present in tea

1.8.10. Volatile compounds (Aroma substances)

The volatile compounds accounts for 0.01% to 0.02% in tea, they play a major role in determining the unique character of tea. More than 600 compounds have been detected in the complex mixture of volatile compounds from tea. These include hydrocarbons, sulfides, carbonyls, acids alcohols, esters, lactones, pyridines, pyrazines, furans, thiazoles, quinolines, amines, amides, etc. It is the combination of these numerous compounds that determines the aroma of any given sample of tea, which provides almost infinite blends of tea aromas. Concentrations of volatiles vary as per climatic conditions and geographical locations.

Besides the world-famous seasonal Dimbula and Uva teas from Sri-Lanka, the second flush teas from Darjeeling in India and Keemun tea from China have outstanding flavour. It was proposed (Wickremasinghe, 1974) that the mechanism in the biogenesis of tea flavour is the desiccation and degradation of the chloroplast structure by the dry and cold conditions, when normal intrachloroplastic bioysnthesis of flavour using acetate as precursor is replaced with extrachloroplastic biosynthesis using leucine as precursor. These two pathways are shown in Scheme 1.1. Evidence supporting these pathways was the production of labelled mevalonic acid (a key intermediate in the biosynthesis of terpenes) from either labelled acetate or labelled leucine during the early stages of processing of black tea (Wickremasinghe, 1978). Scheme 1.1 also shows that hexenal, which detracts from tea flavour, is probably formed during the production of terpenes from acetate but not from leucine. After winter

dormancy, growth resumes, and it is the second harvest (second flush) of the tea in Darjeeling, which has unique flavour. Here, too, it has been proposed that the extra chloroplastic reactions result in good tea flavour.



Scheme1.1. Modified Goodwin's scheme for biosynthesis of terpenoids, showing additional proposed pathways in the tea leaf (Wickremasinghe, 1974)

1.9. BIO-CHEMICAL MOIETIES AND CORRELATION TO QUALITY OF TEA

The standards prescribed for tea by different standards organizations such as ISO, BIS and PFA are the purity standards. These standards do not provide

information about the intrinsic quality. The intrinsic quality of tea depends on number of variable and non-variable factors. Some of the factors can be controlled. With the result the intrinsic qualities of tea vary considerably.

Roberts and Smith (1963) have described a method for determination of polyphenolic oxidation products in black tea liquors for the assessment of quality in tea. Biswas *et al* (1973) reported about the briskness of liquor and intrinsic quality evaluation. Further, Biswas *et al* (1973) had also reported that theaflavin is associated with all the liquor characters, i.e., colour, brightness, strength, briskness and quality. Ullah (1985) had critically discussed some of the intrinsic quality parameters and liquor characters of black tea. The thearubigins mostly contribute to the body of the liquor. Mahanta (1988) had reviewed the chemical basis of liquor characteristics based on the influence of pigments and processing conditions of black tea manufacture. Owuor and Obanda (1998) discussed critically on changes in quality of the South African black teas.

In India, tea is being grown in 38,705 gardens spread over Southern, Northern and North-Eastern States comprising of several regions of variable climate, soil, rainfall, altitude and latitude. This obviously has an effect on the intrinsic quality of tea. The manufacturing practises and methods further add up to the variations in the quality of tea.

Though, the literature on intrinsic qualities of tea is available, the information is scattered and is unorganized. Besides, there are no systematic investigations on fingerprinting of Indian teas. Bio-chemical moieties in tea and their role in quality and health are discussed in the following paragraphs.

The economic value of tea is based on its quality, which is based on taste (non-volatile compounds) and aroma (volatile compounds). The taste characteristic compounds are polyphenolic compounds, amino acids, caffeine, and mixture of volatile compounds such as terpenoids, alcohols and carbonyl compounds. The balanced mixture of astringency, bitterness, brothy taste and sweetness are found in tea infusion.

1.9.1. Catechins

Tsujimura (1929; 1930; 1931; 1934; 1935; 1952; 1955.) added reported tannin I and tannin II respectively, the two gallates having strong bitterness with astringency and the free catechins as having bitterness with a sweet aftertaste.

Nakagawa and Torii (1964) reported the threshold values of galloylated catechins and simple catechins to be approximately 4.1 to 4.4 x 10^{-4} M and 11.5 to 17.2 x 10^{-4} M respectively. Nakagawa (1970), in his studies of the relationships of catechins to the quality of black and green teas, examined the taste characteristics and threshold values of the individual catechins. The results are shown in Table 1.10.

Table 1.10.: Sensory evaluation of five catechins (Nakagawa and Torii,1964)

Catechin	Taste	Threshold values [a]
(+) –Catechin	Bitterness with sweet aftertaste	51.0
(-) – Epicatechin	Bitterness with sweet aftertaste	45.5
(-)- Epigallocatechin	Bitterness with sweet aftertaste	35.2
(-)- Epicatechin gallate	Bitterness and astringency	18.1
(-)-Epigallocatechin gallate	Bitterness and astringency	20.2

[a] Milligrams (mg)/100 milliliters (ml) water.

Millin *et al* (1969) described the contributions of non volatile components of Black tea to the taste characteristics of brewed tea. Five pure flavanols (-) – epicatechin (ECG), (-) epigallocatechin gallate (EGCG), Epigallocatechin (EGC), (-)- epicatechin (EC) and (+)–gallocatechin (GC), which were isolated from dried green leaves of var. *assamica* were tasted and reported that the tastes of these components in water at concentrations found in black tea were slightly astringent and metallic, and that EGC had a very persistent aftertaste of sweetness.

The catechin content in the tea leaves of var. sinensis and var. *assamica* varies considerably (Nakagawa and Torii, 1964; Nakagawa, 1970; Millin *et al*., 1969; Thanaraj and Seshadri, 1990). In Var. *assamica* the catechin content is approximately twice that of var.sinensis, as shown in table 1.11. In general, var. *assamica* is used for black tea and var. *sinensis* is used for green tea.

Table1.11.: Comparison of catechin contents of var. *sinensis* and var. *asamica*

Variety	Clone	EGCG (%)	EGC (%)	ECG (%)	EC (%)	Total (%)
Assamica	1	15.1	6.8	3.4	1.7	27.0
Assamica	2	13.1	7.4	3.1	1.5	25.1
Sinensis	3	6.8	3.7	2.0	1.7	14.2
Sinensis	4	6.3	2.6	2.4	1.0	12.3

Thanaraj and Seshadri (1990) developed a factor called 'theaflavin digallate equivalent' and related it to the black tea quality.

The concentrations of catechins, TF^S, TR^S and high polymerized substances are given in Table 1.12. The concentration of catechins is lower and

that of TF, TR and HPS is much higher in black tea when compared to green and Oolong teas, which is responsible for unique taste of black tea.

Table 1.12.: Concentrations of Catechin, Theaflavins, Thearubigins and Higly Polymerized Substances (HPS) in Black Tea

Compound[a]	Black tea (%)	Reference
(-)-ECG	0.29-0.42	Chamber of Tea Association, Shizuoka prefecture,Shin Chagyo Zensho,1988.
(-)-EC	--	
(-)-EGCG	0.39-0.60	
(-)-EGC	--	
Theaflavins	0.98-2.12	Thanaraj and Seshadri, 1990.
Thearubigins	7.63-8.03	
HPS	7.27-7.66	

1.9.2. Theaflavins and thearubigins

Roberts (1950) considered that the thearubigins are as important to the flavour and quality of black tea as are the theaflavins, and that they are responsible for body richness and fullness of tea brew.

Roberts and Smith (1961) also stated that theaflavins impart the mouth sensation of briskness, freshness and aliveness. Millin *et al* (1969) stated that theaflavin had a distinct black tea like character and was difficult to describe.

Sanderson *et al* (1976) estimated that the amount of polyphenolic compounds was approximately 48.5% of the total solids in a cup of tea and determined the taste of the individual polyphenolic compounds in black tea brew. These results are shown in Table 1.13, along with taste threshold values as to astringency and bitterness for each compound. It was not possible to prepare samples of thearubigins of sufficient sensorial purity for evaluation.

Table 1.13.: Threshold Levels for Astringency and Bitterness of Tea Polyphenols

Phenolic compound	Threshold level (mg/100 ml)	
	Astringency	Bitterness
(-)-EC	Not astringent	60
(-)-ECG	50	20
(-)-EGC	Not astringent	35
(-)-EGCG	60	30
(+)-C	Not astringent	60
Crude theaflavin (natural mixture)	60	70
Theaflavin (Pure)	80	75-100
Theaflavin monogallate (natural mixture)	36	30-50
Theaflavin digallate	12.5	Not determined
Gallic acid	Not astringent	Not bitter
Tannic acid	20	80

Source: Sanderson *et al*., 1976.

Zhang *et al* (1992) reported the influence of catechins and theaflavins on the astringent taste of black tea brew. Five main catechins and four main theaflavins in the tea brew from various regions viz., Darjeeling, Sri Lankan Highlands, Assam, Kenya and China were analysed by High performance liquid chromatagraphy (HPLC) and sensory analysis. Attempts were made to correlate between astringency and the concentrations of total catechins and individual catechins and found no correlation between theaflavins and astringency. The theaflavins and thearubigins content of orthodox and CTC teas are given in table 1.14.

Table 1.14.: Concentrations of Theaflavins and Thearubigins in Black tea

Method of Manufacture	Theaflavins (%)	Thearubigins (%)	Reference
CTC	1.95	13.50	Ullah, (1985)
Orthodox	0.59	5.54	
CTC	1.13 -2.14	15.82 - 20.29	Owuor,(1987)
Orthodox	0.59 - 1.03	9.34 - 11.88	

This clearly indicates that CTC tea may have 2-4 times theaflavins and 1.5-4 times thearubigins as that of orthodox tea.

It was found that theaflavins and total colour are associated with brightness of black tea brew (Lelyveld *et al,* 1990). Mahanta (1988) found that black tea from Assam clones contain high levels of TF and lower levels of TR, whereas China hybird clone had high TF and TR along with positive characteristics of tea.

1.9.3. Caffeine

The caffeine concentration ranges between 2.5 to 5.5 % (dry weight basis) in freshly harvested tea shoots. The tea shoots from assamica are richer in caffeine than those from sinensis variety. Pure caffeine is bitter and the detection threshold is approximately 3 ppm in water (ASTM, 1978). In tea brew, part of the caffeine complexes with flavanols (theaflavins and thearubigins) and play an important role in the tea taste, contributing to briskness, mouthfeel and strength. Interaction between these compounds modifies the taste. It is known that caffeine contributes to one fourth of the bitterness of the brews (Nakagawa, 1975). Removal of caffeine from tea infusion brings a significant effect on the

taste and changes the nature of the astringency from a tangy type to a non-tangy type (Sanderson *et al*, 1976). In Kenyan teas, it was observed (Owuor, 1992), that Group-I VFC levels are inversely related to the caffeine levels; ultimately as caffeine levels increase sensory quality declines.

1.9.4. Amino acids

The major amino acids found in tea are theanin, glutamic acid, aspartic acid, arginine, and serine. These contribute to the characteristic umami or brothy taste (Sanderson, *et al*, 1976). Carbonyl compounds are formed by a non enzymatic route when some amino acids are incubated with flavanols in hot aqueous solutions, during the fermentation process (Nakabayashi, 1958; Saijo and Takeo, 1970; Sanderson, 1972a). Amino acids and sugars are known to react to produce furans, pyrroles and pyrazines (Yamanishi *et al*, 1989) when heated together.

1.9.5. Carotenoids

The role of carotenes in black tea aroma formation by enzymatic reactions has been reported (Sanderson *et al*, 1971). Carotenoids namely α- and β-carotene, zeaxanthine and lutein are found in fresh tea leaves at a concentration of 100 mg percent on dry weight basis (Sanderson and Graham, 1973; Venkatakrishna *et al*, 1976). The ionone related aroma compounds are formed from carotenoids during processing step of black tea manufacture.The formation of isomeric theaspiranes and their different odour properties were reported

(Schmidt *et al*, 1992). More research on chiral compounds found in the tea volatiles could be exploited to find the aroma quality differences in various teas.

1.9.6. Fatty acids:

Linoleic, linolenic and palmitic acids are the principal fatty acids found in tea leaves (Liyanage, *et al*, 1988, Bhuyan and Mahanta, 1989). The linolenic and linoleic acids get degraded to hexanol and (E)-2-hexenal during the tea fermentation (Saijo and Takeo, 1972). Seasonal variations of C_6 volatile compounds viz., [Z]-3-hexenol, (E)-2-hexenal and n-hexanol and their precursors in homogenates of tea leaves of var. sinensis were reported (Hatanaka *et al*, 1976a, 1976b).

1.9.7. Volatile Flavour Compounds

The earliest studies on the volatiles of black tea were carried out by Yamamoto and his co-workers (Yamamoto and Kato, 1934, 1935, 1935a, Yamamoto and Itoh, 1937, Yamamoto *et al*, 1940, 1940a, 1940b). The summary on the quantities of aroma volatiles calculated on the basis of compounds with different functional groups in black tea is presented (Table 1.15).

Table 1.15.: Amounts of Volatiles Isolated from Black Tea

Fraction	Amount (g)	Essential oil (%)	Black tea (ppm)
Acids	32.0	12.8	24.6
Phenols	8.3	3.3	6.3
Bases	0.5	0.2	0.4
Aldehydes	36.4	14.5	28.1
Alcohols	173.2	69.2	133.2

Note:1300 kg black tea yielded 250.4 g (0.02%) crude essential oil

Later, the volatile flavour compounds were analysed by GC-MS and the quantities of the compounds were expressed as the ratio of area of peak to that of internal standard (Baruah *et al*., 1986; Owuor *et al*., 1986a).

Out of the total estimated six hundred compounds contributing to the aroma of black tea, some are recognised to contribute negatively and others positively towards the black tea flavour.

Several quantitative methods have been examined. Yamanishi *et al*., (1989a) used the ratio of the sum of the gas chromatographic peak areas of compounds eluting before linalool to the sum of areas of linalool plus all compounds with gas chromatographic retention time longer than linalool. Since then this ratio was referred to as the *Wickremasinghe-Yamanishi ratio*. It was assumed that compounds with retention times shorter than linalool were deleterious to aroma whereas linalool and the VFCs, with longer retention times than linalool, were desirable for black tea aroma. Thus, smaller the ratios better the aroma and quality.

A ratio based on the sum of gas chromatographic peak areas of terpenoids to non-terpenoids (Baruah *et al*., 1986 and Mahanta *et al*., 1988), called *Mahanta ratio* was developed. The terpenoids were assumed to be desirable while the non-terpenoids were classified as undesirable to tea aroma and quality. However, some non-terpenoids in black tea, e.g., benzaldehyde (Yamanishi *et al*., 1968), phenylacetaldehyde (Matoda, 1979), methyl salicylate (Aisaka *et al*., 1978; Howard, 1978) and benzyl alcohol (Aisaka *et al*., 1978) were demonstrated to possess desired aroma.

Yamanishi *et al*., (1989) developed another ratio based on gas chromatographic peak areas of linalool and E-2-hexenal, which ignored all other VFC. This ratio is known as *Yamanishi-Botheju ratio*. The rationale for the use of Yamanishi-Botheju ratio requires that linalool and E-2-hexenal occur in large amounts in all teas and therefore may have a dominant effect.

Although these ratios (or indices) may seem to work reasonably well, the data must be used with caution. The indices should at best be treated as qualitative since the olfactory perception limits of different VFC are variable. Some VFC may exist at low levels and affect aroma more than those occurring at high levels (Howard, 1978; Kobayashi *et al*., 1988).

Again the contribution of each VFC to flavour is not proportional to gas chromatographic area. It is known that the relationship between stimulant concentration (represented in GC peak area) and neural response (perceived flavour intensity) is not linear.

Changes in black tea quality chemical parameters due to storage were reported for South African teas. Increase in the contents of theaflavins and thearubigins in two to six months of storage was observed and volatile flavour compounds (VFC) were constant except trans-2-hexenal (decline) and phenyl acetaldehyde increased, brought decline in sensory evaluation. This was independent of grade and packaging material (Obanda and Owuor, 1995). Seasonal variations in quality of kangra teas were also studied, six VFCs indentified were more in backend flush compared to main flush (Gulati and Ravindranath, 1996). The sensory attributes of tea aromas such as fresh, floral,

sweet floral, citrus, sweet fruity, fresh green, resinous and roasted had been statistically co-related to the GC profiles of volatile flavour components by multivariate calibration methods (Togari *et al*, 1995).

A parameter for aroma quality of Kenyan black teas was reported called as *flavour index* (FI). FI is the ratio of the sum of peaks of tea volatile compounds imparting superior flavour (Group II VFC) to those contributing to inferior flavour (Group I VFC) (Yamanishi *et al*, 1968a, and Wickremasinghe *et al*, 1973). This is one of important quality parameter for Kenyan teas. Group I VFCs impart inferior green, grassy flavour to black tea. These include hexanol, 1-penten-3-ol, (E)-2-hexenal, 1-pentanol, 2-pentenol, 1-hexanol, (Z)-3-hexenol, (E)-2-hexenol and 2,4-heptadienal and are necessary for characteristic flavour but their high concentration impart an inferior flavour. Group II VFC impart a sweet flowery aroma to black tea and include benzaldehyde, linalool and its oxides, phenylacetaldehyde, methyl salicylate, geraniol, geranic acid, 2-phenyl-ethanol, and β-ionone.

There were negative correlations between Group-I VFC and tasters evaluations and positive correlations in most cases with those in Group II VFC. The FI in all cases positively correlated with tasters' evaluations (Owuor *et al*., 1987a, 1987b). This confirms that FI is the best measure of aroma quality for Kenyan clonal black teas. However, the FI should only be used qualitatively since the olfactory perception limits of individual VFC are different.

The FI for Indian black teas is yet to be explored and limitation is the vide variation in weather conditions compared to countries near equator.

(i) Orthodox black tea

Keemum tea from China, Darjeeling tea from India and Uva tea from Sri Lanka are the three most famous orthodox black teas in the world because of their superior flavours. The variety used in making Keemum tea is predominantly of var. *sinensis*, uva tea of var. *sinensis*, and Darjeeling tea is a hybrid of var. *sinensis* and var. *assamica*. The aroma characteristics can be explained by gas chromatographic analysis of aroma concentrates from these teas. The major differences in aroma patterns are in the concentration of linalool, linalool oxides and geraniol (Takeo, 1983).

The basic aroma characteristics are probably due to different varieties of tea plants as well as the influence of specific growing conditions. To identify the varietal origin of individual teas, Takeo and Mahanta (1983a) proposed a *terpene index* (TI), which is defined as:

$$TI = \frac{\text{Linalool + linalool derivatives}}{\text{Linalool + Linalool derivatives + geraniol}}$$

The terpene index of pure variety *sinensis* approaches zero whereas the TI of pure var. *assamica* approaches unity.

(ii) Crush-tear-curl tea (CTC)

The production of CTC tea is rapidly increasing with the increased use of tea bags throughout the world. The flavour of CTC tea is inferior to that of orthodox black tea because of high level of carbonyl compounds and the low concentration of hexenyl esters, linalool, linalool oxides and other desirable compounds. Compounds like leaf alcohol and aldehyde, which impart tea, an

inferior quality are higher in CTC teas. These differences are presented in Table 1.16.

Table 1.16.: Comparison of aroma compositions of orthodox and CTC black teas

Compound	Orthodox (%)	CTC (%)
(Z)-2-Penten-1-ol	1.0	0.7
(E)-2-Hexenal	13.1	41.1
(Z)-3-Hexen-1-ol	1.4	1.0
(E)-2-Hexen-1-ol	0.9	0.2
Linalool	24.4	6.9
Linalool oxide I	3.4	1.2
Linalool oxide II	9.6	2.3
Geraniol	2.6	1.5
Benzyl alcohol	1.0	1.3
Phenylethylalcohol	0.7	0.7
Methyl salicylate	6.3	2.7
β-ionone, cis-jasmone	2.7	2.1
Nerolidol	3.3	1.9

Source:Yamanishi, 1995

During withering step, in the processing of orthodox black tea, percursors, such as glycosides of linalool, linalool oxides, benzyl alcohol and other alcohols, are hydrolysed. During the rolling step, enzymatic reactions continue along with oxidation. The development of aroma is much more extensive and complicated in orthodox processing to produce black tea, compared to the aroma development in CTC processing. The suppression of the reactions just mentioned explains

why black tea made by the CTC process results in a poorer quality tea with less aroma compared to black tea made by orthodox process (Yamanishi, 1995).

1.10. ROLE OF TEA IN HEALTH

The medicinal use of tea was known long before it was being used as a beverage (Dufresne and Farnworth, 2001). Right from its legendary discovery (2737 BC) by the emperor Sheng Nung to the universally most enjoyed beverage it is today, tea has had a significant role in human heath. The Chinese scholars first reported the medicinal uses of tea in a text written by Pen T'Sao Circa during 24 to 221 AD, and since then effects of tea and its chemical moieties on human health has become a challenge for scientists.

a) Preventive and curative ability

Tea and other plant foods are dietary sources of nutrients as carotenoids, tocopherols, ascorbic acid, and non-nutrient phytochemicals generally classified as flavonoids. According to reports (Steinmetz and potter, 1991; Steinmetz and potter, 1996) increased intake of plant foods reduces the risk of cancers and coronary heart disease (Stensvold *et al,* 1992; Hertog *et al*, 1993; and Knekt *et al* 1996), the main causes of human mortality. Tea is a significant source of flavonoid antioxidants-physiologically active compounds *in vitro* and *in vivo* with a suggested role in prevention of cancer (Dreosti, 1996, Katiyar *et al*, 1994; Yang and Wang, 1993; Weisburger, 1996; shim *et al*, 1995; Xue *et al*, 1992) and coronary heart disease (Salah *et al*, 1995 and Ishikawa *et al*, 1997).

The Chinese value tea for its pleasant flavour and medicinal benefits some of which have possible scientific basis today are given in Table 1.17 (Blofeld, 1985; and Segal 1996).

b) Bioactivity of flavonoids

The flavonoids and methyl xanthines present in tea are unique bioactive compounds contributing to the organoleptic profile of the tea beverage and are being studied to determine their potential role in prevention of chronic diseases such as cancer and cardiovascular diseases. The physiological activity of tea flavonoids is in part due to antioxidant function characterized by redox chemistry and the ability to scavenge reactive oxygen species (ROS). The bioactivity of flavonoids also appears to be mediated by non-antioxidant mechanisms such as modulation of signal transduction pathways (Dong *et al* 1997), proliferation at G1 phase of the cell cycle (khafif *et al*, 1998) and the immune response (Katiyar and Mukhtar, 1996). However the mechanisms of tea bioactivity and its role in human health needs further clarity.

Tea flavonoids are competitive inhibitors of microsomal glucuronidase (Zhu *et al* 1998), slow cell proliferation through modulation of AP 1 activation (Dong *et al*, 1997) and decrease growth of tumors cell lines at the G1 phase of the cell cycle (Khafif *et al*, 1998). These properties of tea flavonoids suggest that the physiological effects of tea on processes involved with chronic diseases such as cancer and coronary heart disease involve more than simple antioxidant mechanisms, which require further research to resolve it.

Table 1.17.: Traditional health claims of tea – scientific basis

S.No	Traditional Claims	Possible scientific basis
1	Improved blood flow	Vasodilatation and decrease platelet activity
2	Elimination of alcohol and toxins	Increased activity of phase I and phase II enzymes
3	Clear urine and improve flow	Diuretic effects
4	Relieves joint pain	Anti- inflammatory activity
5	Improved resistance to diseases	Prevention of cancer and coronary heart disease.

1.10.1. Antioxidant activity

To be regarded as effective in a biological sense, an antioxidant is a substance that when present at low concentrations relative to an oxidizable substrate (e.g. Lipid, protein or DNA molecule), can supress, delay or prevent oxidation of the biological substrate (Halliwell, 1990). Antioxidant potential is determined by chemical reactivity as an electron or hydrogen donar; the ability to delocalize and thus stabilize the unpaired electron; the reactivity with other antioxidants; and the reactivity with molecular oxygen (Rice-Evans *et al*, 1997). Black tea has been identified to act as a powerful chemopreventor of reactive oxygen and nitrogen species (Sarkar and Bhaduri, 2001). Treatment of EGCG to human skin inhibited ultraviolet radiation induced oxidative stress (Katiyar *et al.,* 2001).

a) Reactive oxygen species and interaction of biomolecules

Constant oxidative stress / excess production of reactive oxygen species (ROS) has been linked to the development of chronic diseases such as cancer,

cardiovascular diseases, cataracts (Buring and Hennekens, 1997; Block and Langseth, 1994 and; Marx, 1987) and dementia (Lethem and Orrell, 1997). Where as fiber, vitamins and minerals have been hypothesized as being important, but antioxidants are likely candidates. Derived from plant material and containing numerous biologically active components, tea beverage may also be proposed as a component of a healthy diet (Segal, 1996; Dreosti, 1996; Katiyar *et al*, 1994; Yang and Wang, 1993; Weisburger, 1996). Human cells are constantly exposed to ROS such as superoxide, hydroxyl and peroxyl radicals and hydrogen peroxide. These ROS are mostly produced from endogenous sources, such as electron transport chains, peroxisomes and the cytochrome p-450 system. Other ROS and free radicals are generated by the immune response, for example, macrophages or as a result of smoking, air pollution, or the influence of UV light. Chronic exposure to ROS can damage DNA, membrane lipids, lipoproteins, and functional and structural proteins (Halliwell, 1997 and; Sohal and Weindruch 1996).

b) Defence mechanism

The human body has evolved antioxidant defence mechanisms to minimise the potential for radical damage (Halliwell *et al*, 1995). The extremely reactive hydroxy radical is particularly harmful with a near diffusion controlled rate constant of 10^9 to 10^{10} M^{-1} S^{-1} enabling it to combine with virtually all molecules in living system (Anbar and Neta 1967). Intracellular antioxidant enzymes function as a first line of defense to neutralize ROS. The primary reaction is disputation of superoxide radicals to hydrogen peroxide that is

catalysed by catalase and glutathione peroxidase (Sies, 1993). The endogenous enzymatic defenses against oxidative damage are not completely efficient (Sohal and Weindruch, 1996 and Jacob and Burri, 1996) and a range of endogenous and exogenous free radical scavenging antioxidants act as a second line of defense. Vitamin E, Vitamin C, and the carotenoids are well-recognised antioxidant nutrients (Sies and Stahl, 1995; Olson, 1993; Frei, 1994), but fruits and vegetables also contain flavonoids and other phytochemicals that are potent antioxidants (Namiki, 1990; Okuda, 1993; Namiki *et al*, 1993; Pratt 1992), and may contribute to defenses against oxidative damage.

c) Antioxidant moieties of tea

All tea beverages are rich in flavonoids and their derived compounds particularly catechins, flavonols, theaflavins and thearubigins, which can scavange ROS and free radicals (Graham, 1992; Salah *et al,* 1995; Jovanovic *et al*, 1995). Flavonoids effectively stabilize free electrons through several proposed mechanisms, including delocalisation of electrons, formation of intramolecular hydrogen bonds (Van Acker *et al*, 1996a) and rearrangement of their molecular structure (Jovanonic *et al*, 1995 and Van Acker *et al*, 1996b). Free copper and iron, which may catalyze formation of ROS *in vivo* (Halliwell, 1997) and are used to generate free radicals in some test systems, are chelated by flavonoids (Miller *et al* 1996; Morel *et al*, 1993).

The antioxidant activity of flavonoids may be an important attribute of their proposed beneficial health properties. Tea beverages are major contributes to

the total daily flavonoid intake and elucidation of their antioxidant potential is therefore of interest. The iron-chelating ability of catechins has been ranked as epigallocatechin (EGC) > epicatechin gallate (ECG) = epigallocatechin gallate (EGCG) > epicatechin (EC) (Guo *et al*, 1996).

1.10.1.1. R.O.S. Scavenging

Tea, in particular its flavonoids consistently demonstrate strong *in vitro* scavenging ability against numerous physiologically significant ROS. Black and green teas demonstrate strong antioxidant capacity against both peroxyl and hydroxyl radicals in the oxygen radical absorbing capacity (ORAC) assay. This assay determines the ability of an antioxidant to prevent oxidation of β-phycoerythrin by ROS relative to Trolox, the water soluble form of vitamin-E. A cup of black or green tea was over three times more effective than a serving of most common vegetables (Cao *et al*, 1996) and teas were over two times more effective than a serving of most other common fruits (Wang *et al*, 1986).

a) Scavenging of superoxide radical

Extracts of green and black teas were efficient scavengers of superoxide and hydrogen peroxide. Epicatechin 3-O-gallate (ECG) and epigallotechin 3-O-gallate (EGCG), the main catechins in teas, were good scavengers of the superoxide radical generated by the hypoxanthine -xanthine oxidase system using electron spin resonance (ESR) spectrometry with 5,5-dimethyl -1-pyrroline -1-oxide (DMPO) as a spin-trapping agent (Uchida *et al*, 1987). These data were

confirmed by another study showing that EGCG is a highly efficient scavenger of superoxide radical generated by pulse radiolysis, reacting with a rate constant of 7.2 X IO^5 M^{-1} S^{-1} (Jovanovic *et al*, 1995). The reactivity of EGCG was found to be twice that of either EGC or ECG, possibly due to the presence of two antioxidant gallate moities in the EGCG molecule. However, EGC and EGCG were found to have comparable superoxide scavenging reactivities in a hypoxanthine-xanthine oxidase system (Hatano *et al*, 1989). EGCG was the most potent radical scavenging catechin, and the catechins as a group were more potent than vitamin C and vitamin E (Zhao *et al*, 1989).

Theaflavins were shown to be even better scavengers of the superoxide radical than the gallocatechins. In the case of the theaflavins, gallate substitution appeared to lower the rate of reactivity (Jovanovic *et al*, 1997).

b) Scavenging of singlet oxygen

Catechin was the most efficient quencher of singlet oxygen when a range of flavonoids were compared (Tournaire *et al*, 1993). The rate of quenching by catechin was almost 4 times greater than that of the flavonol quercetin, which generally displays a higher free radical scavenging potency (Paganga *et al*, 1996).

c) Scavenging of hydroxyl radical

The catechins were ranked depending on their ability to protect mitochondrial membranes from oxidation induced by hydroxyl radicals as ECG > EGCG > EC > gallic acid (GA) > gallocatechin (GC) > EGC > C (Hong *et al*,

1994). Generation of the DMPO- OH (5, 5-dimethyl-1-pyrroline-1-oxide-hydroxyl) spin adduct by photolysis of H_2O_2 was inhibited by catechins with hydroxyl radical scavenging activity ranked as ECG > EC > EGCG >> EGC (Guo *et al*, 1996).

d) Scavenging of peroxyl radical

Catechins and quercetin were 5 to 20 times more effective than tocopherol in protecting phosphatidylcholine liposomes from oxidation by peroxyl radicals induced in the aqueous phase by 2, 2-azobis (2-amidinopropane) hydrochloride (AAPH) (Terao *et al*, 1994).

Theaflavins were more effective than vitamin E in protecting erythrocyte membrane lipids from oxidation (TBARS -Thiobarbutyric acid reactive substances, formation) induced by tert-butylhydroxyperoxide (Shiraki *et al*, 1994). The theaflavin inhibited lipid peroxidaion by up to 80%, while an equimolar concentration of vitamin E inhibited formation of TBARS by only 30%. Theaflavin digallate exhibited the strongest antioxidative activity in this system. In a similar system using *tert*-butyl-hydroperoxide induced oxidation of rat liver homogenates, both theaflavin and thearubigin fractions purified from black tea infusions inhibited the formation of TBARS more effectively than glutathione, ascorbic acid (vitamin C), α-tocopherol, and synthetic phenolic antioxidants (Yoshino *et al*, 1994), although ECG, EGC and EGCG were more active. The activity of lyophilized black and green tea was similar.

Catechins are able to effectively inhibit *in vitro* peroxynitrite-induced tyrosine nitration (Pannala *et al*, 1997). Peroxynitrite is a reactive species

generated invivo (eg. during inflammatory responses) and has been shown capable of oxidising LDL to more athrogenic forms (Graham *et al*, 1993). EGCG, ECG and GA were the most efficient scavengers of peroxynitrite (Pannala *et al*, 1997). In a murine macrophage system mimicking inflammation, EGCG reduced lipopolysaccharide and interferon-γ-induced nitrite production (Chan *et al*, 1995). EGCG also effectively scavenged hypochlorite, a product of the enzyme mycloperoxidase that is formed in response to bacterial infection (Sakagami *et al*. 1995).

e) Scavenging of stable free radical

Tea flavonoids are effective scavengers of the stable free radical of ABTS (2, 2' azino bis-[3-ethylbenzthiazoline-6-sulfonic acid] radical cation) in the aqueous phase (Salah *et al*, 1995; Miller *et al* 1996; Rice-Evans *et al* 1996). Black tea and green tea beverages both have a potent antioxidant capacity relative to Trolox (Rice-Evans *et al* 1996). Antioxidants from the tea are significantly more potent radical scavengers in this system than the well recognised antioxidants vitamin E and vitamin C, the carotenes and xanthophylls, presented in Table 1.18. The epicatechins displayed 2.4 to 4.9 times the antioxidant activity of Trolox (Miller *et al* 1996).

In the lypophilic phase, tea flavonoids scavenge the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) radical more effectively than vitamin E (Table 1.19) (Hatano *et al*, 1989; Hong *et al* 1994; Yoshida *et al* 1989; Nanjo *et al*, 1996; Fourneau *et al*, 1996). EGCG is the most effective scavenger of the DPPH radical among other tea flavonoids.

In conclusion, theaflavins and catechin gallates are more effective *in vitro* scavengers of aqueous and lipophilic stable radicals than many other flavonoids and the antoxidant vitamins.

f) Protection of low density lipoprotein from oxidation

Results from *in vitro* studies, using copper, metmyoglobin, or cells as prooxidants show that tea flavonoids (catechins and theaflavins) effectively scavange aqueous and lipophilic radicals and protect LDL from oxidation more effectively than established antioxidants. The gallocatechins were consistently the most protective flavonoids. Gallate substitution of theaflavin enhanced LDL protective activity. The inhibition mechanism of the tea flavonoids did not appear to be dependent on metal ion chelation properties. High LDL *in vitro* plasma concentrations of tea are necessary to recover small amounts of bound tea flavonoids from isolated LDL. The details of prevention of *in vitro* LDL oxidation by tea antioxidants are presented in Table 1.20.

1.10.1.2. *In vivo* antioxidant capacity and protection

Biomarkers of oxidative damage such as oxidized deoxynucleosides [8-hydroxydeoxyguanosine (8-HDG)], plasma and urinary thiobarbutaric acid reactive substances (TBARS) or malondialdehyde (MDA) values, micro-nuclei formation, sister chromatid exchange, F_2-iso-prostanes, and LDL oxidation ex vivo are often employed as surrogates for in vivo antioxidant activity. In biomarker studies, oxidative stress is often induced by radiation, chemical oxidants, tobacco smoke, or diet.

Table 1.18.: Relative antioxidant potentials of vitamins, tea beverage, flavonoids, carotenes, and xanthophylls (Miller *et al*., 1996a.)

Antioxidant	Trolox equivalent antioxidant Capacity (TEAC, mM)[a]
Vitamins	
Vitamin C	1.0 ± 0.02
Vitamin E	1.0 ± 0.03
Tea beverage	
Green tea (1000 ppm)	3.8 ± 0.03
Black tea (1000 ppm)	3.5 ± 0.05
Flavan 3-ols	
Epocatechin	2.5 ± 0.02
Epigallocatechin	3.8 ± 0.06
Epogallpcatechin gallaate	4.8 ± 0.06
Epicatechin gallate	4.9 ± 0.02
Theaflavins	
Theaflavin	2.9 ± 0.08
Theaflavin 3-monogallate	4.7 ± 0.16
Theaflavin 3'-monogallate	4.8 ± 0.19
Theaflavin 3-3'-digallate	6.2 ± 0.43
Flavonols	
Quercetin	4.7 ± 0.10
Kaempferol	1.3 ± 0.08
Rutin	2.4 ± 0.06
Flavones	
Apigenin	1.5 ± 0.08
Luteolin	2.1 ± 0.05

[a] TEAC is the millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to a 1.0 mM solution of the substance under investigation.

Table 1.19.: Scavenging of stable free radicals by tea antioxidants

Stable radical	Ploar/ apolar	Detection Mode	Relative scavenging Capacity	Reference
Fermy's salt	Polar	ESR	EGCG>ECG>EC>EGC>C>GA	Gardner *et al*.1998
Fermy's salt	Polar	ESR	Green tea > black tea	
Galvinoxy	Apolar	ESR	EGCG>ECG>GA>EC=C>EGC	
Galvinoxy	Apolar	ESR	Green tea > black tea	
ABTS $^{\bullet+}$	Polar	abs 734 nm	TF-dg > TF-mg > TF	Miller *et al*. 1996
ABTS $^{\bullet+}$	Polar	abs 734 nm	ECG>EGCG(Q>EGC>GA>EC(C	Salah *et al*. 1995
DPPH$^{\bullet}$	Apolar	ESR	EGCG=ECG>EGC>EC(C>>vitC>vitE)	Nanjo *et al*. 1996
DPPH$^{\bullet}$	Apolar	abs 521 nm	EGCG≈ECG(GA>EC≈C≈rutin>vit E	Fourneau *et al*. 1996
DPPH$^{\bullet}$	Apolar	abs 517 nm	Pouchong tea>green tea>oolong tea>black tea	Yen and Chen 1995
DPPH$^{\bullet}$	Apolar	abs 517 nm	EGCG>ECG>GC>EC>GA>EGC>vitC>Trolox	Hong *et al*. 1994
DPPH$^{\bullet}$	Apolar	abs 520 nm	ECG>EGCG>EGC>GA>EC>C> vitC ≈ vitE	Yoshida *et al*. 1989
DPPH$^{\bullet}$	Apolar	ESR	ECG(tet)>ECG(tri)>ECG(di)≈ EGCG> EGC>EC> vit. C > vit. E	Hatano *et al*. 1989
DPPH$^{\bullet}$	Apolar	ESR	ECG>EGCG	Uchida *et al*., 1987

Note: ABTS $^{\bullet+}$, 2,2′ azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) radical cation; DPPH$^{\bullet}$, 1,1-diphenyl-2-picrylhydrazyl radical; TF-dg, theaflavin digallate; TF-mg, theaflavin monogalate; TF-theaflavin; EGCG,epigallocatechin gallate; EGC, epigallocatechin; ECG, epicatechin gallate; EC, epicatechin; C, catechin; GA, gallic acid; Q, quercetin.

Table 1.20.: Prevention of *in vitro* LDL oxidation by tea antioxidants[a]

Pro-oxidant	Detected parameters	Relative scavenging capacity	Reference
Cu^{2+}	Conjugated dienes (lag phase)	TF-dg>EGCG>TF-mg>ECG>TF> EGC>EC [a]	Ishikawa *et al*. 1997
Cu^{2+}	Conjugated dienes (lag phase)	EGCG> vit E [a]	Ishikawa *et al*. 1997
Cu^{2+}	Conjugated dienes (lag phase)	GT(50mg/ml)>BT(50mg/ml)[a]	Van het Hof *et al*.1997
Cu^{2+}	TBARS	EGC and EC inhibitory in initiation phase, accelerative in propagation phase	Yamanaka *et al*. 1997
Cu^{2+}	Conjugated dienes (lag phase)	GT(vit E > vit C)	Luo *et al*. 1997
Cu^{2+}	TBARS	TF-dg >TF-mg >TF	Miller *et al*. 1996
Cu^{2+}	Conjugated dienes (lag phase)	EGCG> ECG >quercetin >chloro-genic acid > resveratrol > rutin> vitE > hesperetin > genistein	Vinson *et al*. 1995
Cu^{2+}	Conjugated dienes (lag phase)	Sesaminol >Q >EGCG>TF>> BHT >α-tocopherol	Miura *et al*. 1995
Cu^{2+}	apo B fragmentation	EGCG>BHT >α-tocopherol	Miura *et al*. 1995
Cu^{2+}	Conjugated dienes (lag phase)	EGCG>ECG>EC>C>EGC	Miura *et al*. 1994
Cu^{2+}	TBARS	ECG>EGCG>EC>C>EGC>BHT	Miura *et al*. 1994
Cu^{2+}	apo B fragmentation	EGCG>CG	Miura *et al*. 1994
Cu^{2+}	TBARS	Flavanols most effective flavanoids	Vinson *et al*. 1995
Cu^{2+}	TBARS	EGCG>EGC>EC>C>BHT> Vit.C>Vit.E>β-carotene	Vinson *et al*. 1995
Cu^{2+}	TBARS	(+)-C complete inhibition at 20μg/ml	Mangiapane *et al*. 1992
Peroxynitrile	REM	ECG>GA>EC$\approx$EGC$\approx$EGCG	Pannala *et al*. 1997
Metmyoglobin	TBARS/REM	EGCG=ECG$\approx$EC=C>EGC>GA	Salah *et al*. 1995
Macrophages	TBARS	GT polyphenols inhibited TBARs formation	Zenhua *et al*.1991
Macrophages	LDL uptake by scavenger receptor	GT polyphenols inhibited LDL uptake by scavenger receptors	Zenhua *et al*.1991
Macrophages	apo B fluorescence/REM	GT polyphenols inhibited apo B fragmentation	Zenhua *et al*.1991
J774 macrophages*	TBARS	Inhibition by (+)-C at 50mg/ml	Mangiapane *et al*. 1992

Source : Ishikawa et al., 1997, and Miller *et al*., 1996.

Note:TF-dg, theaflavin digallate; TF-mg, theaflavin monogallate; TF-theaflavin; EGCG, epigallocatechin gallate; EGC, epigallocatechin; ECG, epicatechin gallate; EC, epicatechin; C, catechin; GA, gallic acid; GT/BT, green/ black tea; Q, quercetin; BHT, butylated hydroxytoluene; TBARS, thiobarbituric acid reactive substances; REM, relative electrophoretic mobility.

[a] Antioxidants preincubated with plasma prior to LDL isolation.

macrophages***:** vascular endothelial cells, human monocyte-macrophages

(i) Studies on animal models

Tea flavonoids consistently reduced oxidative damage in animals exposed to radiation, chemical oxidants, or dietary stress. Mice fed with several different flavonoids were gamma-irradiated to create hydroxyl radical-induced DNA damage *in vivo*. Treatment with hydroxylated flavan-3-ols and flavonols significantly reduced the formation of micronucleated reticulocyctes, a marker of DNA damage (Shimoi *et al*, 1994). EGCG provided to mice in drinking water for one month duration, significantly blocked radiation-induced oxidative damage to hepatic lipids and improved thirty days survival time after irraditon by 33% (Uchida *et al*, 1992). *Ex vivo* lipid peroxidation determined in rat liver was reduced following feeding of diets containing 3% (w/w) black or green tea for 50 days (Sano *et al*, 1995).

(ii) Studies on human

In a four week intervention trial in non-smokers, consumption of green or black tea (six cups per day) had no effect on plasma levels of malondialdehyde, LDL (Low Density Lipoprotein) lipid hydroperoxide levels, or plasma levels of antioxidant vitamins (ascorbic acid, α-tocopherol, or carotenoids), (Van het Hof *et al*, 1997). However, green tea consumption did result in a small but significant increase in the total antioxidant activity of plasma.

Tobacco smoke contains many ROS that can cause oxidative tissue damage in smokers (Lykkesfeldt, *et al*, 1997). Tea drinking has been shown to reduce oxidative biomarkers in chronic smokers, such as increased sister

chromatid exchange (SCE) and micronucleation in lymphocytes (Shim *et al*, 1995 and Xue *et al*, 1992).

In a case-controlled population study, tea drinking among smokers was associated with a significantly lower level of smoking- induced micronuclei in peripheral-blood lymphocytes (Shim *et al*, 1995). It is evident from the studies that tea beverage is potentially a good dietary source of non-nutrient antioxidants, but needs more studies.

Proteins in the diet may theoretically affect the absorption of tea flavonoids, but the addition of milk to black or green tea does not affect the concentration of quercetin or catechins in plasma. The ability of flavonoids to form insoluble complexes with iron reduces the bioavailability of non-heme iron when tea is combined with a meal.

1.10.2. Tea and cancer

Inhibitory effects have been shown for variety of cancers, including esophagal, gastrointestinal, lung, skin, and other tumors, with blocking of the effects of number of chemical and physical carcinogens. The mechanisms of inhibition are not known, but tea may reduce the metabolism of compounds to known carcinogens and or enhance their detoxification once formed (Dreosti *et al*, 1997). Consumption of tea on a regular basis has been associated with a reduced risk of cancer in human populations, in parts of Asia. How tea prevents cancer by studies in animal carcinogenesis models have been reviewed in detail (Dreosti *et al*, 1997). Evidences suggested that tea may function in multiple ways: as a modulator of carcinogen metabolism, as an antioxidant protecting

DNA from oxidative damage, and as an antiproliferative agent. Animal studies pioneer the development of biomarkers, and that the tea has pronounced effect on these markers, encourages their utility in human studies.

1.10.3. Tea and other bioactivities

a. Modulation of immune function

Tea drinking has been associated with increased cell-mediated immune function (Zvetkova *et al,* 2001). Black tea catechin suppresses the expression of high affinity IgE receptor FC-E RI in human basophillic KU 812 cells (Fujimura *et al*, 2001). Increase in both number and activity of lymphocytes including NK cells has been reported (Lin *et al*, 1999). Tea also possesses the property to stimulate the formation of interleukin 2, which has important immuno-regulatory roles (Zvetkova *et al,* 2001).

b. Anti-inflammatory effect

Application of black tea polyphenols resulted in significant inhibition of TPA (12-O-tetradecanoylphorbol-13-acetate) caused inflammatory responses and suppression of epidermal ornithine decarboxylase (ODC) and of cyclooxygenase (COX) enzyme activities (Katiyar and Mukhtar, 1997). Nitric oxide (NO) radical has a wide biological role in modulating physiological and patho-physiological processes (Moncada *et al,* 1992). During infection and inflammation, the formation of NO is increased, which might promote neoplastic conversion. Tea polyphenols regulate cyclooxygenase and lipoxygenase dependent metabolism of arachidonic acid in human colon mucosa and colon tumor tissues (Hong *et al*,

2001). Both EGCG and TF-3 inhibited the induction of NO in thioglycollate-elicited and lipopoly-saccharide (LPS)-activated peritoneal or RAW2647 macrophages (Lin and Lin, 1997; Chan *et al*, 1997; Lin *et al*, 1999b). EGCG decreased the activity and protein levels of inducible nitric oxide synthase (iNOS) by reducing the expression of iNOS m RNA and that the reduction could occur as a result of prevention of binding of the nuclear factor-kB to the iNOS promoter, thereby inhibiting the induction of iNOS transcription (Lin and Lin, 1997). The phenolic components EGCG and EGC of tea have also been found to regulate the production of pro- and anti-inflammatory cytokines by human leucocytes *in vitro* (Crouvezier *et al*, 2001).

c. Germicidal and antiviral activity

Investigation had proved that tea exhibits germicidal and antiviral activity (Toda *et al*, 1989). Crude catechins, gallated catechins and TF's, TFDG, TF monogallate A and B inhibited the growth of both spore and vegetative cells of C. *botulinum.* ECG and EGCG possessed the inhibitory activity against hemolysin excreted by *Vibro cholera*, the toxin excreted by *Staphylococcus aureus* bacteria and damage to CHO cells included by *Cholera toxin* (Toda *et al*, 1990). Tea polyphenols have been found to retard the infectivity of influenza virus (Nakayama *et al,* 1993). Besides this EGCG has been found to exhibit inhibitory effects on the life cycle of human immunodeficiency virus type1 [HIV-1] (Yamaguchi *et al*, 2002). Black tea extract, thearubigin fraction, counteracts and protects against the adverse effect of tetanus toxin in mice (Satoh *et al,* 2001).

Recent studies indicate that epicatechin gallate in tea may be a valuable therapeutic agent against *H*. *pylori* infection (Yanagawa *et al*, 2003).

d. Regulation of intestinal microflora

Extracts of black tea, Japanese green tea and Chinese tea are known to inhibit the growth of various bacteria causing diarrhoeal diseases (Shetty *et al*, 1994). Tea polyphenols exhibits protection against intestinal disorders as shown by the influence of tea on propagation of *Bifidobacterium* and *Clostridium* (Okubo *et al*, 1991). All tea samples tested showed protective activity against diarrohoea causing bacteria including *Staphylococcus aureus, S.epidermidis, Vibrio cholerae 01, V. cholerae non 01, V. Parahaemolyticus, V. mimicus, Campylobacter jejuni and Plesiomonas shigelloides* (Toda *et al*, 1990).

e. Prevention of dental caries

Tea drinking has been associated with decreased incidence of dental caries (Rasheed and Haider, 1998; Hamilton-Miller, 2001). Experimental studies on rats revealed that black tea administration could prevent and reduce caries, lesions by inhibition of adherence of the pathogenic bacteria on teeth surface and thus exhibiting antiplaque activity (Touyz and Amsel, 2001). Investigations had showed that TF, theaflavin digallate, ECG, GCG and EGCG strongly inhibited water-soluble and insoluble glucan synthesis by glucotransferase enzyme excreted by plaque causing bacteria (Hattori *et al*, 1990).

f. Antimutagenic activity

(i). Microbial system

Antimutagenic activity of tea polyphenols in microbial systems has been extensively investigated. EGCG and ECG showed inhibitory effects against the mutagenicity of MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) in *Salmonella typhimurium* TA 98 and TA 100 with and without rat liver S9 mix (Okuda *et al*, 1984). EGCG also had a strong inhibitory effect against the mutagenicity of BaP diol expoxide in TA 100 strain without S9 mix. Theaflavins, gallate esters and catechins inhibited mutagenicity of PhIP in *Salmonella typhimurium* TA 98 (Apostolides *et al*, 1997). The gallate esters of the catechins EC, ECG and EGCG, theaflavonoids TF, TFMG and TFDG and glucose (tannic acid) had low IC_{50} in the 80-250 μM range against mutagenicity of 10 μM PhIP. Non-polyphenolic fraction of green tea suppressed 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) or mitomycin C (MMC) induced umu C gene expression in *Salmonella typhimurium* TA 1535 / psk 1002 in the presence or absence of S9 metabolizing enzyme mixture (Okai and Higashi-Okai,1997). Antimutagenic activity of green and black tea extracts was also observed towards food mutagen MeIQx in the direct plate assay with *Salmonella typhimurium* in an *in vitro* gastrointestinal model (Krul *et al*, 2001).

(ii). Mammalian cell systems

Crude tea extracts decreased the mutagenic activity of N-methyl-N'-nitro-N-nitrosoguanidine *in vitro* and in intragastric tract of rats (Jain *et al*, 1989). Black and green tea imparted protection against 2-amino-3-methylimidazo[4,5-f]

quinoline-induced DNA adducts and colonic aberrant crypts in the F344 rat (Xu *et al*, 1996). Anticlastogenic effect of black tea and its two active polyphenols theaflavins and thearubigins was identified towards cyclophosphamide (CP) and dimethylbenz(a)anthracene (DMBA) in sister chromatid exchanges (SCE) and chromosomal aberration assay *in vivo* in Swiss albino mice (Gupta *et al*, 2001). Protective effect of green tea has been observed against BaP induced mutations in the liver of big Blue mice (Jiang *et al,* 2001). Tea and its polyphenols inhibited mitomycin induced micronuclei induction in V79 cells (Liu *et al*, 1998). Green or black variety of tea, EGCG, and TFG sharply reduced that mutagenicity of 2-amino-3-methylimidazol [4-5-f] quinoline (IQ) and PhIP, and induced DNA repair in rat hepatocytes (Weisburger *et al*, 1996). ECG and EGCG had inhibitory effects against 6TG-resistant mutations induced by 4NQO in cultured Chinese hamster V79 cells (Kuroda, 1996).

1.11. PRESENT STUDIES

Tea is one of the most important plantation crops and it is very vital to know the physico-chemical and biochemical characteristics of tea which will serve as a quality fingerprint. Tea is grown in a wide range of climate, altitude and soil conditions. Considering the number of variables, it is very vital to have authentic scientific information on the tea which may be called as holistic quality fingerprint of tea. The data on the chemical, physical and biochemical constituents and variations in them will greatly help in getting a wealth of information (knowledge base) that can be termed as holistic quality finger print of tea. It was envisaged

to have such a fingerprint on Indian teas. The importance of fingerprint of Indian teas has been realized by both the industry and researchers for quite sometime. This work reports the results of investigations carried out under this programme.

In this report data on fingerprinting of Indian Teas from different regions of India are presented which includes important physico-chemical and biochemical features of teas from different regions of India. Besides the novel processing approach for utilization, bioactivity evaluation and application of the active conserve derived from otherwise wasted green tea leaf resource (either as coarse or pruned tea leaves) is also presented.

Objectives

1. To study the bio-chemical moieties in tea and their role in quality and activity.
2. Physico-chemical analysis (fingerprint) and processes for green tea and separation of catechin enriched radical scavenging conserve from unused green tea leaves.
3. Analysis for phenolic constituents and volatile flavour compounds (VFC) of tea.
4. Application of the catechin enriched radical scavenging conserve (functional ingredients) in foods.

Scope

- The changes in climate, altitude, the location, the processing and several other factors will have significant influences on the quality of tea.
- Use of sophisticated techniques for chemical and biochemical analysis to determine the changes in flavour profile at molecular levels in order to get data in defining the intrinsic quality of tea to serve as its fingerprint.
- The composition of bio-chemical constituents responsible for strength, taste, colour and flavour will be elucidated so as to generate a fingerprint of Indian teas.
- The novel process for green tea, catechin rich conserve and application for health promotion by incorporating in foods.

1.12. Bibliography

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CHAPTER 2

PROFILING OF INDIAN BLACK TEAS

2.1. BACKGROUND

Tea is one of the most important plantation crops and it is very vital to know the physico-chemical and biochemical characteristics of tea which will serve as a fingerprint. Tea is grown in a wide range of climate, altitude and soil conditions. Considering the number of variables, it is very vital to have authentic scientific information on the tea aptly called as fingerprinting of tea. The data on the chemical, physical and biochemical constituents and variations in them will greatly help in getting a wealth of information (knowledge) that can be termed as finger print of tea. It was proposed to have such a fingerprinting on Indian black teas. The importance of fingerprinting of Indian black teas was realized and work was carried out in this direction. This chapter describes the results of investigations carried out under this area.

India is a leading tea producer, consumer and exporter with 928 million Kgs production during 2005. The world productin of Crush - Tear - Curl (CTC) tea was 1194 million Kgs, and orthodox tea production was 839 million Kgs during 1999 (Ramadurai, 2000). Black tea, manufactured from young tender shoots of *Camellia sinensis* (L.) O. Kuntze is the most widely enjoyed non-alcoholic drink and its flavour quality and taste has been shown to change with variations in geographical (Yamanishi et al., 1968; Takeo and Mahanta, 1983) and climatic (Cloughley *et al* 1982; Howard 1978) conditions. Yamanishi et al. (1968) compared flavour of teas from different parts of the world, while Cloughley *et al* (1982) compared flavour of teas in different season in Malawi, Africa. Indian teas, especially from Darjeeling, Assam and

Nilgiris (Trinitea) are valued for their characteristic aroma and taste. These teas are much sought after and are relished by the beverage consumers throughout the world (Bala Subramaniam, 1995). Number of reports are available about antioxidant, anticancer properties and health benefits of tea (Wiseman, *et al,* 1997; Dreosti, *et al,* 1997; Yang and Wang, 1993; Tijburg, *et al,* 1997; Yang, *et al,* 1998).

The important chemical constituents which influence the taste and flavour in tea brew are polyphenols, caffeine, sugars, organic acids, volatile flavour compounds and amino acids. Phenolic compounds of tea such as theaflavins and thearubigins are very important from intrinsic quality point of view. These are responsible for the colour, flavour and brightness of tea. Caffeine is responsible for the briskness. The physico-chemical parameters such as TSS and viscosity of the brew and bulk density (packed/loose) of the tea are important quality indicators. The volatile flavour compounds of tea and their variation in composition due to geographical and other process variables is of paramount importance from the quality point of view. Though considerable work has been done (Gulati, *et al,* 1999; Gulati and Ravindranath, 1996 and Ullah, 1985) on the quality aspects of tea a comparative study covering Indian regions has not been done so far. Keeping in view the complexity of the intrinsic tea quality a study has been carried out to fingerprint the Indian black teas obtained from different regions. The data generated based on the studies will help to trace back the origin of the teas.

The standards prescribed for tea by different standards organizations such as ISO, BIS and PFA are the purity standards. These standards do not

provide information about the intrinsic quality. The intrinsic quality of tea depends on number of variable and non-variable factors. Some of the factors can be controlled. With the result the intrinsic qualities of tea vary considerably.

India grows her tea from the 38700 gardens approximately spread in South, North and North-East States comprising of several regions of variable climate, soil, rainfall, altitude and latitude. This obviously has an effect on the intrinsic quality of tea. The manufacturing practices and methods further add up to the variations in the quality of tea. Though, the literature on intrinsic qualities of tea is available the information is scattered and is unorganized. Besides, there are no systematic investigations reported on fingerprinting of Indian black teas.

This chapter reports the data on fingerprinting of Indian Black Teas from various regions of India in different seasons, which includes the analyses of physico-chemical parameters as well as biochemical components viz., Physico-chemical characteristics such as moisture, viscosity, total soluble solids, bulk density, caffeine, phenolic constituents (i.e., theaflavins and thearubigins) and volatile flavour compounds (VFC) of tea.

Roberts and Smith (1963) have described a method for determination of polyphenolic oxidation products in black tea liquors for the assessment of quality in tea. Biswas and Biswas (1971) has also reported about the briskness of liquor and intrinsic quality evaluation. Further, Biswas et al (1973) reported that theaflavin is associated with all the liquor characters, i.e.,

colour, brightness, strength, briskness and quality. The thearubigins mostly contribute to the body of the liquor. Ullah (1984) has critically discussed some of the intrinsic quality parameters and liquor characters of black tea. Mahanta (1988) had reviewed the chemical basis of liquor characteristics based on the influence of pigments and processing conditions of black tea manufacture. Owuor and Obanda (1998) have critically discussed the changes in quality of the South African black teas.

2.2. SCOPE

- The changes in climate, altitude, the location, the processing, packaging and several other factors will have significant influences on the quality of tea.

- Use of sophisticated techniques for chemical and biochemical analysis to determine the changes in flavour profile at molecular levels in order to get data in defining the intrinsic quality of tea to serve as its fingerprint.

- The composite of biochemical constituents responsible for strength, taste, colour and flavour will be elucidated so as to generate a fingerprint of Indian black teas.

2.3. MATERIALS AND METHODS

2.3.1. Materials

In India, tea cultivation is spread over the hilly and plain areas of North, South and North-East. Tea is also grown in Kangra valley, Kumaon Hills and Ranchi. In South India, tea is cultivated in Western Ghats of Kerala, Nilgiris of Tamilnadu and Karnataka. The important tea growing regions of South India are:

1. Nilgiris
2. Nilgiris-Waynad-Mysore
3. Anamallais
4. High range
5. Central Travancore
6. South Travancore

The Main three tea growing regions in North and North-East India are:

1. Darjeeling and Dooars (Darjeeling)
2. Brahmaputra valley (Assam)
3. Surma valley (Palampur)

Collection of Samples

Samples were selected cutting across all indian tea producing regions and parts of the season. The regions, parts of season and gardens were so selected as to represent a range of climatic, topographical, agronomical, processing and management factors. Samples of tea from the following Regions (gardens) spread over four seasons (1.April-June; 2.July-

September; 3.October-December; 4.January-March) of the year were collected.

1. Parajulie (Tamilnadu)
2. Pandiar (Tamilnadu)
3. AFTL (Assam - A AFTL)
4. Magor (Assam - A Mag)
5. Darjeeling premium (DP)
6. Darjeeling medium (DM)
7. Chinchula (Dooars)
8. Aibheel (Dooars)
9. Nilgiris (Tamilnadu)
10. Cachar best (Assam)
11. Cachar medium (Assam)
12. Palampur G_1 (PG_1) – (Surma valley)
13. Palampur G_2 (PG_2) – (Surma valley)
14. Assam BOP
15. Assam OP
16. Nilgiris high grown (Nil Hg) - (Tamilnadu)
17. Nilgiris high grown CTC-BOP (Nil Hg CTC) - (Tamilnadu)
18. Nilgiris Waynad - (Tamilnadu)
19. Annamalai - (Tamilnadu)
20. Rosekandy (Dibrugarh - Assam)
21. Kurti (Darjeeling)

2.3.2. Methods

a. Analysis for physico-chemical characteristics such as :

   i) Moisture

   ii) Viscosity of liquor

   iii) Total soluble solids

   iv) Bulk density

   v) Colour of liquor

   vi) Brightness

b. Quantitative estimation of samples for caffeine, theaflavins, thearubigins, colour and theaflavin digallate

c. Quantitative determination for major volatile flavour compounds such as :

   i) n-Hexanal

   ii) Leaf aldehyde

   iii) Leaf alcohol

   iv) *trans/cis*-2 Hexenol

   v) n-Hexanol

   vi) n-Heptanal

   vii) Benzaldehyde

   viii) Nonanal

   ix) Linalool

   x) Methyl salicylate

   xi) β-ionone, etc.

2.3.2.1. Determination of Moisture

It is the measurement of the quantity of moisture present in the tea sample apart from dry matter present (AOAC, 2000a). The tea sample was ground in a suitable grinder (Maharaja, India) to pass through 30-mesh sieve and mixed thoroughly to get a homogenous sample. A clean aluminium dish dried at 110°C in hot air oven for one hour, cooled and stored in a desiccator. Prepared tea sample weighed using Mettler balance into a suitable (dried and cooled) aluminium dish. Dried to constant weight at 100-106°C (Approximately 6 hrs) in a hot air oven (Lynx, Lawrence and Mayo, India) having accurate temperature control. The loss in weight of sample is noted as moisture and percentage of moisture content calculated on dry weight basis (DB).

2.3.2.2. Determination of Viscosity of Tea Liquor

Measurement of viscosity of tea liquor may provide some basis for the body of the liquor. Viscosity is defined as the measurement of the resistance offered by the test material to the rotating spindle of the viscometer (or) it is defined as resistance to flow. Viscosity measurement is based on measurement of resistance offered to rotation of a spindle immersed in the test material. This instrument can also be classed as a torsion viscometer, since the results are obtained by measuring torque on the rotary part of instrument. The measurement of torque, by a calibrated spring on a spindle with constant speed of rotation in the material is the basis of operation of the above viscometer. The viscosity in milli Pascals can be read directly (AOAC, 2000b).

Tea sample (2 g) was added to a beaker containing boiling distilled water (140 ml). The boiling was continued for 4 mins. Brew filtered using

Whatman No.5 filter paper placed in a Buchner funnel under vacuum. Brew (30 ml) was taken in a beaker and placed under the spindle of the viscometer (Rheology international Ltd., Shennon, Ireland) and by trial and error selected a suitable spindle keeping the RPM constant. Readings were noted in triplicate and average of the readings was taken as final viscosity of the tea brew.

2.3.2.3. Determination of Total Soluble Solids (Hanan *et al,* 2001)

The total soluble solids content which contributes to the characteristic taste and body of a cup of tea apart from other constituents was determined. It is the measure of the soluble solids that dissolve in water upon brewing of the tea (AOAC, 2000c).

Tea sample (2 g) was added to a beaker containing boiling distilled water (140 ml) and the boiling was continued for 4 mins. Brew filtered using Whatman No.5 filter paper in a Buchner funnel under vacuum. Brew was mixed well and a drop of cooled brew was placed on the prism of a pre-calibrated refractometer (Atago,Japan). Illumination and colour compensation were adjusted. Temperature was maintained at 20^{o}C (by circulating water through the provided channel next to the prism). The value for total soluble solids was noted while adjusting the measurement knob. The average value of three readings was taken as TSS (%).

2.3.2.4. Determination of Bulk Density (Ramaswamy, 1995)

Bulk density indicates the weight of substance held in a unit volume. The bulk density of tea varies with grade and method of manufacture. Also this gives idea on cuppage and packaging of tea.

a. Loose Bulk Density

The tea sample was filled upto 500 ml level in a glass cylinder using a hopper suspended 3 cm away from it. Weight of the tea sample noted using a balance. The weight of tea per unit volume (g/ml) is loose bulk density.

b. Compacted or Packed Bulk Density

The tea sample was filled upto 500 ml level in a glass cylinder using a hopper suspended 3 cm away from it. Filled cylinder was tapped five times the cylinder using soft rubber tubing. The weight and volume of tea is noted. Weight of tea per unit volume is the compacted or packed bulk density (g/ml).

2.3.2.5. Estimation of Soluble Caffeine in Tea by HPLC Method (Pura Naik and Nagalakshmi, 1997)

Caffeine is an alkaloid present in tea to the extent of 1-5%. Chemically it is trimethyl xanthine. The amount of water-soluble caffeine in tea brew is less than the total caffeine content of tea.

Reagents: Methanol, Chloroform, (both GR Grade), Acetronitrile (HPLC grade) and double distilled water (glass), standard caffeine. All solvents distilled and filtered through a 0.5 μ filter and de-gassed under vacuum prior to use.

a. Calibration Curve for Standard Caffeine

Standard stock solution was prepared by dissolving 80 mg caffeine (Sigma-Aldrich chemie GMBH, Steinheim, Germany) in 100 ml water. Working standard was prepared by diluting 10 ml of stock solution to 100 ml to give a concentration of 0.08 μg / μl. Working standard solution was analysed on HPLC (Shimadzu LC-6A and system controller SCL-6A,) equipped with the μ - Bondapak C_{18} column (3.9 mm x 15 cm, Waters, Milford,USA), using the mobile phase (Acetonitrile: water = 20:80 v/v) at a flow rate of 1 ml / min. Detection was by a UV-visible spectrophotometric detector (SPD-6AV) set at a sensitivity of 0.08 AUFS and wavelength of 276 nm.

Working standard solution (5-25 μl) was injected on the HPLC after initial equilibrium time (10 min), and peak area responses were obtained. A standard graph for caffeine was prepared by plotting concentration versus area.

b. Sample Preparation:

Black tea (2 g) weighed into a 250 ml beaker and 177 ml of boiling water was added, brewed for 6 min on boiling water bath (80°C) filtered the brew through Whatman No.44 filter paper and 2 ml filtrate was subjected to analysis for caffeine content. A SEP-PAK C_{18} cartridge (Millipore, Waters Associates, Maple Street / Milford, MA, USA) was activated by first passing methanol (2 ml), followed by double distilled water (2 ml) by means of a glass syringe. Tea extract (brew, 2 ml) was then passed through the cartridge and elute was rejected. Air was passed to expel any remaining water. Caffeine

was eluted from the cartridge with 6 ml of chloroform (drop by drop), into an evaporating flask. The chloroform was removed on a water bath under vacuum. The residue in the flask was dissolved in the water and made upto 4 ml. An aliquot (5-10 μl) of this solution was analysed on the HPLC under the earlier conditions (described for standard caffeine solution). The percent caffeine is calculated by comparing the peak area responses with standard caffeine curve (Calibration curve).

C. Clean Up of sep-pak C_{18} Cartridge for Re-use

The used cartridge is cleaned up with 35% (v/v) methanol in water (5 ml) followed by 80% (v/v) methanol in water (4 ml) and finally with 2 ml of methanol for re-use. Cartridge could be re-used 25 times.

2.3.2.6. Spectrophotometric analysis for theaflavins (TFs) and thearubigins (TRs), Total colour and Brightness

Biochemical assessment of black tea quality was done from estimation of TFs and TRs of tea brew. In the present studies, a rapid procedure for estimating theaflavins and thearubigins of black tea was adopted (Ullah, 1986). The absorbances were measured on a UV-visible spectrometer, Cintra 10 (Australia). The tea sample (9 g) added to 375 ml of boiling water in a conical flask and the boiling continued for 10 min using an air condenser on a water bath. The tea infusion was filtered through cotton cloth and cooled to room temperature. The infusion (6 ml) was mixed with 6 ml of 1% (w/v) aqueous solution of anhydrous disodium hydrogen phosphate and the mixture extracted with 10 ml of ethyl acetate by quick repeated inversion for 1 min. The separated bottom layer drained, remaining was the ethyl acetate layer

(the TF fraction) and diluted with 5 ml ethyl acetate. Optical densities, E_1, E_2, E_3; were obtained on extracts prepared as follows:

E_1 - TF extract (10 ml) were diluted to 25 ml with methanol;

E_2 - Infusion (1 ml) diluted to 10 ml with water and made up to 25 ml with methanol;

E_3 - Infusion (1 ml) was mixed with aqueous oxalic acid (10% w/v, 1 ml), and water (8 ml) and made up to 25 ml with methanol;

Optical densities of E_1, E_2, and E_3 were measured at 380 and 460 nm.

At 380 nm

% TF = 2.25 XE_1

% TR = 7.06 (4 E_3 -E_1)

At 460 nm

Total colour = 6.25X 4E_2

% Brightness = E_1 / 4E_2 X 100

2.3.2.7. HPLC Profile of Theaflavins (Bailey *et al*, 1991)

Theaflavins (TFs) and Thearubigins (TRs) are the enzymatic oxidation products of tea flavanols which are formed during manufacture of tea.

Sample Preparation and analysis

Black tea (4 g) was taken in boiling water (100 ml) and infused for 10 min on water bath at 80^{o}C. Brew was filtered through whatman No.5 filter paper using Buchner funnel by applying vacuum and volume was made up to 100 ml with double distilled water and used for HPLC analysis. The sample solution was analysed on HPLC (Shimadzu LC-6A) equipped with the µ-

Bondapak C_{18} column (3.9 mm x 15 cm, Waters, Milford, USA) using the mobile phase [A -1% citric acid solution (pH adjusted to 2.8 using NaOH); B - Acetonitrile; Gradient, linear 8% to 31% B (organic) in A over 50 min] at a flow rate of 1.5 ml / min. Detection was by a photodiode array detector, SPD-M10 AVP at wavelengths 280, 380, 460 nm, software-class 10 (Shimadzu, Kyoto, Japan) communication bus module-CBM-10A.

By comparing the retention time responses with literature values the Theaflavins (TF_s) were evaluated and classified according to Retention times (Bailey *et al*, 1991).

2.3.2.8. Analysis for Volatile Flavour Compounds from Black Tea by GC-MS.

Flavour Compounds (VFCs) are the volatiles from black tea which are responsible for characteristic aroma of the black tea (Group-I and II, VFCs).

a. Sample Preparation

Simultaneous distillation and solvent extraction (SDE), using a Likens–Nickerson apparatus (1964) for isolation of volatiles, was carried out. Black tea sample (50 g) was added to a 2 Liter round-bottom flask containing 1 Liter distilled water, along with 0.2 ml of internal standard (15 μl of cumene in 10 ml of petroleum ether). Into another flask (200 ml), 25 ml of petroleum ether (40–50^{o}C fraction), 0.25 ml of ethanol and 25 ml of diethyl ether with magnetic bit were placed. These two R.B. flasks were attached to two arms of the Likens–Nickerson apparatus and extracted for 2.5 h. After extraction the solvent containing VFCs was evaporated on a water bath to 0.5 ml volume, which was then transferred to a test tube and stored at 4^{o}C for GC–MS analysis.

b. GC-MS analysis for VFCs

A Shimadzu GC-17A equipped with QP-5000 (Quadrupole) mass spectrometer was used. A fused silica capillary column SPB TM-1, coated with polydimethylsiloxane of 30 m length and 0.32 mm internal diameter and film thickness 0.25 μm, was used. Helium was the carrier gas with a flow rate of 1 ml/min. Split ratio was 1:50 and ionisation voltage was 70 eV. The injection port temperature and detector port temperature were maintained at 220°C. Oven temperature programme was: 40°C (3)-2°C/min, 100°C-4°C/ min, 220°C (7); a sample of 1 μl was injected for each analysis. Total ion chromatogram (TIC) for the samples and mass spectrum of each peak are obtained. Identification of compounds was achieved by comparison mass spectra (NIST library and Adams, 2001) and Kovats indices (Davies, 1990; Jennings and Shibamoto, 1980).

$$\text{Kovat's Index} = 100\,N + 100\,n \frac{[\text{Log } t_R(A) - \text{Log } t_R(N)]}{[\text{Log } t_R(N+n) - \text{Log } t_R(N)]}$$

Where:

$t_R(A)$ = Retention Time (RT) of unknown compound peak.

$t_R(N)$ = RT of smaller hydrocarbon eluted before the compound peak.

$t_R(N+n)$ = RT of larger hydrocarbon eluted after the compound peak.

N = Carbon number of smaller standard hydrocarbon.

(N+n) = Carbon number of larger standard hydrocarbon.

$$\text{Amount of Unknown} = \frac{\text{Area Response of Unknown}}{\text{Area Response of Standard}} \times \text{Weight of Standard}$$

2.3.2.8.1. Calculation of terpene index, Mahanta ratio, Yamanishi Botheju- ratio and Borse-Rao quality index

The basic aroma characteristics are probably due to different varieties of tea plants as well as the influence of specific growing conditions. To identify the varietal origin of individual teas, Takeo *et al* (1983a) proposed a terpene index (TI), which is defined as:

$$\text{Terpene Index} = \frac{\text{Linalool + Linalool derivatives}}{\text{Linalool + Linalool derivatives + geraniol}}$$

The terpene index of pure variety sinensis approaches zero whereas the TI of pure var. assamica approaches unity.

A ratio based on the sum of gas chromatographic peak areas of terpenoids to non-terpenoids (Baruah *et al*, 1986 and Mahanta *et al*, 1988), called *Mahanta ratio* was developed. The terpenoids were assumed to be desirable while the non-terpenoids were classified as undesirable to tea aroma and quality.

$$\text{Mahanta ratio} = \frac{\text{Terpenes (desirable)}}{\text{Non-terpenes (Undesirable)}}$$

Yamanishi *et al*., (1989) developed another ratio based on gas chromatographic peak areas of linalool and E-2-hexenal, which ignored all other VFC. This ratio is known as *Yamanishi-Botheju ratio*. The rationale for the use of Yamanishi-Botheju ratio requires that linalool and E-2-hexenal occur in large amounts in all teas and therefore may have a dominant effect.

$$\text{Yamanishi-Boteju ratio} = \frac{\text{Linalool}}{\textit{E}\text{-2-Hexenal}}$$

A new approach in terms of novel quality index for tea has been innovated and proposed through this work. The sum of TF/TR ratios of tea and the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) added together is proposed for the first time as a new and novel quality index, hence forth referred to as Borse-Rao quality index (unpublished data), considered to be an overall quality indicator of tea as both the non-volatiles/volatiles are given due consideration in this quality index.

2.4. RESULTS AND DISCUSSION

Samples were selected cutting across all Indian tea producing regions and four seasons. The regions, seasons and gardens were so selected as to represent a range of climatic, topographical, agronomical, processing and management factors. Samples of tea from the given regions / gardens spread over four seasons of the year (April-June, July- Sept., Oct.- Dec., Jan.- March) were procured, studies were carried out for fingerprinting. The results are presented below in terms of regional and seasonal fingerprint.

2.4.1. Profiling of the tea samples from first season (April- June)

2.4.1.1. Physico-Chemical Constituents

The results of the analyses of the physico-chemical parameters for the black tea samples from Season-1 (April-June) are presented in Table 2.1.

Table 2.1. Season 1 (April- June) - Physico chemical analysis of black tea samples

Sample	Caffeine* (%)	TSS (%)	Viscosity (mPa)	Moisture (%)	Bulk density (g/ml)		TF (%)	TR (%)	Total colour	Brightness (%)
					Loose	Packed				
Darj. Medium	2.28	0.05	11.3	8.27	0.25	0.31	0.25	8.37	2.36	9.03
(±) S.D.	0.04	0.0	0.00	0.05	0.01	0.01	0.02	0.05	0.09	0.02
Darj. Premium	3.36	0.05	11.40	6.47	0.26	0.34	0.24	8.58	1.33	9.90
(±) S.D.	0.03	0.0	0.0	0.06	0.01	0.01	0.03	0.03	0.04	0.05
Parajulie	1.65	0.25	8.6	3.53	0.39	0.44	0.42	13.69	3.85	6.81
(±) S.D.	0.01	0.0	0.0	0.10	0.01	0.01	0.04	0.10	0.09	0.07
Pandiar	2.63	0.15	8.6	4.13	0.37	0.43	0.43	11.91	2.59	12.57
(±)S.D.	0.06	0.0	0.0	0.02	0.01	0.01	0.05	0.08	0.06	0.08
Assam AFTL	4.03	0.1	4.1	6.87	0.38	0.36	1.50	15.06	4.78	24.73
(±)S.D.	0.03	0.0	0.0	0.04	0.0	0.01	0.05	0.06	0.04	0.02
Assam Magor	4.09	0.15	10.8	6.87	0.36	0.39	1.72	13.54	6.09	25.85
(±) S.D.	0.08	0.0	0.0	0.02	0.0	0.01	0.06	0.08	0.02	0.10
Aibheel	2.46	0.25	10.8	5.13	0.38	0.42	1.15	14.78	4.96	21.49
(±) S.D.	0.05	0.0	0.0	0.08	0.01	0.0	0.02	0.02	0.01	0.08
Chinchula	3.00	0.25	12	7.93	0.42	0.45	0.91	14.81	4.77	16.26
(±)S.D.	0.01	0.0	0.0	0.03	0.01	0.01	0.05	0.06	0.02	0.04
Cachar Med	2.58	0.15	7.4	7.07	0.37	0.41	0.69	11.29	3.88	15.08
(±)S.D.	0.08	0.0	0.0	0.02	0.01	0.01	0.03	0.01	0.09	0.09
Cachar Best	3.01	0.25	4.1	5.07	0.35	0.39	0.50	11.63	3.46	12.01
(±)S.D.	0.01	0.0	0.0	0.02	0.01	0.01	0.01	0.04	0.03	0.06
Nil-HG	2.51	0.25	11	6.07	0.31	0.38	0.50	9.37	1.63	16.97
(±) S.D.	0. 01	0.0	0.0	0.10	0.01	0.0	0.03	0.06	0.08	0.07

Values expressed are mean ± S.D. of five experiments; * Soluble caffeine

Moisture content in teas varied from 3.5% to 8.2%. The total soluble solids were determined using Atago refractometer (Method 2.3.2.3). The total soluble solids varied from 0.05 to 0.25%. Teas from Parajulie, Niligiri high grown, Aibheel, Chinchula and Cachar best had highest total soluble solids of 0.25% each. Teas from Pandiar, Assam Magor and Cachar medium had moderate total soluble solids of 0.15% each. Tea from Assam AFTL alone had lower TSS of 0.1%. Teas from Darjeeling (Premium and Medium) had lowest TSS of 0.05% each. It is clear from these results that some of the teas from North and South have good TSS content 0.25% and few have moderate of 0.15%, whereas the Darjeeling teas can be characterized by their lowest TSS of 0.05%.

The soluble caffeine content (Method 2.3.2.5) in the samples varied from 1.65 to 4.09%. Assam AFTL and Assam Magor samples contained highest soluble caffeine 4.03 and 4.09 percent. Darjeeling premium, Chinchula and Cachar best teas also contained high soluble caffeine 3.35, 3.21 and 3.01 percent respectively. Pandiar, Darjeeling medium, Nilgiris high grown, Aibheel and Cachar medium teas had moderate quantities of soluble caffeine 2.63, 2.28, 2.51, 2.46 and 2.58 % respectively. Only teas from Parajulie had the lowest soluble caffeine content of 1.65%. It can be seen that Assam teas have the highest caffeine content and so the briskness. Also the best teas or quality teas had 2 to 3 % of soluble caffeine content.

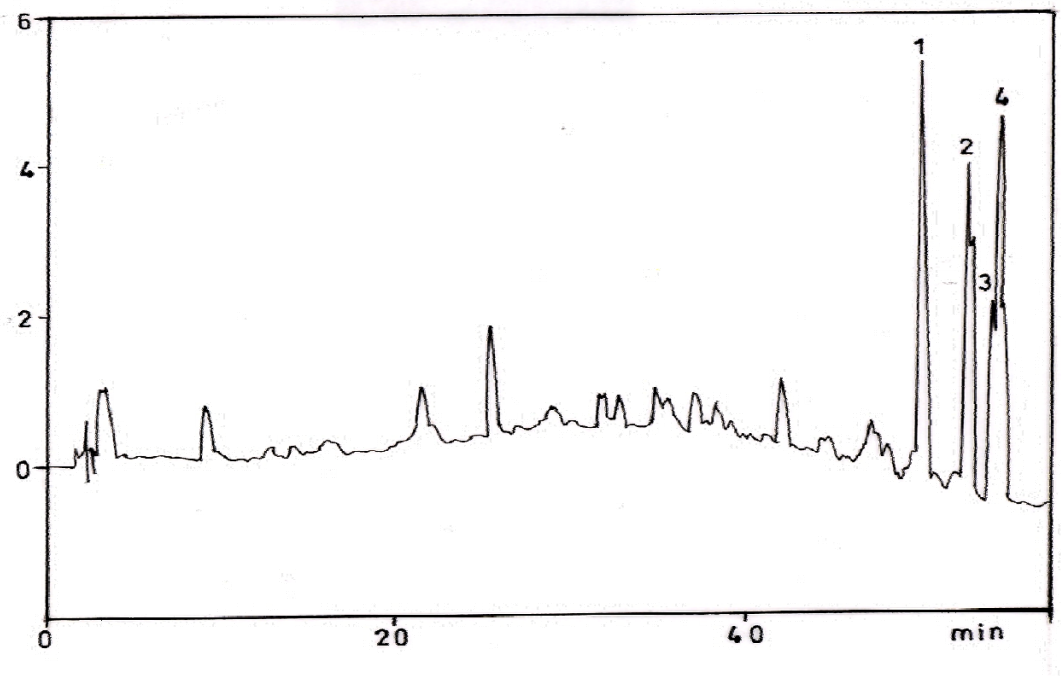
Viscosity of the brew (Method 2.3.2.2) varied from 4.1 to 12 milli Pascals. Tea brew from Chinchula alone had highest viscosity 12 mPa and Darjeeling (Medium and Premium), Nilgiri high grown, Assam Magor and

Aibheel had medium viscosity 11.3, 11.4, 11.0, 10.8 and 10.8 mPa respectively. Tea brews from Parajulie and Pandiar had viscosity 8.6 mPa each and Cachar medium tea brew had viscosity of 7.4 mPa. The brew from Assam AFTL and Cachar best had lowest viscosity 4.1 mPa. Darjeeling teas' brews showed very good amount of viscosity even though they looked thin.

Bulk density (BD-method 2.3.2.4) varied from 0.25 to 0.42 g/ml (Loose B.D.) and 0.31 to 0.45 g/ml (Packed BD). Darjeeling (Premium and Medium) and Nilgiri high grown teas have lowest loose bulk densities (0.25, 0.26 and 0.31 g/ml) as well as compacted bulk densities (0.31, 0.34 and 0.38 gm/ml). Chinchula tea had the highest loose and packed BD (0.42 and 0.45 g/ml).

2.4.1.2. Theaflavins and Thearubigins

The results of analysis for theaflavins, thearubigins, total colour and brightness for black tea samples from season-1(April-June) are presented in Table 2.1 and figure 2.1 (HPLC profile). It is evident from the results (Method 3.8) that theaflavin (TF) content varied from 0.24 to 1.72 %. Darjeeling teas can be characterized by lowest TF of 0.24 and 0.25% whereas Assam AFTL and Assam Magor can be characterized by highest TF of 1.5 and 1.72 % respectively. But Nilgiris high grown possesses almost double the TF content compared to Darjeeling. Other south Indian teas (Parajulie, Pandiar – 0.42% and 0.43%) had lower TF content as compared to North Indian teas (Aibheel (1.15%), Chinchula (0.91%), Cachar medium (0.69) and Cachar best (0.50%).



Peaks: 1 = theaflavin
2 = theaflavin-3-gallate
3 = theaflavin-3`-gallate
4 = theaflavin-3.3`-digallate

Fig.2.1. HPLC chromatogram of tea

The results indicate that thearubigin (TR) content ranged from 8.37% to 15.06%. Assam AFTL (15.06%), Aibheel (14.78%) and Chinchula (14.81%) had highest TR content. Parajulie and Assam Magor had TR content 13.69% and 13.54% respectively. Cachar teas (Medium and Best) and pandiar teas had moderate TR content of 11.29%, 11.63% and 11.91% respectively. Darjeeling (Medium and Premium) teas can be marked with lowest TR content of 8.37% and 8.56% and Niligiri high grown by slightly higher content of TR (9.37%) than Darjeeling.

The results indicate that Assam Magor have highest total colur (6.09) and brightness (25.85%). Assam AFTL (4.78, 24.73%), Aibheel (4.96, 21.49%), Chinchula (4.77, 16.26%) had the high total colour and brightness

values, when compaed to Cachar teas, which have moderate total colour (3.88 and 3.46) and brightness (15.08% and 12.01%). Parjulie and Pandiar have total colour 3.85 and 2.59 respectively. Parajulie has the lowest brightness (6.81%) and Pandiar has almost double the brightness as to Parajulie (12.57%). Darjeeling premium can be marked with lowest total colour (1.33) and brightness (9.90%). But Darjeeling medium has total colour slightly highest (2.36) and brightness lower (9.03) than premium. Nilgiri high grown tea can be marked by lower total colour (1.63) and moderate brightness (16.97%).

2.4.1.3. Volatile flavour compounds (VFC)

Volatile flavour compounds were isolated and analysed as per 2.3.2.8 Identification of compounds was achieved by comparison mass spectra (NIST library and Adams, 2001) and Kovats indices (Davies, 1990; Jennings & Shibamoto, 1980) Twenty-five volatile flavour compounds (VFCs) are identified and taken as markers and presented Table 2.2. Their quantities were calculated with reference to internal standard in different tea samples. The teas from different origins can be marked by the composition of VFCs identified. Gulati and Ravindranath (1996) explained seasonal variation of the VFCs in Kangra teas. Gulati *et al* (1999) studied the aroma profiles with respect to clonal variations in Kangra teas. Ullah (1985) studied aroma constituents, of Assam and China hybrid teas and their manifestation during tea processing. He identified eight aroma constituents, concluding that aroma constituents of tea are mainly inherent in the leaf and their manifestation is largely governed by black tea processing methods.

Table 2.2. Identification of major volatile flavour compounds from black tea

S.No	Compound	KI cal	M^+	m/z
1	n-Hexanal	772	100	41,55,42,69,57
2	E- 2- hexenal	825	98	41,42,55,69,57
3	Z-3- Hexenol	842	100	41,67,82,55,69
4	2-hexenol	848	100	57,82,41,43,44
5	n-hexanol	853	102	56,43,41,55,42
6	n-heptanal	875	114	43,41,70,44,55
7	Cumene	911	120	105,120,77,51,79
8	Benzaldehyde	939	106	77,106,105,51,50
9	(*E*,*Z*)-2,4-Heptadienal	975	110	81,41,53,67,110
10	(*E*,*E*)-2,4-Heptadienal	984	110	81,41,53,67,110
11	Nonanal	1006	142	41,57,43,55,56
12	Phenyl acetaldehyde	1017	120	91,65,92,120,51
13	Benzyl alcohol	1022	108	79,108,77,107,1
14	*cis*-Linalooloxide	1058	170	59,43,55,94,68
15	*trans*- Linalooloxide	1073	170	59,43,55,94,68
16	Linalool	1085	154	71,41,93,55,43
17	Phenyl ethyl alcohol	1097	122	91,92,65,122,51
18	α-terpineol	1173	154	59,43,93,81,121
19	Methyl salicylate	1180	152	120,92,152,121,65
20	*cis*-Geraniol	1232	154	41,69,93,67,53
21	Indole	1291	117	117,89,90,63,118
22	Geranyl acetate	1358	196	41,43,69,68,67
23	β-Ionone	1455	192	177,43,41,44,178
24	Dihydroactinidiolide	1471	180	111,43,109,137,67
25	Nerolidol	1518	222	41,69,43,93,71
26	Phytol	2010	296	71,43,57,69,123

The results of the fingerprinting with regard to VFC for season-1 (April-June) are presented in Table 2.3 and figure 2.2 – 2.28. It is clear from the results that:

1. Darjeeling premium quality tea possesses highest quantity (13.49 mg %) of total volatiles, where as Cacher best has lowest (3.18 mg %). Darjeeling medium quality, parajulie and Pandiar contained higher amounts of total volatiles.
2. Darjeeling teas contained highest n-hexanal (1241-3702 μg / 100g) and leaf alcohol (967 -1018 μg / 100g).

3. Darjeeling premium quality tea can be marked by the presence of nonanol (60 - 70 μg / 100g).
4. Nilgiris high grown can be marked by absence of phenyl ethanol and highest content of linalool (2345 μg / 100g) and methyl salicylate (721 μg / 100g).
5. Parajulie can be marked by highest content of Nerolidol (160 - 200 μg / 100g) and β - ionone (260 – 300 μg / 100g) and absence of trans/cis-2-hexanol.
6. Pandiar can be characterized with highest content of benzyl alcohol (560 - 590 μg / 100g) and trace of benzaldehyde, and higher content of leaf aldehyde (5900 – 5930 μg / 100g).
7. Assam AFTL and Assam Magor can be characterized by lowest β - ionone content (20 μg / 100g)
8. Assam AFTL has only trace of Trans/cis -2-hexanol and Assam Magor has highest content of leaf aldehyde (6070 – 6100 μg / 100g).
9. Chinchula tea can be marked by the absence of geranyl acetate and Aibheel by lowest content of geranyl acetate (19 μg / 100g).
10. Chinchula and Aibheel can be marked by trace and lower (54 μg / 100g) content of linalool
11. Cachar Best and medium can be characterized by trace of both heptadienals and traces of nerol and cis-geraniol.
12. Cachar Best has lowest content of n-hexanal (50 μg / 100g), while it is absent in Nilagiri high grown.

Table 2.3. Season 1(April- June)- Black tea samples-volatile flavour compounds (VFC) μ g / 100 g

Sl. No.	Compound name	Darjeel Premi.	Darjeel Medim.	Parajulie	Pandiar	Assam AFTL	Assam Magor	Aibheel	Chinchula	Cachar best	Cachar med.	Nilgiris HG
1	n-hexanal	3702	1241	562	448	257	203	293	89	50	149	A
2	Leaf aldehyde (E-2-hexenal)	3436	1692	3136	5912	2478	6088	799	1677	2133	4312	958
3	Leaf alcohol (Z-3-hexenol)	1018	967	560	740	34	223	154	236	158	151	662
4	*trans* / *cis*-2-hexenol	168	531	A	183	T	86	40	62	50	63	115
5	n-hexenol	151	459	25	320	T	49	44	158	138	T	91
6	n-heptanal	58	320	185	169	100	133	55	64	38	95	63
7	Benzaldehyde	254	216	204	T	122	57	62	57	49	92	73
8	(*E*,*Z*)-2,4-Heptadienal	27	T	185	T	T	14	18	T	T	T	12
9	(*E*,*E*)-2,4-Heptadienal	37	T	185	T	T	63	62	T	T	T	19
10	Nonanal	66	A	A	A	T	T	T	T	A	T	A
11	Phenyl acetaldehyde	310	928	951	355	836	716	898	682	330	2061	322
12	Benzyl alcohol	310	77	31	577	252	111	157	150	0	147	68
13	*cis*-linalool oxide	516	805	142	T	44	55	128	29	T	65	268
14	*trans*-linalool oxide	890	1611	574	128	29	95	427	90	27	206	1136
15	Linalool	1166	2052	1642	128	103	48	54	T	T	206	2345
16	Phynyl ethyl alcohol			25	137	166	35	602	112	28	286	A
17	4-terpineol			A	579	199	173	0	112	78	540	A
18	α-terpineol	165	270	154	T	131	51	157	48	19	92	80
19	Methyl salicylate	314	475	130	180	96	76	145	50	40	212	721
20	Nerol		44			18		26	36			A
21	*cis*-Geraniol	723	987	43	369	19	17	44		T	T	14
22	Geranyl acetate	46	110	37	T	20	26	19	A	T	32	35
23	β-Ionone	57	54	278	76	20	20	70	34	26	103	42
24	Nerolidol	76	87	179	T	52	17	26	31	12	55	101
	Total	13490	12926	9228	10301	4976	8356	4280	3717	3176	8867	7125
	Terpene Index	0.78	0.81	0.98	0.41	0.90	0.92	0.93	1.0	1.0	1.0	0.99
	Yamanishi Botheju ratio	0.34	1.21	0.52	0.02	0.04	0.007	0.07	-	-	0.05	2.44
	Mahanta ratio	0.41	1.10	0.60	0.16	0.20	0.07	0.60	0.15	0.06	0.27	2.01

Values expressed are mean of three experiments, Where A=Absent, T= Trace

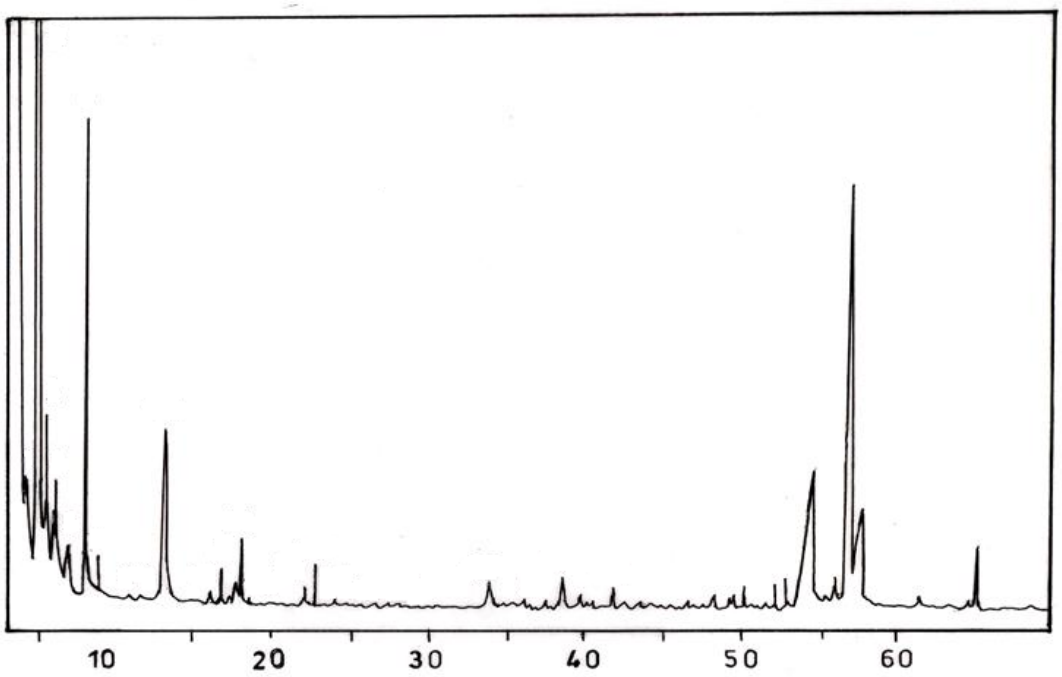


Fig.2.2. Total ion chromatogram of black tea volatiles (VFC)

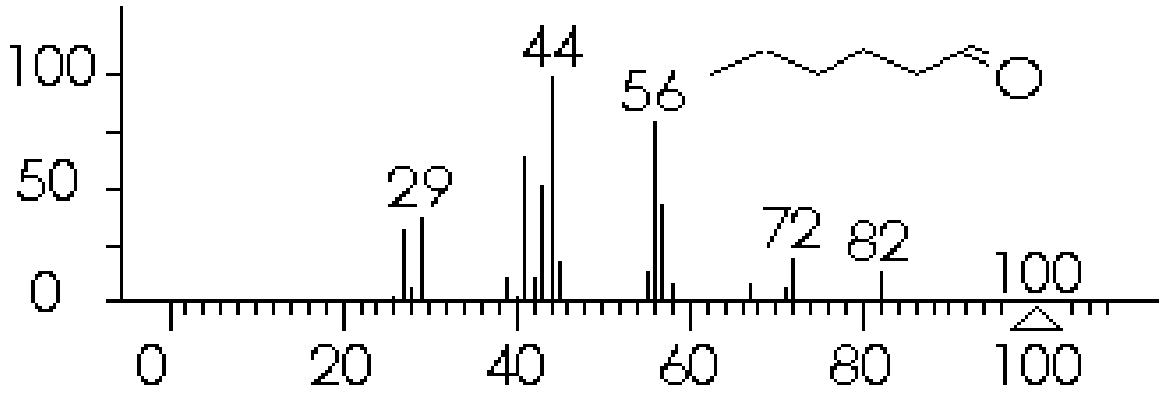


Fig.2.3. Mass spectrum of n-Hexanal

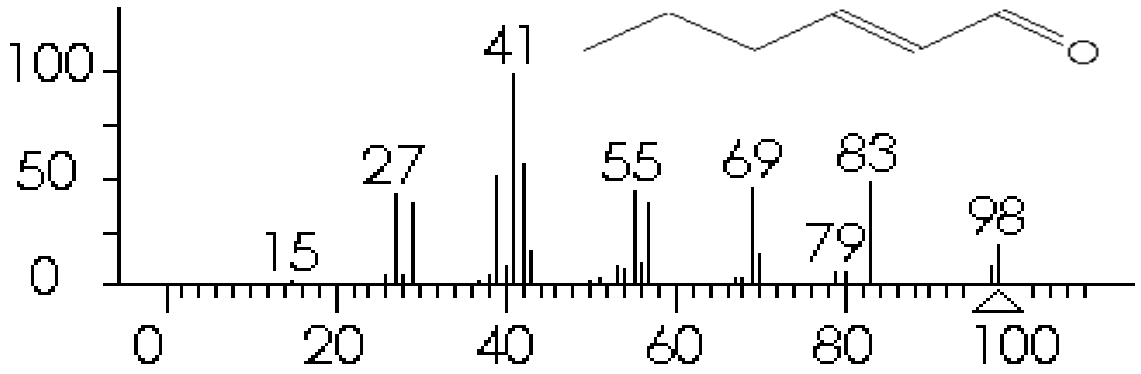


Fig.2.4. Mass spectrum of Leaf aldehyde (E-2-Hexenal)

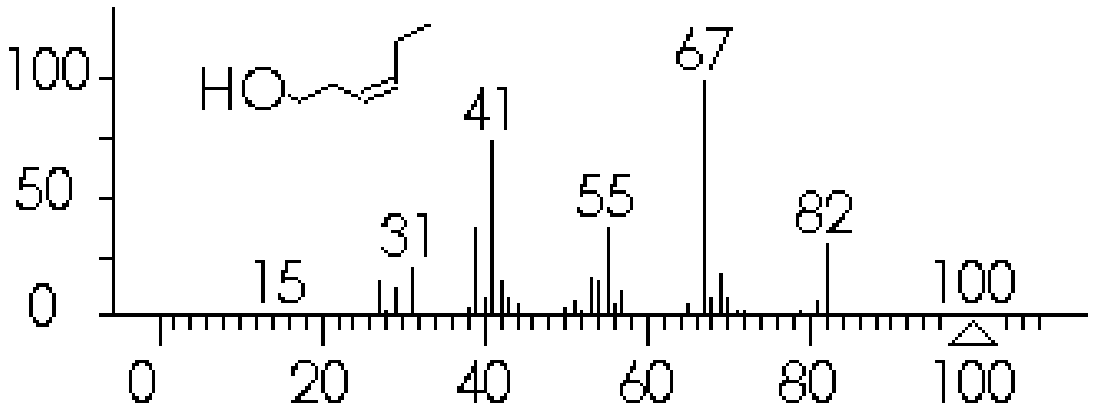


Fig.2.5. Mass spectrum of Leaf alcohol (Z-3-Hexenol)

2-Hexen-1-ol, (E)-

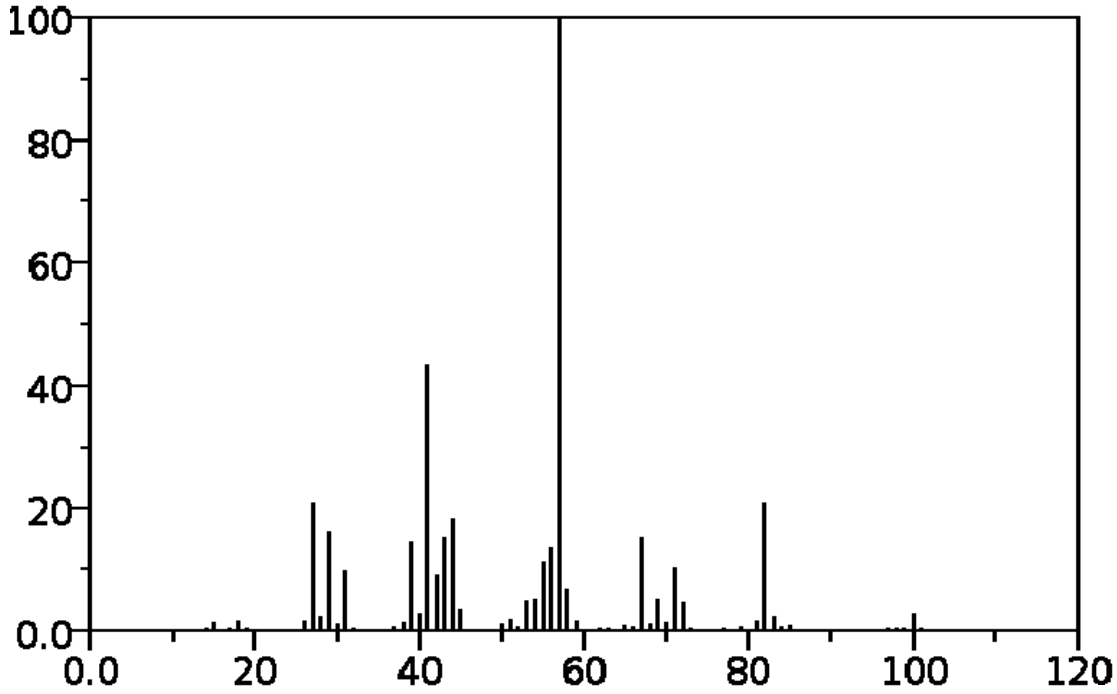


Fig.2.6. Mass spectrum of *trans*-2- hexenol

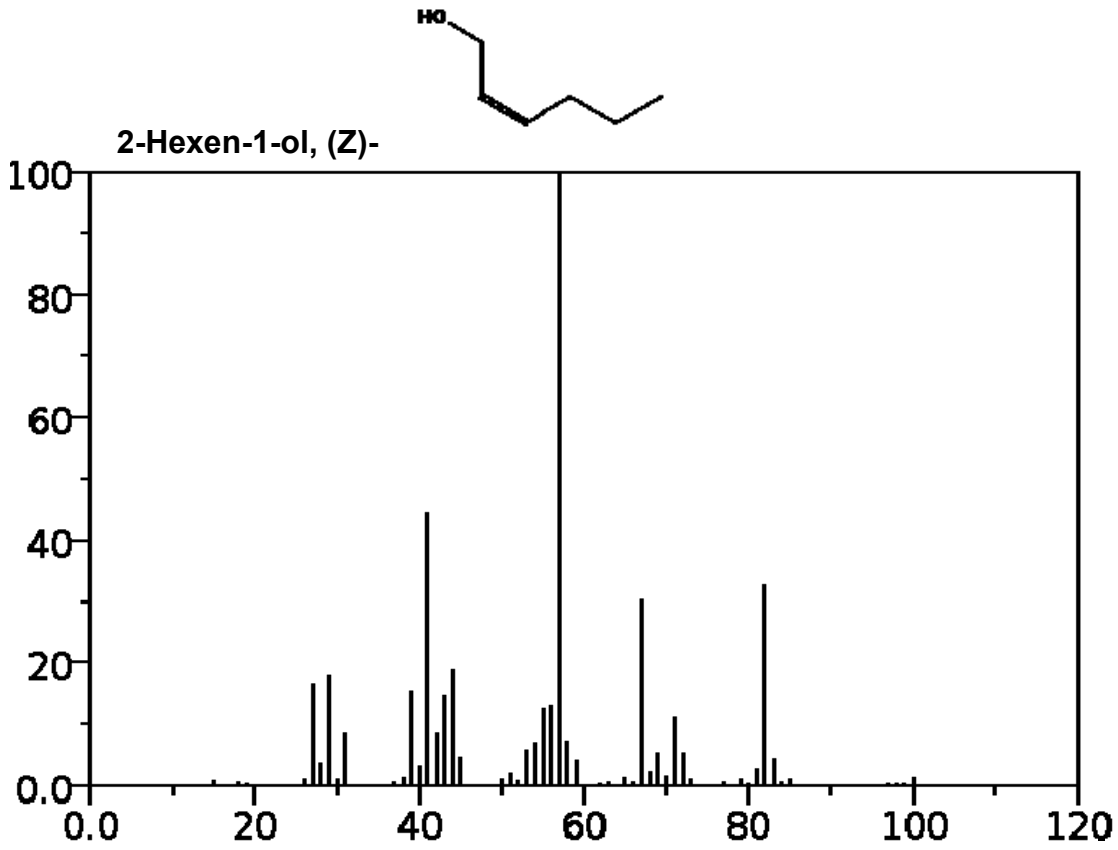


Fig.2.7. Mass spectrum of cis-2- hexenol

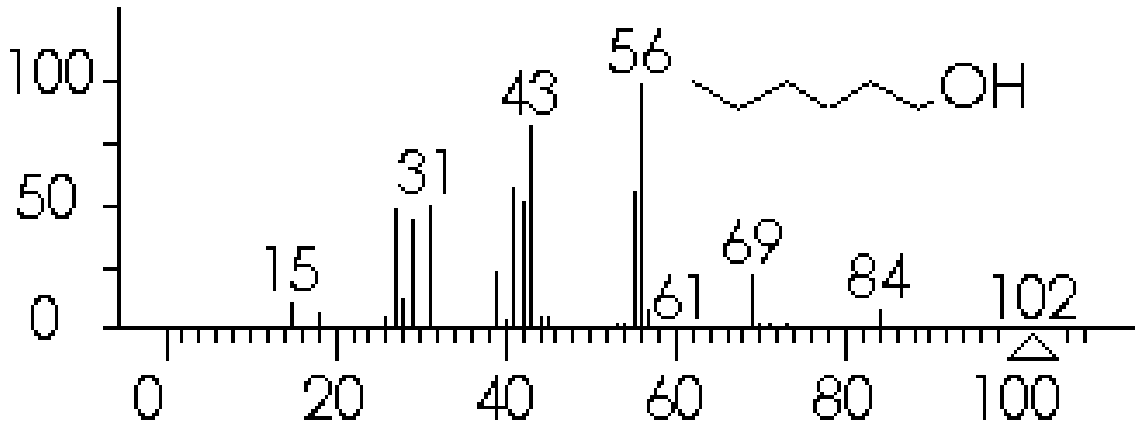


Fig.2.8. Mass spectrum of n-Hexanol

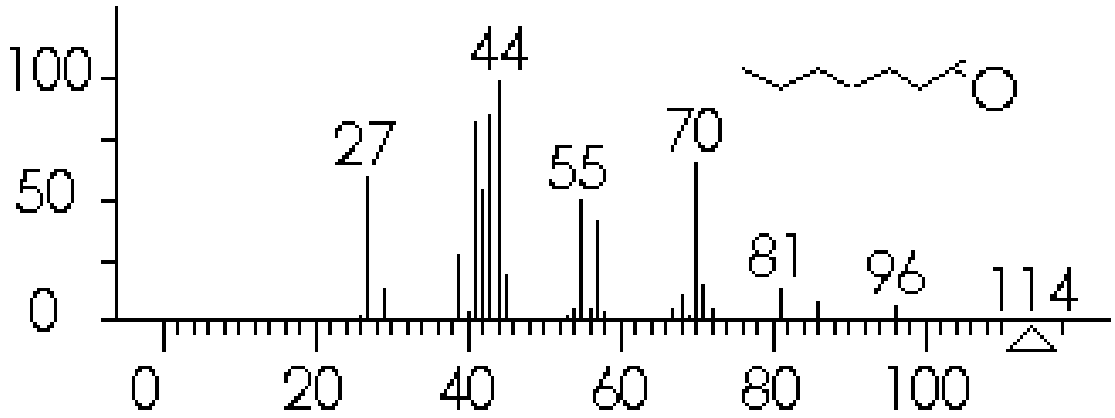


Fig.2.9. Mass spectrum of n-Heptanal

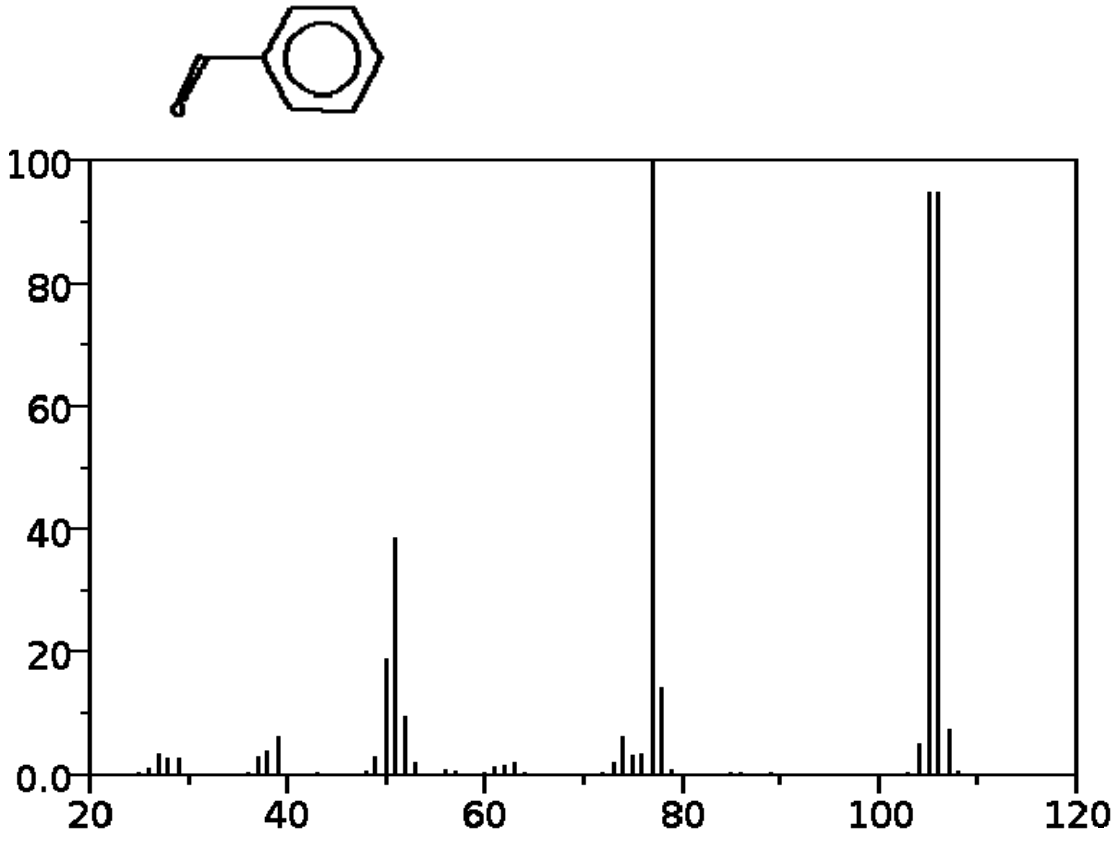


Fig.2.10. Mass spectrum of Benzaldehyde

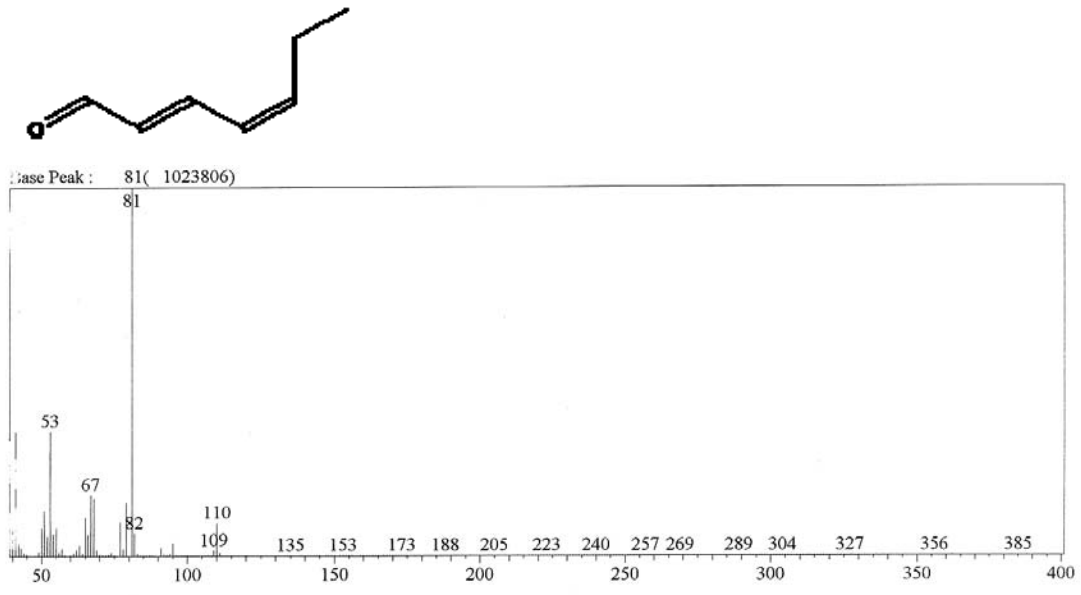


Fig.2.11. Mass spectrum of (E,Z)-2,4-Heptadienal

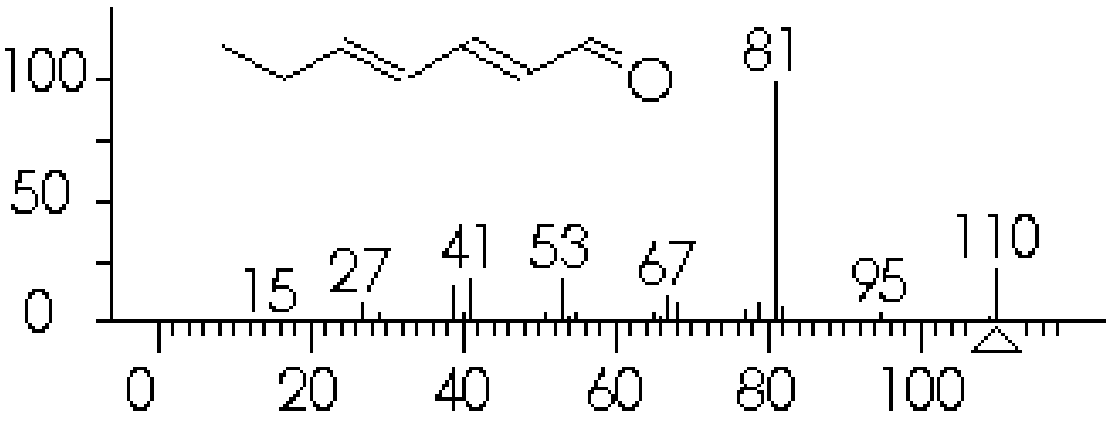


Fig.2.12. Mass spectrum of (E,E)-2,4-Heptadienal

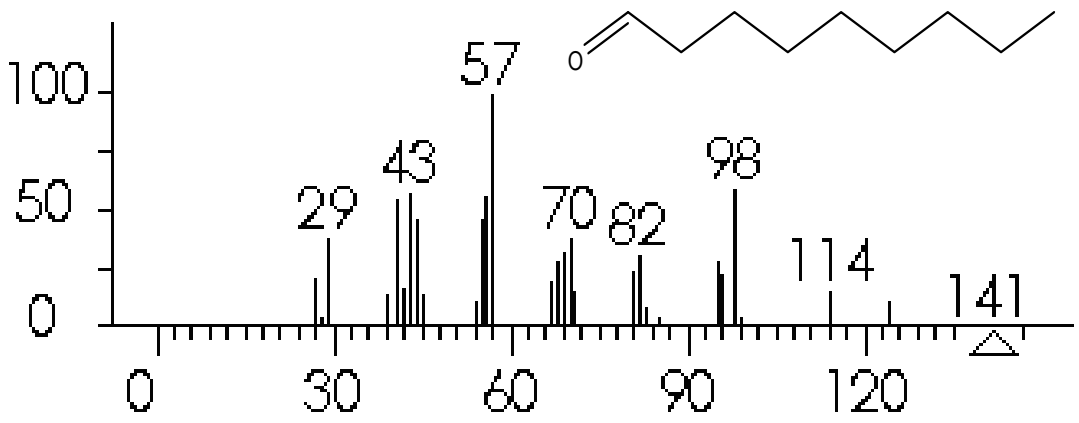


Fig.2.13. Mass spectrum of Nonanal

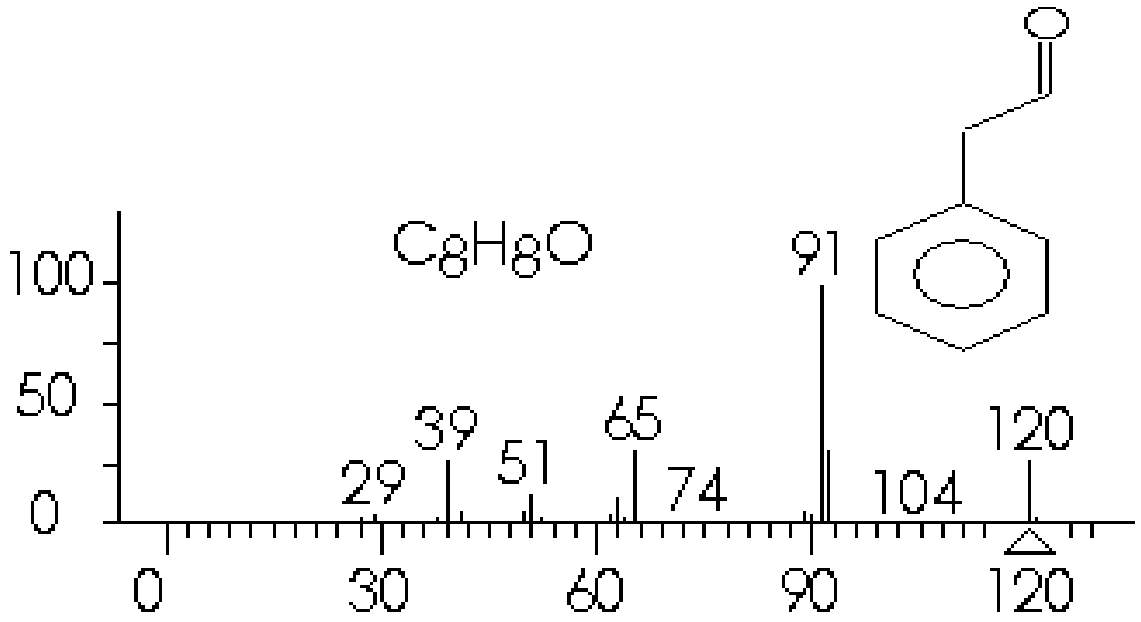


Fig.2.14. Mass spectrum of Phenyl acetaldehyde

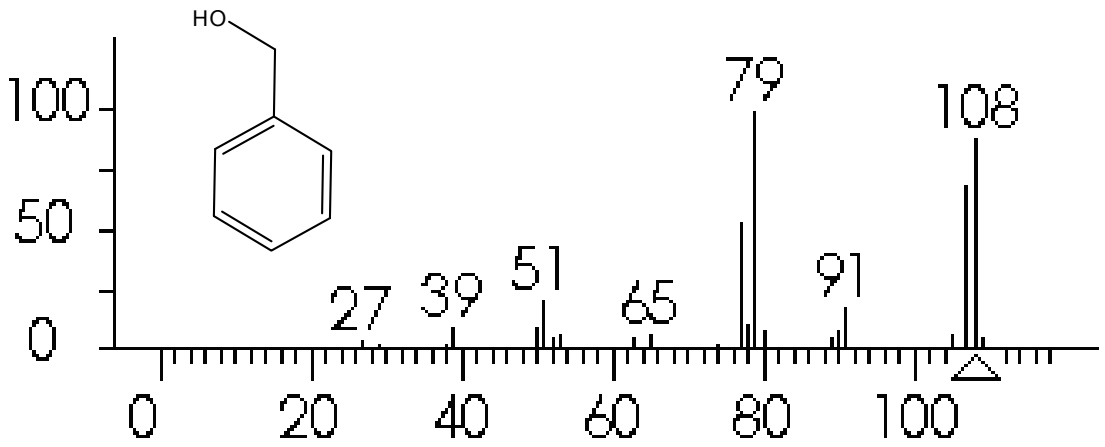


Fig.2.15. Mass spectrum of Benzyl alcohol

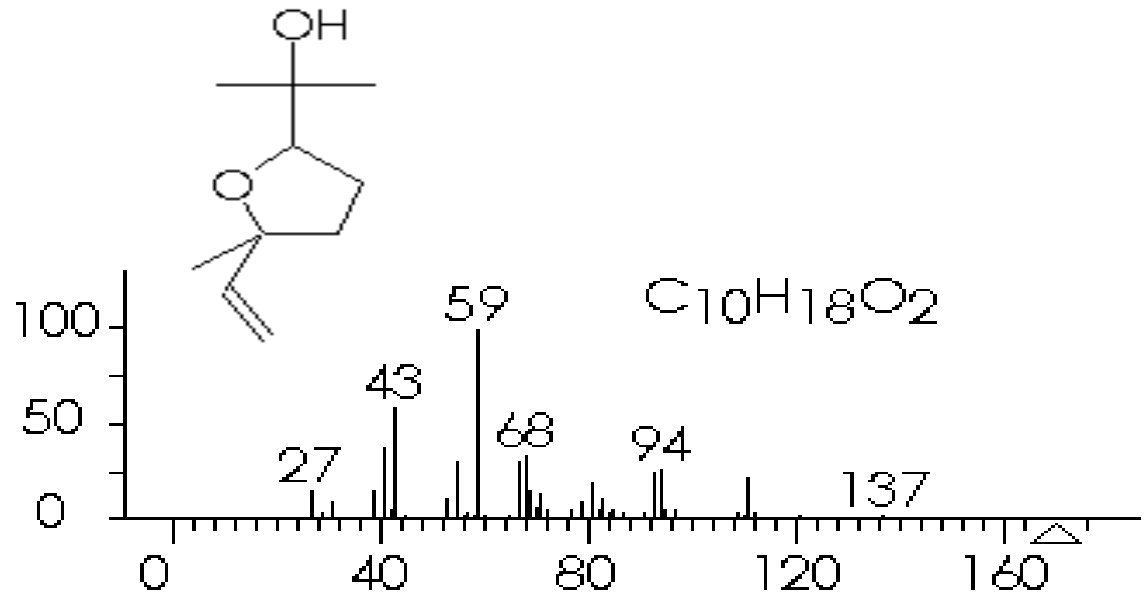


Fig.2.16. Mass spectrum of *cis*-Linalool oxide

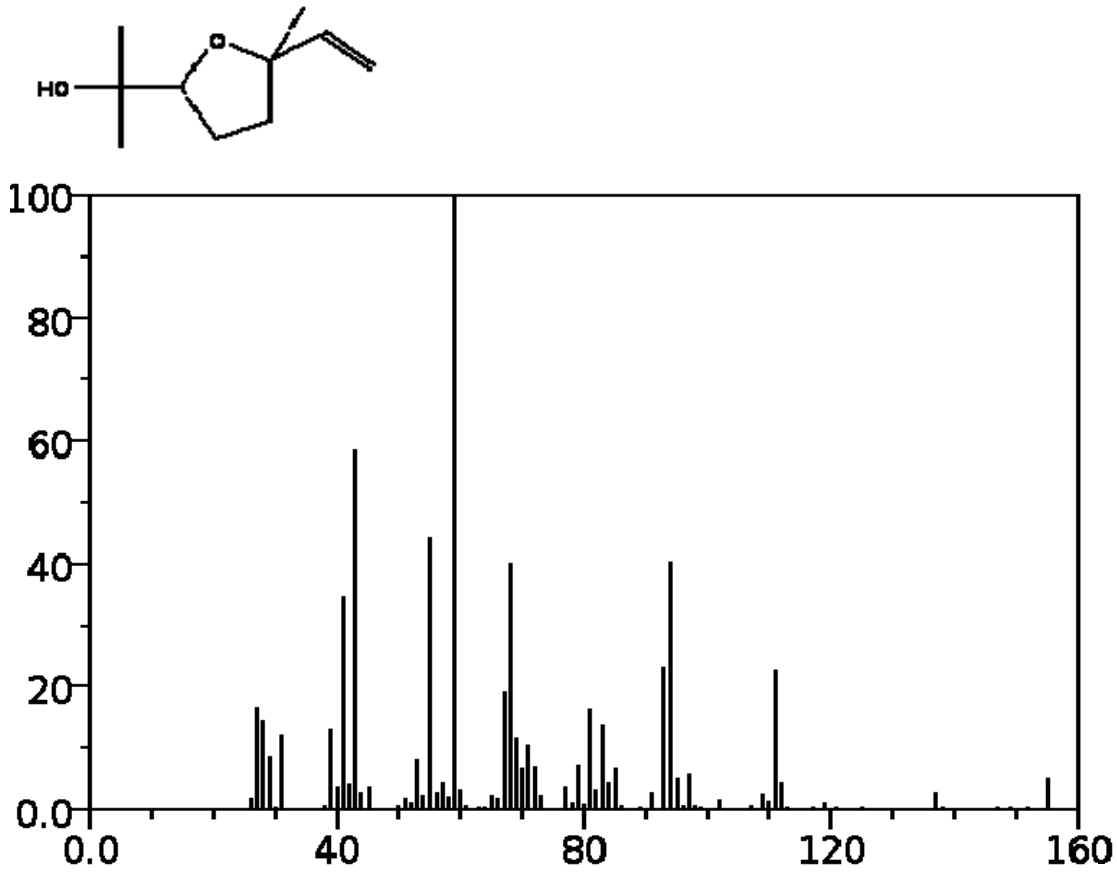


Fig.2.17. Mass spectrum of *trans*-Linalool oxide

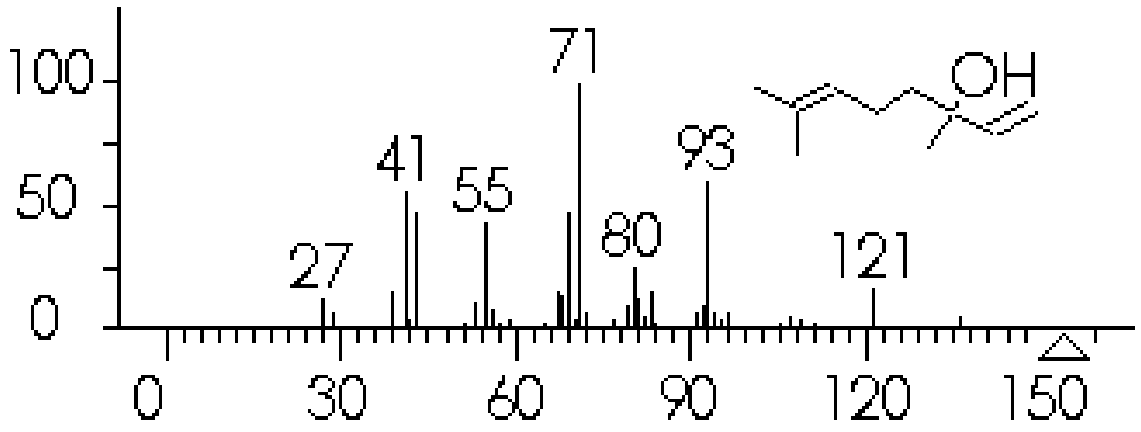


Fig.2.18. Mass spectrum of Linalool

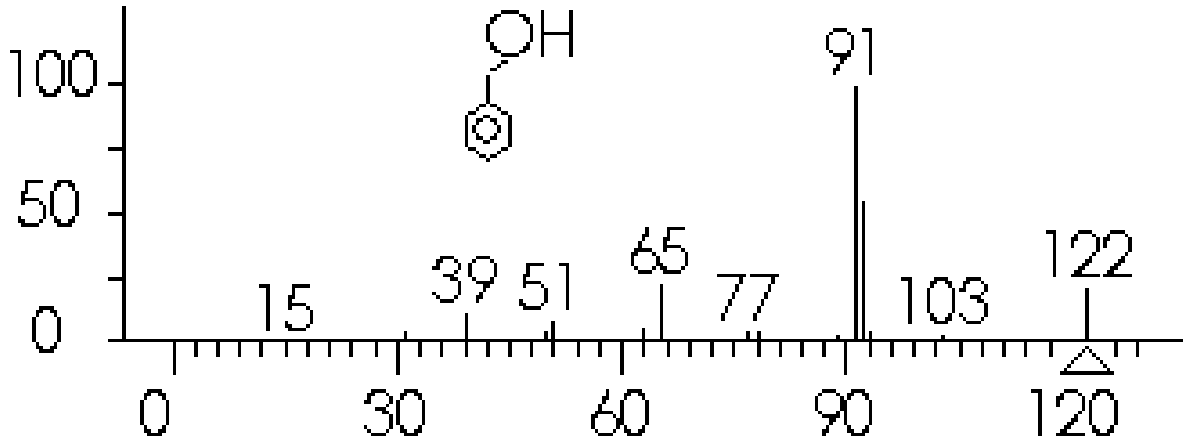


Fig.2.19. Mass spectrum of phenyl ethyl alcohol

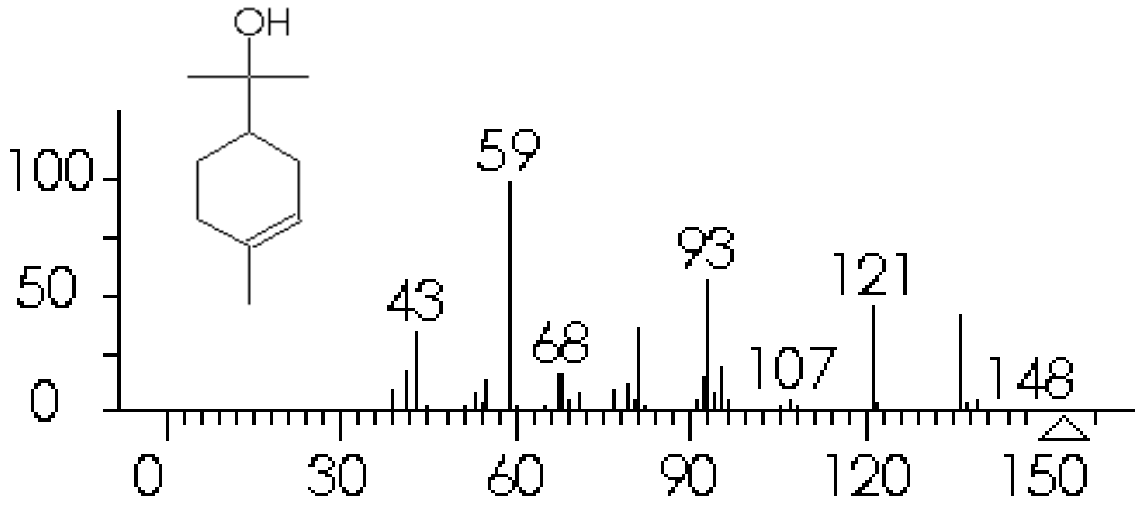


Fig.2.20. Mass spectrum of α-terpineol

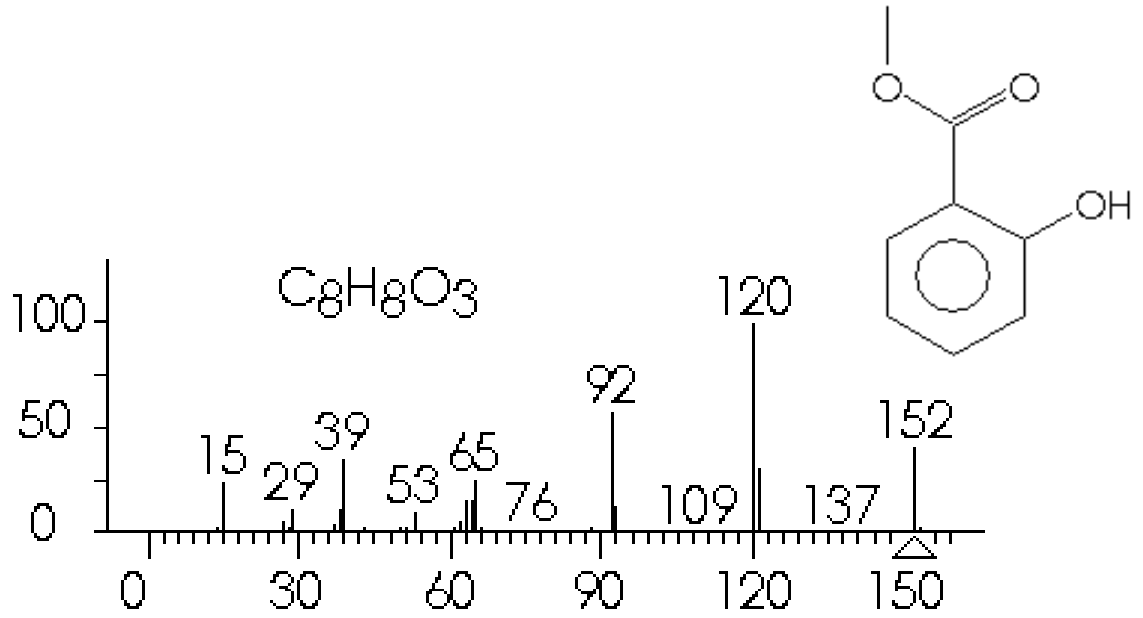


Fig.2.21. Mass spectrum of Methyl Salicylate

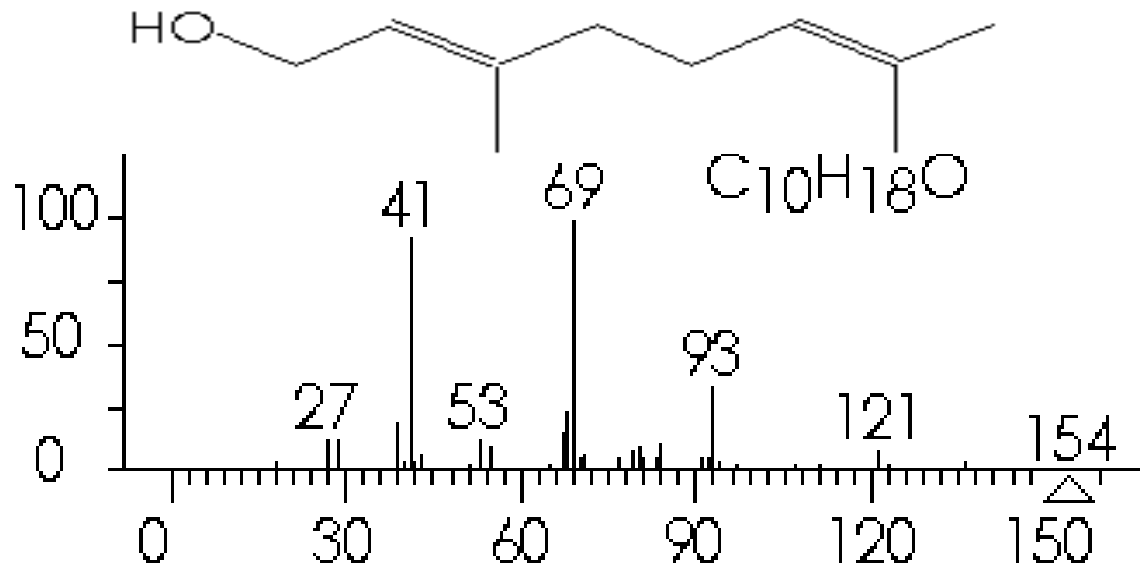


Fig.2.22. Mass spectrum of cis-Geraniol

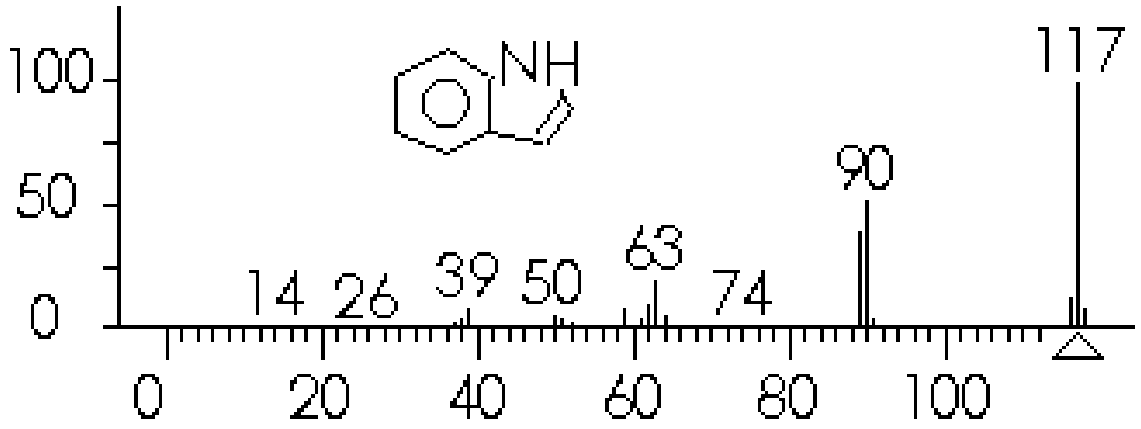


Fig.2.23. Mass spectrum of Indole

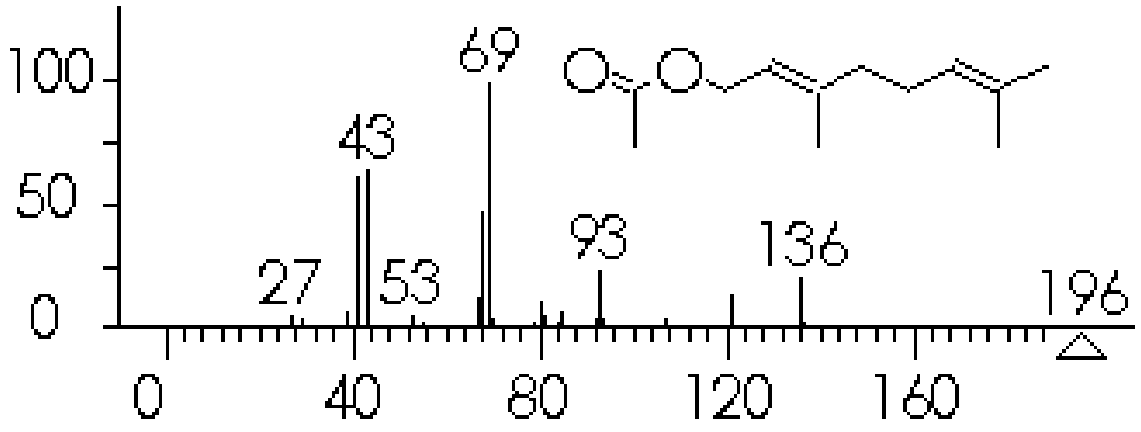


Fig.2.24. Mass spectrum of Geranyl acetate

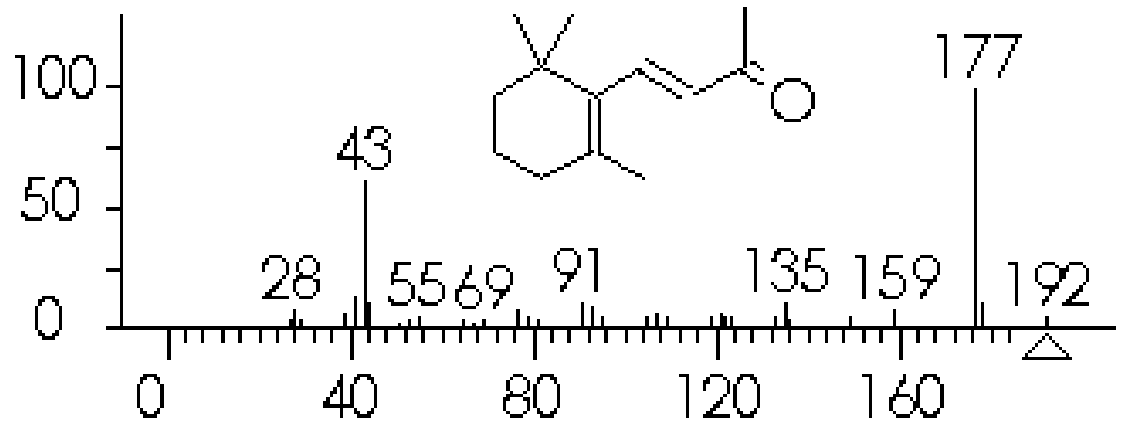


Fig.2.25. Mass spectrum of β- Ionone

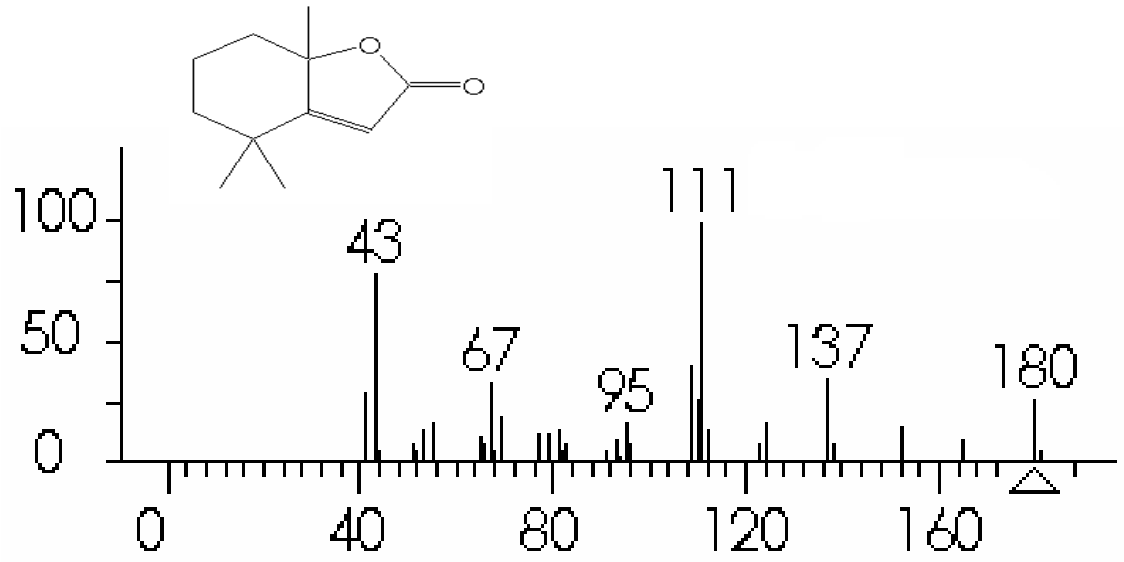


Fig.2.26. Mass spectrum of Dihydroactinidiolide

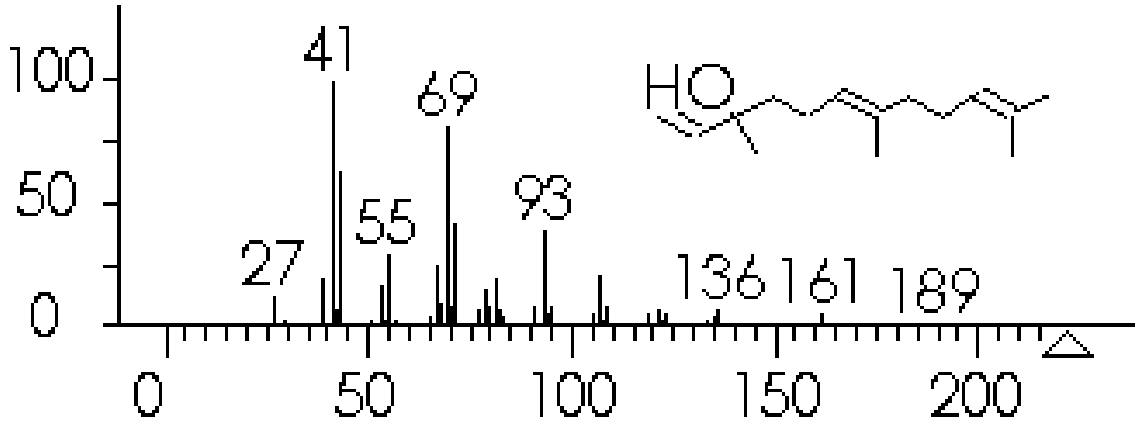


Fig.2.27. Mass spectrum of Nerolidol

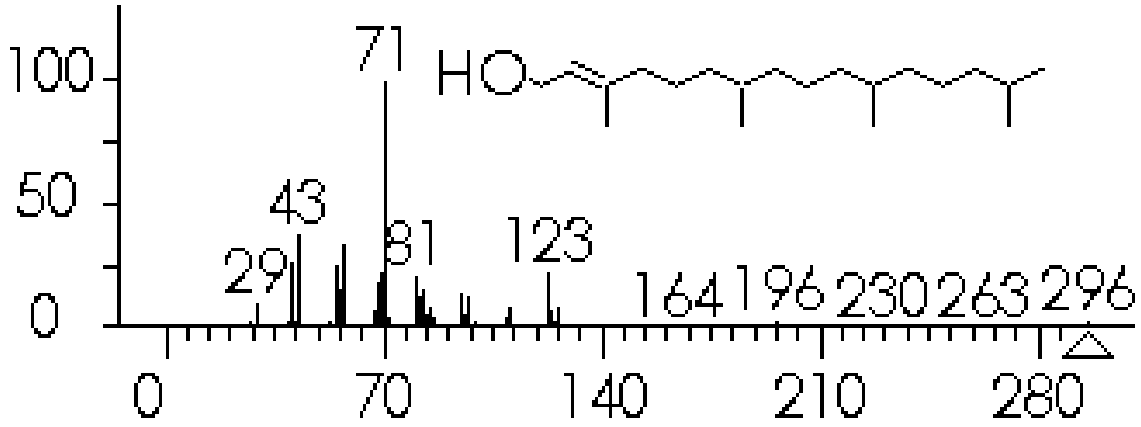


Fig.2.28. Mass spectrum of Phytol

Terpene index, Yamanishi-Botheju ratio and Mahanta ratio for the volatile flavour compounds are calculated and presented in table 2.3. The terpene indices of Chinchula, Cachar Best and medium teas are unity and indicating these to be pure *assamica* varieties, where as that of Nilgiris high

grown, parajulie, Aibheel, Assam AFTL and Assam Magor are approaching unity (0.90 – 0.99) and indicating these to be hybrids dominated by *assamica.* Darjeeling premium and medium quality, pandiar teas are found to be hybrids of *assamica and sinensis,* from their terpene indices (0.78, 0.81 and 0.41 respectively).

Nilgiris high grown tea showed highest Mahanta ratio for VFCs indicating the presence of more quantities of desirable VFC, which indicate better quality of flavour. Darjeeling premium and medium, parajulie and Aibheel teas are of moderate quality with regard to flavour. Teas from Pandiar, Assam AFTL and Assam Magor, Chinchula, Cachar Best and medium possess lowest Mahanta ratio and an indication that flavour is low. Yamanishi-Botheju ratios also support the above findings.

2.4.2. Profiling of the tea samples from second season (July-September)

2.4.2.1. Physico-Chemical Constituents

The results of the physico-chemical analyses for the black tea samples from season-2 (July-september) are presented in Table 2.4.

It is clear from the results that the moisture content varied from 3.2 to 6.4% and TSS content (Method 2.3.2.3) ranged from 0.05 to 1.0%. Tea brews from Palampur G_1 contained highest TSS (1.0%), followed by Rosekandy (0.6%) and and Nilgiris high grown CTC BOP (0.4%). Tea brews from Parajulie, Nilgiris high grown, Aibheel and Chinchula contained moderate TSS 0.25% each. Tea brew from Pandiar contained TSS 0.15%. Tea brew from Assam AFTL and Assam Magor had lower TSS of 0.10%. Darjeeling (medium / premium) tea can be marked by lowest TSS of 0.05% in its brew.

Table 2.4. Season 2 (July-September) – Physico-chemical analysis of black tea

Sample	Soluble Caffeine (%)	TSS (%)	Viscosity (mPa)	Moisture (%)	Bulk density (g/ml)		TF(%)	TR (%)	Total colour	Brightness (%)
					Loose	Packed				
Parajulie	2.26	0.25	12.00	4.53	0.38	0.44	0.62	11.86	2.61	16.90
(±)S.D.	0.02	0.00	0.00	0.02	0.01	0.01	0.01	0.01	0.10	0.08
Pandiar	1.91	0.15	12.00	4.93	0.38	0.42	0.77	6.88	1.90	19.41
(±)S.D.	0.04	0.00	0.00	0.01	0.01	0.0	0.01	0.02	0.03	0.09
Assam AFTL	3.84	0.10	10.50	4.53	0.37	0.42	1.02	12.36	4.08	21.33
(±)S.D.	0.01	0.00	0.00	0.03	0.0	0.01	0.02	0.01	0.03	0.07
Assam Magor	2.74	0.10	10.50	4.47	0.36	0.40	0.78	13.65	5.21	36.11
(±)S.D.	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.01	0.04
Darj. Medium	2.60	0.05	11.00	5.00	0.24	0.30	0.20	5.47	1.08	9.91
(±)S.D.	0.02	0.00	0.00	0.00	0.01	0.01	0.01	0.14	0.05	0.03
Darj. Premium	2.31	0.05	12.80	6.07	0.25	0.34	0.51	8.30	1.92	17.25
(±)S.D.	0.03	0.00	0.00	0.02	0.01	0.01	0.00	0.02	0.01	0.07
Nil-HG	2.43	0.25	10.70	4.80	0.30	0.38	0.60	6.61	1.43	21.59
(±)S.D.	0.03	0.0	0.0	0.0	0.01	0.01	0.01	0.03	0.07	1.05
Aibheel	2.29	0.25	11.70	3.20	0.42	0.46	0.64	8.58	3.24	16.53
(±)S.D.	0.03	0.0	0.0	0.0	0.01	0.01	0.01	0.02	0.02	0.08
Chinchula	2.72	0.25	10.50	4.13	0.42	0.45	0.62	9.54	3.19	15.12
(±)S.D.	0.02	0.0	0.00	0.12	0.00	0.01	0.01	0.03	0.03	0.05
PalampurG1	3.69	1.00	11.0	3.40	0.27	0.35	0.10	9.48	1.78	7.41
(±)S.D.	0.05	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.03
Nil-HG-CTC-BOP	2.08	0.40	6.30	6.40	0.40	0.46	0.80	14.82	3.85	16.27
(±)S.D.	0.03	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.04
Rosekandy*	3.46	0.60	8.50	6.40	0.36	0.41	0.88	12.46	4.85	16.57
(±)S.D.	0.06	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.01

Values expressed are mean ± S.D. of five experiments; * Dibrugarh, Assam

The soluble caffeine content varied from 1.91 to 3.84%. Assam AFTL contained highest soluble caffeine (3.84%) followed by Palampur G_1 (3.69%), Rosekandy (3.46%), Assam Magor (2.74%) and Chinchula (2.72%). Darjeeling medium (2.6%), Niligiris high grown (2.43%), Darjeeling best (2.31%), Aibheel (2.29%) and Parajulie (2.26%) teas contained moderate soluble caffeine. Nilgiris high grown CTC-BOP and Pandiar teas had lowest soluble caffeine (2.08 % and 1.91%).

Determination of viscosity of the tea liquor (Method 2.3.2.2) was done with a view that it may provide information about body of the tea liquor. The viscosity ranged from 6.3 to 12.80 m Pa. It was again noted that Darjeeling best tea brew had highest viscosity 12.80 m Pa and Nilgiris high grown CTC-BOP had lowest viscosity 6.3 m Pa.

The bulk densities ranged from 0.24 to 0.46 g/ml. The packed (compacted) bulk density of Darjeeling teas (Medium and Premium, 0.30 and 0.34 g/ml respectively) as well as loose bulk density (Medium and Premium, 0.24 and 0.25 g/ml respectively) is lowest compared to other teas. Teas from Aibheel and Nilgiris high grown CTC-BOP have the highest packed (0.46 g/ml) and highest loose (0.42 and 0.40 g/ml) bulk densities.

2.4.2.2. Theaflavins and Thearubigins

The results of the fingerprinting with reference to theaflavins, thearubigins, total colour and brightness for black tea samples from season-2 (July-Sept.) are presented in Table 2.4. and figure 2.1.

It is evident from the results that theaflavin (TF) content varied from 0.10 to 1.02%. Assam AFTL has the highest TF (1.02%), besides Rosekandy (0.88%), Nilgiris high grown CTC-BOP (0.80%), Assam Magor (0.78%) and Pandiar (0.77%). Aibheel (0.64%), Parajulie (0.62%), Chinchula (0.62%) and Niligiri high grown (0.60%) have moderate TF content. Darjeeling premium (0.51%), Darjeeling medium (0.20%) and Palampur G_1 (0.10%), can be marked with lowest TF conent.

Thearubigins (TR) content varied from 5.47 to 14.82%. Nilgiris high grown CTC-BOP (14.82%), Assam Magor (13.65%), Rosekandy (12.46%) and Assam AFTL (12.36%) can be characterized by highest TR contents besides the highest TF content. Parajulie have slightly lower TR (11.86%) than Assam teas. But Pandiar (6.72%) and Nilgiris high grown (6.61%) have much lower TR content. Aibheel (8.58%), Chinchula (9.54%) and Darjeeling best (8.30%) had moderate TR content. But Darjeeling medium tea can be marked by lowest TR (5.47%).

The results for total colour varied from 1.08 (Darjeeling medium) to 5.21 (Assam Magor). Interestingly the trend was found similar for brightness also i.e. 9.91% (Darjeeling medium) to 36.11% (Assam Magor). In sum total Assam Magor can be marked by highest colour and brightness whereas Darjeeling medium by lowest colour and brightness. Assam AFTL (4.08 and 21.23%), Rosekandy (4.85, 16.57%), Nilgiris high grown CTC-BOP (3.85 and 16.27%), Aibheel (3.24 and 16.53%) and Chinchula (3.19 and 15.12%), had highest total colour and brightness followed by Assam Magor. South Indian teas Parajulie (2.61 and 16.90%) and Pandiar (1.89 and 19.41%) along with

Palampur G_1 (1.78, 7.41%) had marginally more or less colour and brightness than Darjeeling teas. But Nilgiri high grown had less total colour (1.43) and more brightness (21.29%) as compared to Darjeeling best (1.92 and 17.25%).

2.4.2.3. Volatile flavour compounds (VFCs)

The results of fingerprinting with respect to volatile flavour compounds for season-2 (July-Sept.) are presented in Table 2.5. and figure 2.2. – 2.28. It is evident from the results that:

1. The total quantity of volatiles are in the range of 2.145 – 18.630 mg / 100 g of tea sample during this season. Aibheel possesses highest quantity (18.63 mg%) of total volatiles, where as Nilgiri high grown CTC has lowest (2.15 mg%). Palampur G1, Parajulie, Nilgiri high grown, Pandiar and Assam magor contained higher amounts of total volatiles. Content of phytol is not considered for total volatiles, as it is product derived from chlorophylls during degradation.
2. Marker for Darjeeling (Medium/Premium) and Nilgiris high grown teas is absence of β - ionone content and higher content of methyl salicylate (519 - 761 μg / 100g)
3. Darjeeling teas can be marked by lower content of leaf aldehyde (708-736 μg / 100g) and lower to modest content of leaf alcohol (124-522 μg / 100g) and absence of both heptadienals and traces of nonanal
4. Palampur G_1 and Nilgiris HG (non CTC) tea has highest and higher content of leaf alcohol (2781μg and 728 μg / 100 g) and Nilgiris HG (non CTC) tea has highest content of linalool (2542 μg / 100g).

5. Parajulie and Pandiar can be marked by lowest content of n-hexanal (88 - 95μg / 100g) and either absence or trace of phenyl ethyl alcohol.
6. Assam AFTL and Assam Magor can be marked by lowest content of hexanal (61-101 μg / 100g) and benzaldehyde (57 - 104 μg / 100g).
7. Assam magor can be marked by highest content of leaf aldehyde (6024 μg / 100g) and Assam AFTL by lowest content of *cis*-geraniol (24 μg / 100g) and (E,E)-2,4-heptadienal (22 μg / 100g), besides above .
8. Aibheel and Chinchula can be marked by highest content of benzaldehyde (447 and 500 μg / 100g respectively) and highest content of phenyl acetaldehyde (5609 and 965 μg / 100g) respectively.
9. Aibheel can be marked by highest (27370 μg / 100g) phytol and β - ionone (902 μg / 100g) content.
10. Aibheel and Chinchula can be characterized by trace of trans-linalool oxide.

Terpene index, Yamanishi-Botheju ratio and Mahanta ratio for the volatile flavour compounds are calculated and presented in table 2.5. The terpene indices of Niligiri high grown, Chinchula, Assam Magor teas are approaching to unity (0.91 – 0.97) and indicating these to be dominated with *assamica.* Remaining teas are found to be hybrids of *assamica and sinensis,* from their terpene indices, which is in the range of 0.63- 0.88.

Table 2.5. Season 2 (July-September)- Black tea samples-volatile flavour compounds (VFC) μ g / 100 g[@]

Sl. No.	Compound	Parajulie	Pandiar	Darjeelg. Medium	Darjeel. Premim	Assam AFTL	Assam Magor	Nilgiris HG	Aibheel	Chinch-ula	Palam pur G1	Nilgiris HG-CTC	Rose kandy
1	n-hexanal	88	95	232	338	101	61	650	380	182	A	199	128
2	Leaf aldehyde (E2–hexenal)	1307	1150	736	708	406	6024	755	3196	1444	3871	1230	2071
3	Leaf alcohol (Z-3-hexenol)	136	160	522	124	314	118	728	543	140	2781	63	84
4	Trans / cis-2-hexenol	A	62	134	A	73	A	192	T	T	272	43	48
5	n-hexenol	57	58	A	T	A	891	272	163	47	565	50	44
6	n-heptanal	85	A	115	120	T	T	A	T	752	147	47	55
7	Benzaldehyde	143	T	315	328	104	57	139	447	500	125	88	31
8	(E,Z)-2,4-Heptadienal	T	A	A	A	A	A	A	A	A	170	A	T
9	(E,E)-2,4-Heptadienal	45	A	A	A	22	A	T	A	A	275	A	11
10	Nonanal	73	A	T	T	A	57	T	250	42	114	A	A
11	Phenyl acetaldehyde	600	569	1076	914	783	891	419	5609	965	147	99	797
12	Benzyl alcohol	67	193	229	119	130	A	T	1043	131	806	31	T
13	Cis-linalool oxide	77	252	1047	833	192	160	767	359	206	719	20	15
14	Trans-linalool oxide	196	A	1516	1552	0	0	1614	0	0	1745	60	55
15	Linalool	469	869	1510	1838	A	528	2542	1326	500	1313	96	A
16	Phynyl ethyl alcohol	T	A	A	533	556	0	575	2217	0	210		122
17	Alfa-terpineol	T	T	A	A	A	A	617	A	A	455	17	10
18	Methyl salicylate	99	314	761	519	146	168	614	1402	292	1495	47	40
19	Cis-Geraniol	170	657	1764	1509	24	66	147	348	40	822	34	20
20	Geranyl acetate	T	A	302	124	T	A	T	206	93	181	A	A
21	β-Ionone	182	A	A	A	T	123	A	902	203	47	6	14
22	Nerolidol	T	248	A	A	A	A	A	A	A	453	A	14
23	Phytol*	2164	7245	A	A	1768	9009	1386	27370	5572	A	256	589
24	Indole	A	A	A	A	A	A	A	239	A	T	8	9
25	dihydroactinidiolide	A	A	A	A	A	A	A	A	A	A	7	10
	Total	3794	4627	10259	9559	2851	9144	10031	18630	5537	16713	2145	3578
	Terpene Index	0.81	0.63	0.69	0.73	0.89	0.91	0.97	0.82	0.94	0.82	0.83	0.77
	Yamanishi Botheju ratio	0.36	0.75	2.05	2.59	-	0.08	3.36	0.41	0.34	0.33	0.07	-
	Mahanta ratio	0.56	1.32	2.98	3.61	0.21	0.12	2.07	0.64	0.28	0.68	0.13	0.06

* Phytol is not included in the total volatiles as well as in mahanta ratio (refer results and discussion for details)

[@]Values expressed are mean of three experiments, Where A=Absent, T= Trace

Darjeeling medium, Darjeeling premium and Nilgiris high grown teas showed highest Mahanta ratios (2.07–3.61) for VFCs indicating the presence of more quantities of desirable VFC, which indicate better quality of flavour during the second season. Pandiar, Parajulie, Palampur G1 and Aibheel teas showed medium Mahanta ratios (0.56–1.32) indicating moderate quality with regard to flavour during this season. Chinchula, Assam AFTL, Nilgiris high grown CTC, Assam Magor and Rosekandy possess lowest mahanta ratio and indicate flavour is low. Yamanishi-Botheju ratios of these teas also support the above findings.

2.4.3. Profiling of the tea samples from third season (October-December)

2.4.3.1. Physico Chemical Constituents

The results of the physico-chemical fingerprint for the black tea samples from season-3 (October-December) are presented in Table 2.6.

The moisture content in teas varied from 2.8 to 7.7% and soluble caffeine content in tea brews of samples from third season ranged from 1.33 to 4.07%. Aibheel (1.33%) and Chinchula (1.39%) tea brew had lowest and that of Darjeeling premium (4.07%) had highest soluble caffeine content. Tea brews from Pandiar (3.94%), Assam BOP (3.92%), Palampur G_2 (3.72%), Kurti (3.54%), Assam AFTL (3.37%) and Parajulie (3.15%), had slightly lower soluble caffeine contents compared to Darjeeling premium. Tea brews from Darjeeling Medium (2.96%), Niligiri high grown (2.71%) and Assam Magor (2.24%) have the moderate soluble caffeine content.

Table 2.6. Season 3 (October-December) - Physicochemical analysis of black tea

Sample	Soluble Caffeine (%)	TSS (%)	Viscosity (mPa)	Moisture (%)	Bulk density (g/ml)		TF (%)	TR (%)	Total colour	Brightness (%)
					Loose	Packed				
Parajulie	3.15	0.25	11.9	4.86	0.38	0.42	0.74	12.27	2.76	16.18
(±)S.D.	0.04	0.0	0.0	0.01	0.02	0.0	0.04	0.05	0.01	0.04
Pandiar	3.94	0.15	10.4	5.0	0.38	0.42	0.61	8.55	1.83	18.92
(±)S.D.	0.03	0.0	0.0	0.0	0.03	0.01	0.02	0.04	0.02	0.05
Assam AFTL	3.37	0.15	10.2	7.0	0.39	0.45	1.36	16.29	4.90	23.46
(±)S.D.	0.01	0.0	0.0	0.0	0.0	0.0	0.07	0.06	0.03	0.03
Assam Magor	2.24	0.15	10.3	4.8	0.37	0.42	1.86	15.24	6.56	26.97
(±)S.D.	0.01	0.0	0.0	0.0	0.01	0.02	0.05	0.03	0.04	0.02
Darj. Medium	2.96	0.05	11.6	7.73	0.25	0.32	0.70	9.15	2.04	9.05
(±)S.D.	0.02	0.05	0.0	0.01	0.01	0.0	0.02	0.01	0.01	0.01
Darj. Premium	4.07	0.05	11.8	6.06	0.25	0.33	0.29	8.00	2.06	11.85
(±)S.D.	0.02	0.0	0.0	0.01	0.03	0.01	0.02	0.02	0.03	0.03
Nil-HG	2.72	0.25	11.1	5.06	0.25	0.32	0.54	8.42	1.38	18.58
(±)S.D.	0.02	0.0	0.0	0.01	0.0	0.02	0.03	0.04	0.04	0.05
Aibheel	1.33	0.25	11.5	4.40	0.45	0.49	1.26	15.00	5.45	21.84
(±)S.D.	0.01	0.0	0.0	0.0	0.02	0.0	0.04	0.05	0.04	0.03
Chinchula	1.39	0.25	10.3	5.80	0.42	0.46	0.98	16.80	5.23	17.03
(±)S.D.	0.01	0.0	0.0	0.0	0.0	0.03	0.02	0.03	0.03	0.02
Palampur G2	3.72	1.0	10.2	2.8	0.20	0.27	0.14	13.50	1.56	5.23
(±)S.D.	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02
Assam BOP	3.92	0.8	8.1	5.6	0.35	0.42	0.45	8.57	2.30	8.53
(±)S.D.	0.04	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.01
Kurti	3.54	0.6	6.8	6.4	0.39	0.43	0.82	13.80	4.82	14.04
(±)S.D.	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.05

Values expressed are mean ± S.D. of five experiments

The TSS in the tea brews during third season varied from 0.05 to 1.0%. Palampur G_2 (1.0%), Assam BOP (0.8%) and Kurti (0.6%) had higher TSS in tea brew. While Parjulie, Nilgiri high grown, Aibheel and Chinchula had moderate (0.25%) TSS in tea brew; whereas tea brews from Pandiar, Assam AFTL and Assam Magor had lower TSS of 0.15%. Darjeeling Medium and premium had lowest TSS (0.05%) in tea brew.

The results for viscosity showed that it had a narrow range of variation during the third season, ie. 6.80 to 11.90 mPa. Tea brews from Parajulie (11.9 mPa), Darjeeling premium (11.8 mPa), Darjeeling Medium (11.6 mPa), Aibheel (11.5 mPa) and Nilgiri high grown (11.1 mPa) had higher viscosities; whereas tea brews from Pandiar (10.4 mPa), Assam Magor (10.30 mPa), Chinchula (10.3 mPa), Assam AFTL (10.20 mPa) and Palampur G_2 (10.2 mPa) had comparatively lower viscosities. Tea brews from Assam BOP (8.1 mPa) and Kurti (6.8 mPa) had lowest viscosities respectively.

Bulk densities (Loose and compacted or packed) of the tea samples from third season ranged from 0.20 to 0.45 and 0.27 to 0.49 g/ml respectively. Aibheel and Chinchula teas can be marked by highest bulk densities (Loose and compacted) of 0.45 and 0.49 g/ml and 0.42 and 0.46 g/ml respectively, whereas Darjeeling Medium (0.25 and 0.32), Darjeeling premium (0.25 and 0.33) and Nilgiri high grown (0.25 and 0.33) can be characterized by their lower bulk densities (g/ml, loose and compacted); Palampur G_2 (0.20 and 0.27) can be characterized by the lowest bulk densities (g/ml, loose and compacted). On the contrary Assam BOP (0.35 and 0.42), Kurti (0.39 and 0.43), Parajulie (0.38 and 0.42), Pandiar (0.38 and 0.42), Assam AFTL (0.39

and 0.45) and Assam Magor (0.37 and 0.42) teas had more or less similar and moderate bulk densities (g/ml, loose and compacted).

2.4.3.2. Theaflavins and Thearubigins

The results of fingerprinting with respect to theaflavins, thearubigins, total colour and brightness for black tea samples from season-3 (Oct. – Dec.) are presented in Table 2.6. and figure 2.1.

It is evident from the results that theaflavin (TF) content varied from 0.14% (Palampur G_2) to 1.86% (Assam Magor) and thearubigin (TR content) from 8.0% (Darjeeling premium) to 16.80% (Chinchula). Palampur G_2 (0.14% and 13.50%), Assam BOP (0.45% and 8.57%), Darjeeling premium (0.29% and 8.00% and Niligiri high grown (0.54% and 8.42%) teas have the lowest TF and TR contents; whereas Darjeeling Medium (0.70% and 9.15%), Parajulie (0.74% and 12.27%), Kurti (0.82% and 13.80%) and Pandiar (0.61% and 8.55%) had the marginally higher TF and TR content. Teas from Aibheel (1.26% and 15.00%) and Chinchula (0.98% and 16.80%) had further high TF and TR content; whereas the Assam teas, Assam AFTL (1.36% and 16.29%) and Assam Magor (1.86% and 15.24%) can be marked by their highest TF and TR content.

Almost similar trend is observed in the results for total colour and brightness. Assam Magor (6.56 and 26.97%) and Assam AFTL (4.90 and 23.46%) can be marked by highest colour and brightness followed by Aibheel (5.45 and 21.84), Chinchula (5.23 and 17.03%) and Kurti (4.82 and 14.04%). Teas from Palampur G2 (1.56 and 5.23%) had lowest colour and brightness. Nilgiri high grown (1.38 and 18.58%), Parajulie (2.76 and 16.18%) and

Pandiar (1.83 and 18.92%) contained lower colour and moderate brightness. Assam BOP (2.30 and 8.53%), Darjeeling Medium (2.04 and 9.05%) and Darjeeling premium (2.06 and 11.85%) have more or less moderate colour and brightness.

2.4.3.3. Volatile flavour compounds (VFCs)

The results of the fingerprinting with respect to volatile flavour compounds for season-3 (Oct. – Dec.) are presented in Table 2.7 and figure 2.2 – 2.28. It is clear from the results that

1. The total quantity of volatiles is in the range of 3.30 – 22.61 mg / 100 g of tea sample during this season. Darjeeling premium and medium possess highest quantity (22.61 and 18.50 mg% respectively) of total volatiles, where as Kurti has lowest (3.30 mg %). Palampur G2, Nilgiri high grown, Assam AFTL, Pandiar and Chinchula contained higher amounts of total volatiles. Content of phytol is not considered for total volatiles, as it is product derived from chlorophylls during degradation.
2. Marker for Parajulie is lowest leaf aldehyde content (65 – 85 μg / 100g)
3. Marker for Pandiar is lower leaf alcohol content (150 – 180 μg / 100g) and lowest (40 – 60 μg / 100 g) benzaldehyde content.
4. Marker for Darjeeling premium is highest linalool content (3201 μg / 100g) and methyl salicylate (2870 – 2900 μg / 100 g) content.
5. Marker for Darjeeling Medium is highest α-terpineol content (2050 – 2090 μg / 100 g) and n-heptanal (620 - 650 μg / 100g) content
6. Marker for Nilgiris high grown is highest n-hexanal (2800-2830 μg / 100g) content.

7. Marker for Assam AFTL and Assam magor is the lowest leaf alcohol (0-20 μg / 100 g) content.
8. Marker for Aibheel is the lower content of geranyl acetate (70 - 90 μg /100 g).
9. Marker for Chinchula is the lowest (10 -30 μg/100g) content of n - hexenol.
10. Special marker for Kurti is the presence of Xylene (1375-1425 μg/100g).
11. Markers for Assam BOP are the lowest content of geraniol (0-20 μg / 100 g) and geranyl acetate (0-20 μg / 100 g) content.

Terpene index, Yamanishi-Botheju ratio and Mahanta ratio for the volatile flavour compounds are calculated and presented in table 2.7. The terpene index of Aibheel, Assam AFTL, Kurti, Assam BOP and Assam Magor is approaching unity (0.90 – 0.95) and indicating these to be hybrids dominated by *assamica.* Nilgiris high grown, Palampur G2, Chinchula, Darjeeling premium, Pandiar, Parajulie and Darjeeling Medium teas are found to be hybrids of *assamica and sinensis,* from their terpene indices (0.83, 0.80, 0.76, 0.69, 0.69, 0.66 and 0.60 respectively).

Table 2.7. Season 3 (October - December)- Black tea samples-volatile flavour compounds (VFC) μ g / 100 g[@]

Sl. No.	Compound	Para julie	Pan diar	Darjeelng Medium	Darjeel Premim	Assam AFTL	Assam Magor	Nilgiris HG	Aibh eel	Chin chula	Palam pur G2	Kurti*	Assam BOP
1	n-hexanal	362	1133	1227	1175	A	121	2817	A	216	A	31	168
2	Leaf aldehyde (E-2-hexenal)	75	1617	1662	1225	703	1112	413	453	1341	2581	1349	1959
3	Leaf alcohol (Z-3-hexenol)	1978	165	777	300	A	2	1710	90	1084	1890	97	12
4	*trans* / *cis*-2-hexenol	233	91	176	110	267	A	382	95	33	244	102	A
5	n-hexenol	68	A	205	830	T	T	321	A	22	503	A	43
6	n-heptanal	70	94	633	T	139	92	52	56	T	234	A	A
7	Benzaldehyde	66	49	72	145	167	131	78	63	57	165	39	38
8	(E,Z)-2,4-Heptadienal	A	A	A	A	A	A	A	T	130	323	T	A
9	(E,E)-2,4-Heptadienal	A	A	A	A	A	A	T	74	178	381	11	T
10	Nonanal	A	32	54	T	114	123	A	119	163	129	A	A
11	Phenyl acetaldehyde	1285	766	464	715	1106	646	329	696	506	133	1072	378
12	Benzyl alcohol	197	782	158	205	539	379	T	453	367	754	T	24
13	*cis*-linalool oxide	120	198	4705	5755	1194	821	1565	290	400	503	34	24
14	*trans*-linalool oxide	360	0	0	0	0	0	0	822	0	1287	105	55
15	Linalool	93	727	906	3201	1106	900	2071	1052	635	851	45	13
16	Phynyl ethyl alcohol	A	0	295	1350	A	0	A	0	152	511	200	133
17	α-terpineol	A	A	2072	T	203	A	A	A	A	285	10	41
18	Methyl salicylate	457	374	658	2885	1241	500	592	1225	295	1257	95	34
19	*cis*-Geraniol	292	409	3658	3925	128	177	714	138	316	657	15	9
20	Geranyl acetate	118	101	201	A	164	198	A	81	120	201	A	9
21	β-Ionone	188	188	176	230	103	77	T	162	A	105	33	18
22	Nerolidol	A	A	396	560	A	A	136	A	A	245	26	19
23	Phytol	3009	5075	A	A	1361	1102	796	3928	1177	A	1308	493
24	Indole	A	A	A	A	A	A	A	A	A	T	10	23
25	dihydroactinidiolide	A	A	A	A	A	A	A	A	A	A	24	26
	Total	5962	6726	18495	22611	7174	5279	11180	5869	6015	13239	3298	3026
	Terpene Index	0.66	0.69	0.60	0.69	0.95	0.90	0.83	0.94	0.76	0.80	0.92	0.91
	Yamanishi Botheju ratio	1.24	0.45	0.54	2.61	1.57	0.80	5.01	2.32	0.47	0.33	0.03	0.07
	Mahanta ratio	0.41	0.51	2.52	3.61	2.08	1.37	0.78	2.68	0.46	0.64	0.18	0.10

* Phytol is not included in the total volatiles as well as in mahanta ratio (refer results and discussion for details)

@ Values expressed are mean of three experiments, Where A = absent, T= trace

* Special marker-Xylene is present upto 1400 µg/100g tea

Darjeeling premium, Aibheel, Darjeeling medium and Assam AFTL teas showed highest Mahanta ratios (3.61 – 2.09) for VFCs indicating the presence of more quantities of desirable VFC, which indicate better quality of flavour during the third season. Assam Magor, Nilgiris high grown, Palampur G2 and Pandiar teas showed medium Mahanta ratios (1.37 – 0.56) and are moderate quality with regard to flavour during this season. Chinchula, Parajulie, Kurti and Assam BOP possess lowest mahanta ratio and indicating flavour is low. Yamanishi-Botheju ratios of these teas also support the above findings.

2.4.4. Profiling of the tea samples from fourth season (January-March)

2.4.4.1. Physico-chemical Constituents

The results of the physico-chemical analyses for Black Tea Samples from season-4 (January-March) are presented in Table 2.8.

It is clear from the results that the moisture content of teas varied from 4.6% to 7.1% and soluble caffeine content of the tea samples from fourth season ranged from 2.03% to 5.28%. Assam Magor (5.28%) had highest soluble caffeine content followed by Chinchula (5.23%) and Assam AFTL (4.67%). Darjeeling Premium, Parajulie, Aibheel and Nilgiri high grown have comparatively moderate quantity (in the range of 3.60-4.50%) of soluble caffeine content; whereas Pandiar (3.28%) and Darjeeling Medium (3.21%) have still lower soluble caffeine content. Assam OP, Nilgiris Waynad CTC-BOP and Annamalai teas had lowest soluble caffeine content (2.03-2.84%).

Table 2.8. Season 4 (January-March) - Physicochemical analysis of black tea

Sample	Soluble Caffeine (%)	TSS (%)	Viscosity (mPa)	Moisture (%)	Bulk density (g/ml)		TF (%)	TR (%)	Total colour	Brightness (%)
					Loose	Packed				
Parajulie	4.16	0.15	12.3	5.13	0.38	0.43	0.83	10.20	2.48	15.43
S.D.(±)	0.02	0.00	0.00	0.02	0.01	0.01	0.02	0.04	0.06	0.05
Pandiar	3.28	0.15	10.60	4.73	0.39	0.43	0.64	7.80	1.91	17.36
S.D.(±)	0.01	0.00	0.00	0.01	0.02	0.02	0.03	0.05	0.05	0.04
Assam AFTL	4.67	0.15	10.3	6.93	0.39	0.43	1.39	16.26	4.55	22.81
S.D.(±)	0.02	0.00	0.06	0.03	0.01	0.01	0.02	0.06	0.04	0.06
Assam Magor	5.28	0.15	10.10	4.67	0.40	0.44	1.77	14.25	6.35	27.94
S.D.(±)	0.02	0.00	0.00	0.02	0.01	0.01	0.04	0.03	0.03	0.03
Darj. Medium	3.21	0.15	11.60	7.07	0.24	0.30	0.61	9.20	2.33	10.11
(±)S.D.	0.01	0.00	0.00	0.02	0.00	0.01	0.03	0.02	0.01	0.01
Darj. Premium	4.50	0.25	12.00	6.40	0.25	0.32	0.39	9.28	2.18	10.87
(±)S.D.	0.02	0.00	0.00	0.00	0.01	0.00	0.02	0.02	0.01	0.02
Nil-HG	3.67	0.15	11.4	4.8	0.33	0.38	0.65	8.94	1.77	15.90
S.D.(±)	0.01	0.00	0.00	0.01	0.01	0.00	0.03	0.03	0.03	0.05
Aibheel	3.88	0.25	11.00	4.80	0.44	0.48	1.50	16.56	6.16	21.78
(±)S.D.	0.01	0.00	0.00	0.00	0.01	0.01	0.04	0.04	0.02	0.01
Chinchula	5.23	0.25	10.60	5.20	0.41	0.45	0.88	15.78	4.10	15.45
(±)S.D.	0.02	0.00	0.00	0.00	0.00	0.01	0.03	0.05	0.02	0.01
Assam OP	2.84	0.6	5.6	5.8	0.24	0.31	0.27	14.56	3.94	2.52
(±)S.D.	0.02	0.01	0.02	0.02	0.03	0.01	0.01	0.03	0.01	0.01
Nil.Wynad CTC-BOP	2.03	0.4	6.2	6.0	0.41	0.47	0.75	12.69	3.60	16.61
(±)S.D.	0.01	0.01	0.01	0.03	0.02	0.01	0.03	0.01	0.01	0.03
Annamalai	2.78	0.4	4.8	4.6	0.43	0.49	0.64	11.83	3.49	10.24
(±)S.D.	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.02

Values expressed are mean ± S.D. of five experiments

Tea brews of Assam OP, Nilgiris Waynad CTC-BOP and Annamalai teas had the highest total soluble solids content (0.4-0.6%) compared to Darjeeling Premium, Aibheel and Chinchula which have the higher total soluble solids (0.25%) content; whereas tea brews from Parajulie, Pandiar, Assam AFTL, Assam Magor, Darjeeling Medium and Nilgiri high grown have the lowest total soluble solids (0.15%) content.

The results for viscosity of tea brews ranged between 4.8 and 12.3 mPa. Parajulie (12.30 mPa) and Darjeeling Premium (12.0 mPa) had highest viscosities. Darjeeling Medium (11.60 mPa), Nilgiri high grown (11.40 mPa) and Aibheel (11 mPa), have higher viscosities. Pandiar, Chinchula, Assam AFTL and Assam Magor had moderate viscosity in the range of 10.60-10.00 mPa. Assam OP, Nilgiris Waynad CTC-BOP and Annamalai teas had the lowest (4.80 -6.20 mPa) viscosities.

Darjeeling Medium (0.24 and 0.30 g/ml), Darjeeling Premium (0.25 and 0.32 g/ml), Assam OP (0.24 and 0.31 g/ml) and Nilgiri high grown (0.33 and 0.38) have the lowest bulk densities (Loose and packed). Aibheel (0.44 and 0.48 g/ml), Chinchula (0.41 and 0.45 g/ml), Nilgiris Waynad CTC-BOP (0.41 and 0.47 g/ml) and Annamalai teas (0.43 and 0.49 g/ml) had the highest bulk densities (Loose and compacted). On the contrary Assam AFTL (0.39 and 0.43 g/ml), Assam Magor (0.40 and 0.44 g/ml), Pandiar (0.39 and 0.43 g/ml) and Parajulie (0.38 and 0.43 g/ml) had more or less same and moderate bulk densities (loose and packed).

2.4.4.2. Theaflavins and Thearubigins

The results of fingerprinting of theaflavins, thearubigins, total colour and brightness for black tea samples from season-4 (January-March) are presented in Table 2.8 and figure 2.1.

It is clear from the results that the TF and TR contents of the tea samples from fourth season (January-March) ranged between 0.27-1.77% and 7.80-16.56% respectively. Assam Magor (1.77 and 14.25%), Assam AFTL (1.39 and 16.26%) and Aibheel (1.50 and 16.56%) teas can be marked by highest TF and TR contents. Nilgiris Waynad CTC-BOP, Parajulie and Chinchula have comparatively moderate quantities for TF (0.75, 0.83, 0.88%) and TR (12.69, 10.20, 15.78%) respectively. Darjeeling Premium, Darjeeling Medium, Annamalai and Nilgiri high grown, can be marked by lower values of TF (0.39, 0.61, 0.64, 0.65 %) and TR (9.28, 9.20, 11. 83, 8.94, %) respectively. Assam OP tea had the lowest TF (0.27%) of all teas and moderate content of TR (14.56%). Pandiar tea had the lowest TR (7.80%) of all teas and moderate content of TF (0.64%).

Total colour varied in the range of 1.77-6.35 and brightness varied in the 2.52-27.94%. Not only for TF and TR but also for total colour and brightness the three teas viz., Assam Magor - 6.35 and 27.94%; Assam AFTL - 4.55 and 22.81%; and Aibheel 6.16 and 21.78% showed the presence of higher values. Assam OP had showed the lowest brightness (2.52%) and moderate colour (3.94), where as Nilgiri high grown and Pandiar showed lowest colour (1.77 and 1.91 respectively) and moderate brightness (15.90 and 17.36% respectively). Parajulie, Nilgiris Waynad CTC-BOP and Chinchula have

comparatively moderate values for total colour (2.48, 3.60 and 4.10) and brightness (15.43, 16.61 and 15.45%) respectively. Although, Annamalai teas has moderate total colour (3.49) the brightness (10.24 %) is lower. Darjeeling Medium and Darjeeling Premium can be marked by comparatively lower values of total colour (2.33 and 2.18) and brightness (10.11 and 10.87 %) respectively.

2.4.4.3. Volatile flavour compounds

The results of the fingerprinting with respect to volatile flavour compounds for black tea samples from season-4 (January-March) are presented in Table 2.9 and figure 2.2. – 2.28.

It is clear from the results obtained for fingerprinting of volatile flavour compounds that

1. The total quantity of volatiles are in the range of 1.8 – 61.49 mg / 100 g of tea sample during this season. Nilgiri high grown possesses highest quantity (61.49 mg %) of total volatiles, where as Assam OP has lowest (1.8 mg%).

Darjeeling Medium and Premium, Parajulie and Pandiar teas contained higher amounts of total volatiles. Content of phytol is not considered for total volatiles, as it is product derived from chlorophylls during degradation.

2. Marker for pandiar is highest (1700-1720 μg / 100 g) content of n-hexanal.
3. Marker for Darjeeling medium is highest (3800-3900 μg / 100 g) content of linalool.
4. Marker for Annamalai is lowest (30 – 50 μg / 100 gm) content of n – hexanal and leaf alcohol (18 μg / 100 gm).

5. Marker for Assam AFTL is lower (200 μg / 100 g) content of leaf alcohol.
6. Marker for Assam magor is the highest (11840 –11880 μg / 100 g) content of phytol.
7. Marker for Nilgiris highgrown is the highest content of leaf aldehyde (17480
    a. 17520 μg / 100 g), phenyl acetaldehyde (2850 –2890 μg / 100 g) and the phenyl ethyl alcohol (7410 – 7450 μg / 100 g).
8. Markers for Aibheel are the lowest contents of trans/cis –2-hexenol (10 –30 μg / 100 g), n – heptanal (15 – 35 μg / 100 g) and nonanal (25 –65 μg / 100 g)
9. Marker for chinchula is the lowest (245 –285 μg / 100 g) content of leaf aldehyde.

Table 2.9. Season 4 (January - March)- Black tea samples-volatile flavour compounds (VFC) μ g / 100 g@

Sl. No.	Compound	Para julie	Pan diar	Darjeel Medium	Darjeel Premium	Assam AFTL	Assam Magor	Nilgiri HG	Aibh eel	Chin chula	Assam OP	Ana malai	Nilgiris Waynad
1	n-hexanal	1259	1707	A	507	A	A	1333	1066	1399	T	35	A
2	Leaf aldehyde (E2–hexenal)	2108	2457	1197	1467	1435	1026	17500	533	265	434	889	1581
3	Leaf alcohol (Z-3-hexenol)	238	344	1094	667	200	90	1893	81	146	94	18	51
4	Transicis-2-hexenol	62	T	175	180	96	A	5190	21	253	A	A	A
5	n-hexenol	A	165	491	A	A	A	1440	T	A	A	15	79
6	n-heptanal	96	125	111	153	300	276	2357	25	280	A	55	A
7	Benzaldehyde	117	T	20	300	127	82	440	131	122	104	35	62
8	(E,Z)-2,4-Heptadienal	A	A	A	A	A	A	A	A	A	A	5	8
9	(E,E)-2,4-Heptadienal	151	122	149	333	119	A	428	72	A	7	9	14
10	Nonanal	117	T	308	313	211	194	452	45	A	A	A	A
11	Phenyl acetaldehyde	1352	793	1440	1780	1327	750	2869	908	908	382	355	350
12	Benzyl alcohol	605	518	684	167	A	A	2107	221	299	37	31	T
13	Cis-linalool oxide	654	558	2624	2827	1108	534	9917	468	582	31	28	22
14	Trans-linalool oxide			5141	5393						79	89	89
15	Linalool	1513	1357	3850	1873	1138	655	A	762	875	19	256	438
16	Phynyl ethyl alcohol		A	1513		A	A	7429	A	A	365	256	408
17	Alfa-terpineol	A	A	A	A	A	A	3702	A	A	36	26	36
18	Methyl salicylate	413	457	1739	2273	373	202	1238	216	329	36	25	41
19	Cis-Geraniol	639	698	4158	3120	A	A	1667	131	A	18	74	149
20	Geranyl acetate	346	113	145	A	177	A	A	21	A	19	8	7
21	β-Ionone	417	271	222	380	181	276	1524	138	134	33	52	44
22	Nerolidol	A	A	A	A	A	A	A	A	A	27	19	29
23	Phytol	799	5628	3205	3407	7285	11858	A	3101	8598	A	443	888
24	Indole	A	A	A	A	A	A	A	A	A	23	10	11
25	Dihydroactinidiolide	A	A	A	A	A	A	A	A	A	68	22	43
	Total	10087	9685	25061	21733	6792	4085	61486	4839	5592	1805	2312	3462
	Terpene Index	0.77	0.73	0.73	0.76	1.0	1.0	0.85	0.90	1.0	0.88	0.83	0.79
	Yamanishi Botheju ratio	0.72	0.55	3.22	1.28	0.79	0.64	-	1.43	3.30	0.04	0.29	0.27
	Mahanta ratio	0.86	0.61	4.55	3.47	1.05	0.88	0.54	0.77	0.65	0.52	0.54	0.48

* Phytol is not included in the total volatiles as well as in mahanta ratio (refer results and discussion for details)

@ Values expressed are mean of three experiments, Where A = absent, T= trace

2.4.5. Novel approach for overall quality based on Seasonal, regional variations and bio-chemical quality fingerprint

The following codes are given for each region / garden / location and season codes are given for the part of the year for convenience.

Table 2.10.Codes for Region / Grade / Garden

Region / Grade / Garden	Code
Tamilnadu, Parajulie	A
Tamilnadu, Pandiar	B
Darjeeling Medium	C
Darjeeling Premium	D
Assam AFTL	E
Assam Magor	F
Nilgiris HG	G
Dooars, Aibheel	H
Dooars, Chinchula	I
Palampur G1	J
Nilgiris HG-CTC	K
Dibrugarh, Rose kandy	L
Palampu G2	M
Assam, Cachar best	N
Assam, Cacher Med.	O
Darjeeling, Kurti	P
Assam BOP	Q
Nilgiris Waynad	R
Annamalai	S
Assam OP	T

Table 2.11.Season code for part of the year

Period	Season
April - June	S_1
July-September	S_2
October - December	S_3
January - March	S_4

2.4.5.1. Seasonal variation of TF/TR ratio over tea producing region / grade and quality

The TF content of a tea or the ratio TF/TR is considered to be a good quality indicator of tea. Accordingly Fig. 2.29 presents seasonal variations of TF/TR ratios over the coded tea producing regions / grades in all the four

seasons (s_1, s_2, s_3, s_4). Teas having TF/TR ratios upto 0.04, >0.04-0.08 and >0.08 can be considered to be good, better and best quality indicator of tea quality respectively. Teas from the region/grade A-I are the better (TF/TR ratios >0.04-0.08) to best (TF/TR ratios >0.08) quality teas over all the four seasons except for the teas from region A, C, D (s_1), which fall under good quality category considering their TF/TR ratios (upto 0.04).

Also the teas from region/grade K-L (s_2), region N, O (s_1), region PQ (s_3) and region RS (s_4) are the better (TF/TR ratios >0.04-0.08) quality teas except for the teas from region J (s_2), M(s_3) and T(s_4) teas, which fall under good quality category considering their TF/TR ratios (upto 0.04).

The teas from region/grade JKL (s_1, s_3), M –T (s_2), ORST (s_3), MP (s_1), Q (s_4) are also falling under good quality category teas, considering their TF/TR ratios (upto 0.04).

2.4.5.2. Seasonal variation of sum of Yamanishi-Botheju and Mahanta ratio over tea producing region/grade and tea quality

The VFC (Volatile Flavour Compounds) content of a tea or the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) is considered to be a good quality indicator of tea. Accordingly Fig. 2.30 presents seasonal variations of or the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) over the coded tea producing regions / grades in all the four seasons (s_1, s_2, s_3, s_4). Accordngly based on the sum of the VFC ratios (i.e.Yamanishi-Botheju ratio and Mahanta ratio) the teas can be categorized as a good (upto 1), better (>1-4) and best (>4) quality indicator of tea respectively.

The teas from regions/grade A-J (all seasons) have better (>1-4) to best (>4) quality as indicated by sum of the VFC ratios (i.e.Yamanishi-Botheju ratio and Mahanta ratio) except for teas from regions/grade AEFHIJ(s_2),K-T (s_2), BDFHIJ(s_1), BIJ(s_3) which are good (upto 1) quality teas, as indicated by sum of the two VFC ratios. Also the teas from regions/grade M (s_3), O (s_1), P (s_3), RST (s_4) are good (upto 1) quality teas as indicated by sum of the two VFC ratios.

2.4.5.3 Seasonal variation of Borse-Rao quality index over tea producing region/grade and tea quality

The sum of TF/TR ratios of tea and the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) added together is proposed for the first time as a new and novel quality index, hence forth referred to as Borse-Rao quality index, considered to be an overall quality indicator of tea as both the non-volatiles/volatiles are given due consideration in this quality index. Accordingly Fig. 2.31 presents seasonal variations of the Borse-Rao quality index over the coded tea producing regions / grades in all the four seasons (s_1, s_2, s_3, s_4). Accordingly based on the the Borse-Rao quality index teas can be categorized as good (upto 1), better (>1-4) and best (>4) quality tea respectively.

The teas from regions/grade having Borse-Rao quality index more than four are C (s_2, s_4), D (s_3, s_4), G (s_1, s_2, s_3), H (s_3) and I (s_4) are the best (>4) quality teas.

The teas from regions/grade having Borse-Rao quality index ranging from one to four are A (s_1, s_2, s_3, s_4), B (s_2, s_3, s_4), C (s_1, s_3), E(s_3, s_4), F(s_3, s_4),

H (s_2, s_4), I (s_3, s_4), J(s_2), M (s_3) and RS (s_4) and indicate that these are better (>1-4) quality teas.

The rest of the teas from regions/grade having Borse-Rao quality index upto one are good quality teas which are BDFH (s_1), EF (s_2), I (s_1, s_2), JKL (s_3), K-T (s_2) and P-T (s_3).

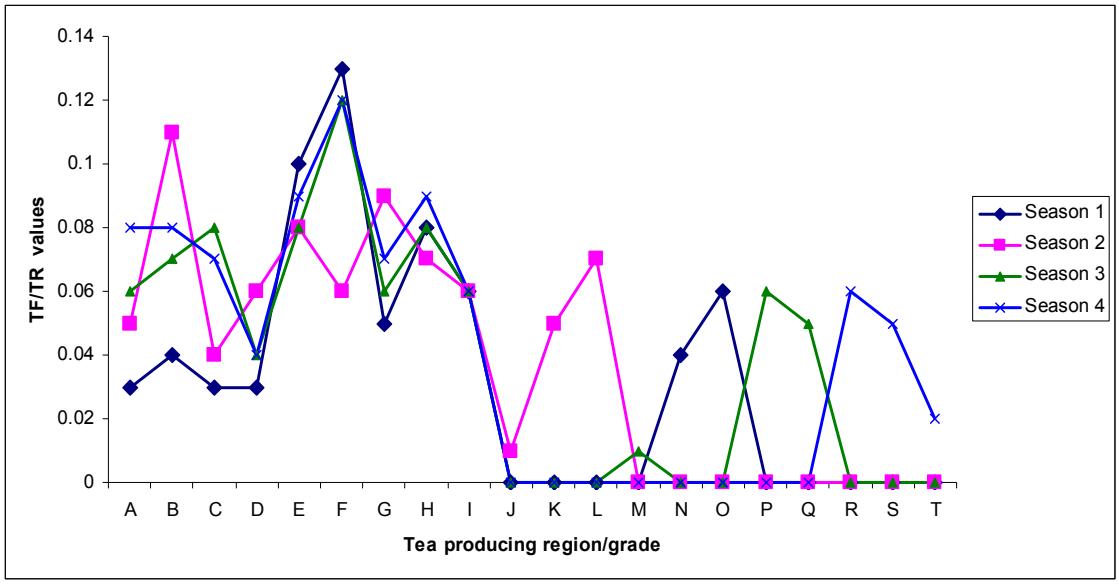


Fig. 2.29. Seasonal variation of TF/TR ratio over tea producing region/grade

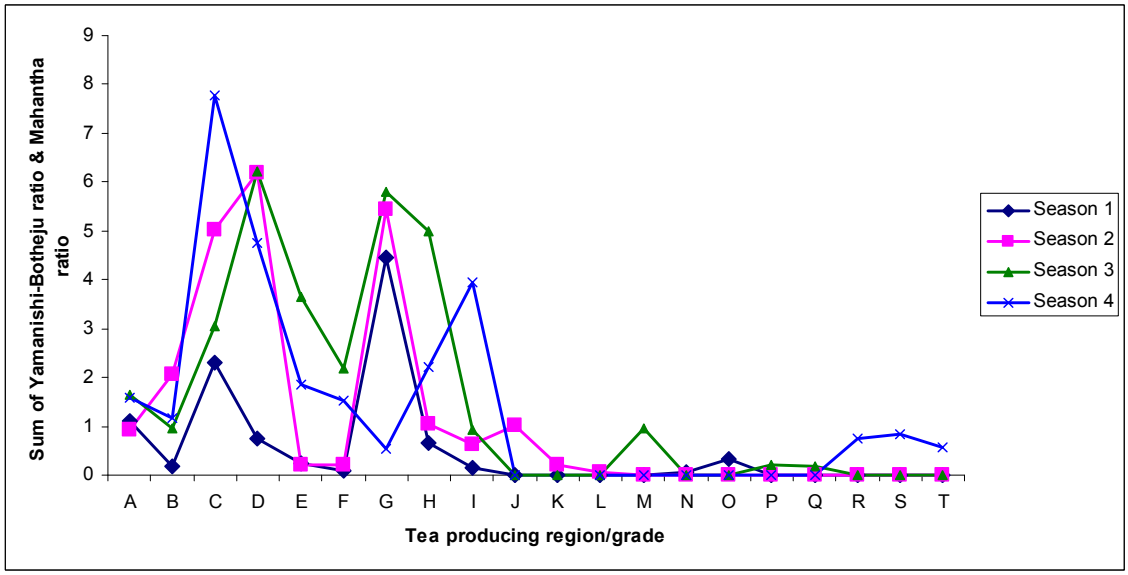


Fig. 2.30. Seasonal variation of sum of Yamanishi-Botheju and Mahantha ratio over tea producing region/grade

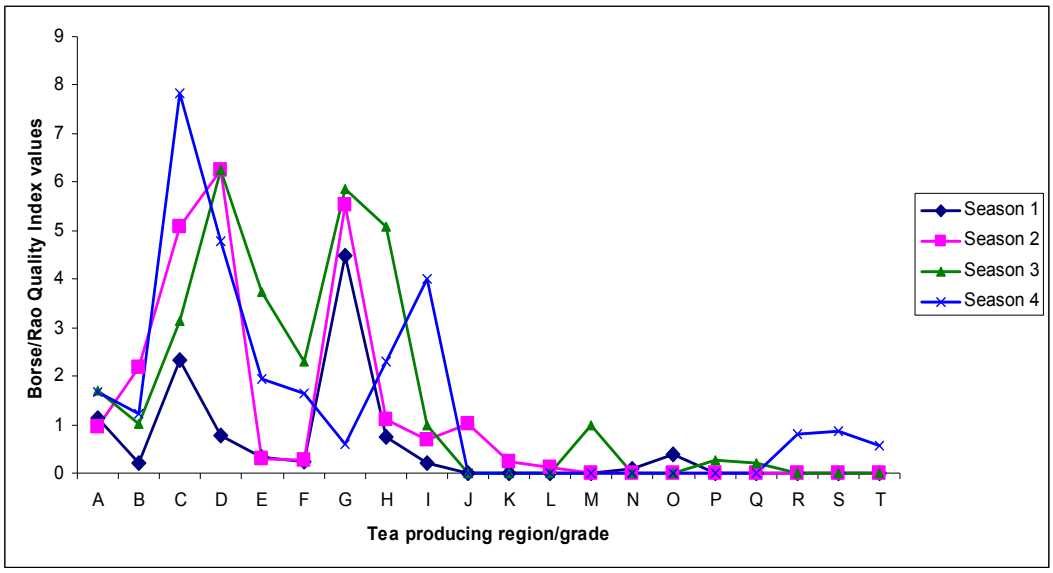


Fig. 2.31. Seasonal variation of Borse-Rao quality index over tea producing region/grade

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CHAPTER 3

FUNCTIONAL INGREDIENTS FROM UNUSED GREEN TEA LEAVES: ACTIVITY AND APPLICATIONS

3.1. INTRODUCTION

Green tea is one of the most popular and widely consumed beverages in Japan and China since several centuries. Drinking tea is a culture by itself and is performed as tea ceremony in these countries. The characteristic aroma of a green tea is an important attribute which determines its acceptability coupled with the other attributes such as colour, taste and appearance. These attributes are governed by the source and quality of tea leaves used besides the method of processing. The typical process of green tea manufacture includes pan firing or steaming by which the inactivation of the enzymes in tea is achieved while retaining the green colour. In general, Japanese process uses steaming whereas Chinese process uses pan firing resulting in green teas with characteristic aroma, taste and colour attributed to the respective processing method. A number of volatile compounds in green tea have been identified.

Tea is one of the widely used beverages. Green teas and their extracts, which consist of polyphenols (principally catechins) possess lot of health benefits (i.e., prevention of hypertension, cardiovascular diseases, dental caries and some types of cancer; strengthening the walls of blood vessels and regulating their permeability). Tea is also a foreign exchange earning commodity for our country. However, in the recent years, the prices for Indian CTC teas are decreasing due to various reasons such as low quality and competition from other countries. There is a need for research / study to find

out alternate uses and value added products, besides utilizing the tea agro waste. In this context, attempts are being made to utilize the coarse and pruned teas leaves for the purpose of preparation of green teas and their conserves.

Tea obtained from processed shoots of *Camellia sinensis* is one of the most popular non-alcoholic beverages in the world. The shoots, consisting of the tender apical bud and subtending two leaves, are processed to give the tea beverage. The main constituents, catechins, which constitute up to 30% on a dry weight basis (Millin, 1987), possess medicinal properties, namely, antioxidative, anticancerous, and antibacterial (Matsuzaki and Hara, 1995; Ding *et al*, 1992, Jankun *et al*, 1997, Yang, 1997). The main compounds present in tea leaves are (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate(ECG), (-)-epicatechin (EC), (+)-gallocatechin gallate (GCG), (+)-gallocatechin (GC), and (+)-catechin (C). During the green tea manufacturing process, some of the catechins undergo isomerization at the C-2 position of flavan-3-ol. For example, (-)-EGCG, (-)-EGC, (-)-ECG, and EC isomerize to (+)-GCG, (+)-GC, (+)-catechin gallate (CG), and (+)-C, respectively. These catechins, belonging to the flavanol group of phenols, remain un-oxidized in the green tea, as the enzymes are deactivated during the processing.

An antioxidant can be defined as any substance that when present at low concentration compared to that of substrate significantly delays or inhibits the oxidation (Precival, 1998). Green tea extracts are powerful antioxidants, mainly due to the presence of the above flavanols (Salah *et al*, 1995; Zandi and Gordon, 1995). These compounds are believed to have physiological

effects by acting as free radical scavengers, which are generated by metabolic pathways within body tissue or introduced by external sources such as foods, drugs, and environmental pollutants (Salah, *et al* 1995*;* Zhao, et al 1989; Quartley *et al*, 1994). Tea catechins are effective scavengers of free radicals and catechins having a galloyl moiety at C-3 (Rice-Evans and Miller, 1996) and a tri-hydroxy structure in ring B (Nanjo *et al*, 1996) are more effective. Chlorophyll present in organic extract from green tea also affects the antioxidant activity of the extracts (Gutierrez Rosales *et al*, 1992). Besides these, caffeine, theophylline, and theobromine are the main methyl xanthines constituting the tea alkaloids and are important factors in determining the quality of green teas. Many epidemiological and preclinical studies strongly suggest that drinking green tea may lower the risks of cancer and cardiovascular disease. Moreover, other health beneficial effects including anti-inflammation and anti-obesity were reported (Dreosti *et al*, 1997, Tijburg *et al*, 1997). Despite several reports on the radical scavenging activity of green tea from two leaves and a bud, the radical scavenging activity of green tea from coarse and pruned leaves in particular is not studied, and this material is presently being used as compost. The pruned and coarse tea leaves are tea plantation waste, after using up the two leaves and bud which are used for the manufacture of various types of teas. A huge quantity of this waste is available in India for value addition, because India is one of the largest producers of tea. Therefore, testing of its radical scavenging properties is of interest primarily in order to find new promising sources for natural antioxidants for functional foods and nutraceuticals as well as utilization of plantation waste. Use of natural antioxidants as food additives for inactivating

free radicals has received much attention recently due to the health consciousness of recent generations, as these consumers are more exposed to environmental pollutants such as those released from various types of vehicles and industries. The present chapter describes the preparation of green teas from the pruned or coarse tea leaves and optimization of extraction conditions to obtain radical scavenging conserve (Jagan Mohan Rao *et al,* 2005). Here, this waste (unused tea leaves) was also utilized to prepare the catechin rich conserve, and its radical scavenging activity was evaluated. The radical scavenging activities of these conserves are evaluated using the DPPH model system, and the results are presented along with the HPLC profiles of the active conserves for identification and quantification of composition. The nutraceutical characteristics of the polyphenols of coarse/pruned (low-grade) tea leaves were carried out with the following objectives.

- ❖ To isolate active conserves from coarse and pruned green tea leaves.
- ❖ To use the active conserves for food applications

3.2. MATERIALS AND METHODS

3.2.1. Chemicals and Reagents

The analytical grade solvents (viz., methanol, ethyl acetate, and formic acid), Folin-Ciocalteu's reagent, and sodium carbonate were procured from Merck (India). Tea catechins [viz., (+)-catechin (Cat, 98%), (+)-epicatechin (EC, 98%), (-)-epigallocatechin (EGC, 98%), (-)-epicatechin gallate (ECG, 98%, (-)-epigallocatechin gallate (EGCG, 95%), (-)-gallocatechin gallate (GCG, 98%)], alkaloids [viz., theophylline (TP, 98%), theobromine (TB, 98%),

caffeine (C, 99%), gallic acid, and 1,1-di-phenyl-2-picrylhydrazyl radical (DPPH) were procured from Sigma-Aldrich Chemical Co (St. Louis, MO, USA).

3.2.2. Plant Material

Coarse, pruned (low grade) and normal green tea leaves were collected from a tea estate in the Nilgiri-waynad region (Tamilnadu, India). Normal (control) leaves are plucked as one bud and two leaves from the tea plant, whereas coarse material comprised the remaining leaves of the shoot.

3.2.3. Moisture

The amount of moisture in the tea samples was measured using a vacuum oven (ISO 1573, 1980) to confirm the drying process to a moisture content of 4-6%. The samples were dried (70°C, 100 mm Hg, 6-7 hr.) to a constant weight and loss in weight (moisture) was calculated and reported as percentage moisture content.

3.2.4. Enzyme Inactivation

Enzymes (including polyphenol oxidase) of the fresh tea leaves were inactivated immediately by drying using two different kinds of dryers, namely, crossflow and infrared dryers, having different drying conditions (Borse, *et al*. 2004, 2005). Pruned, normal and coarse fresh tea leaves were procured and subjected to enzyme inactivation using cross flow dryer (CFD), Precision products, Vatva, Ahemadabad, India (CFD; 80-120^{o}C, 4 – 8h; (Fig.3.1) and continuous infra red dryer (IRD), designed and developed at CFTRI, Mysore, India (IRD; 70-120^{o}C, 0.5-1.5h; Fig.3.2) at different temperature and time intervals.

3.2.5. Size Reduction

The dried green teas were then subjected to size reduction by grinding in a mixer to a particle size of 30 µm and stored in suitable polypropylene bags at 4°C under refrigerated conditions until further use.

3.2.6. Chemical composition of volatiles from Green teas

3.2.6.1. Isolation of the volatiles

Double distilled water (75-80ºC, 2L) was added to 100g of green tea in a round bottom flask (3L) along with an internal standard viz., ethyl caproate (250 µg). In another round bottom flask (250 ml), petroleum ether (40-60 fraction) and diethyl ether with 1 ml methanol was added and connected to both arms of Likens - Nickerson apparatus. The simultaneous distillation and solvent extraction (SDE) were carried out by heating both the round bottom flasks and by condensing the vapours using chilled water circulation for 3h. The contents of the flask containing solvent along with aroma volatiles were concentrated by distillation using chilled water condenser followed by concentration using nitrogen gas flush to 100 µl and preserved at 4^{o}C for further analysis. The volatiles were isolated from all the green tea (CRD and IRD) samples and concentrated similarly.

3.2.6.2. Gas chromatographic-mass spectrometric (GC-MS) analysis of volatile constituents.

Shimadzu GC-17A equipped with QP-5000 (Quadrupole) mass spectrometer equipped with fused silica capillary column SPB-1, coated with polydimethylsiloxane of 30 M length and 0.32 mm internal diameter and film thickness 0.25 µm was used for the separation of the green tea volatiles. Helium was the carrier gas with a flow rate of 1 ml / min. The injection port

temperature and detector port temperature were maintained at 220ºC. Oven temperature programme: 40ºC (3)-2ºC/min-100ºC-4ºC/min-220ºC (7); Split ratio was 1:50 and ionisation voltage was 70 ev. A sample of 1µl was injected for each analysis. Identification of compounds was achieved by comparison of mass spectra and Kovats indices from literature (Adams, 2001; Davies, 1990; Jennings & Shibamoto, 1980), and fragmentation patterns in mass spectra were matched with those of the NIST62-LIB library and published mass spectra (Adams, 2001; Ten Noever de Bravw, *et al*, 1988).

3.2.7. Extraction of green teas

Dried and powdered green tea leaves (200g) were extracted with various solvents (e.g., acetone, ethyl acetate, methanol, ethanol, and their aqueous mixtures) for a period of 15 h using the Soxhlet apparatus. The material to solvent ratio used was 1:12. The extract was desolventized using a rotavapor at 50°C under reduced pressure. These extracts were evaluated for their polyphenol content and radical scavenging activity.

3.2.8. Determination of total phenolic content

Samples were analyzed for total phenolics content using the Folin-Ciocalteu's phenol reagent (Singleton and Rossi, 1965). Powdered tea samples and extracts (0.5 g) were introduced in test tubes, and methanol + de-ionized water (70:30) solution was added, heated on a water bath maintained at 70 °C for 10 min. The samples were cooled to room temperature and subjected to centrifugation. The supernatant was mixed with saturated sodium carbonate and Folin-Ciocalteu's reagent. The mixture was diluted to 10 mL with deionized water; tubes were mixed and incubated in the

dark for 60 min for colour development. The absorbance of this solution was measured using a UV-visible spectrophotometer (GBC Cintra 10, Australia) at 765 nm. The total phenol content of each extract in triplicate was estimated by comparison with a calibration curve generated from the analysis of gallic acid solutions and expressed as mean (SD) percent of gallic acid equivalents.

3.2.9. Determination of Radical Scavenging Activity

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) method, which has been widely used to evaluate the free radical scavenging ability of various samples (Jayaprakasha *et al*, 2001), was adopted here. Tea extract samples were dissolved in distilled methanol, and solutions of different concentrations (25, 50, 100, and 200 ppm) were prepared in different test tubes. Four milliliters of a 0.1 mM methanol solution of DPPH was added to these test tubes and shaken vigorously. The tubes were then incubated in the dark at room temperature for 20 min. A DPPH blank sample was prepared without any extract, and methanol was used for the baseline correction. Changes (or decrease) in the absorbance at 517 nm were measured using a UV visible spectrophotometer. The DPPH solution was freshly prepared daily, stored in a flask covered with aluminium foil, and kept in the dark at 4°C between measurements. All experiments were carried out in duplicate and repeated three times. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decrease in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

Radical scavenging activity= [control OD-sample OD/ control OD] x 100

The radical scavenging activity of BHA was also measured and compared with that of the various green tea leaf extracts.

3.2.10. Fractionation of the Extract

The extract was subjected to solvent-solvent extraction using water and solvent (low molecular weight ester) for 20-25 h using an all glass liquid-liquid extraction unit. Both aqueous and solvent-partitioned portions were desolventized in a rotavapor at 50°C under reduced pressure.

3.2.11. Identification of Catechins in the Solvent-Solvent Extract by HPLC

Several studies were reported for determining tea catechins and alkaloids separately using HPLC following either isocratic or gradient elution methods (Zhu and Chen, 1999, Wang, *et al* 2000). To determine the composition of the active conserves a simple HPLC isocratic elution method was developed, which efficiently separates various tea bio-chemicals / functional ingredients, namely, catechins and gallic acid.

Tea extract (10 mg) was dissolved in 10 mL of methanol, from this aliquot about 0.25 mL was taken and made up to 4 mL by methanol, and from this, 10µL of the sample was analysed by HPLC under the following conditions (Terasawa *et al*, 2001). The column used was a Spherisorb S10 OD52 (4.6 x 250 mm, Waters). The mobile phase was water/methanol/formic acid (19.5:80.2:0.3 v/v) under isocratic conditions at a flow rate of 1.0 mL/min using the Waters 515 HPLC pump. Peaks were observed at a wavelength of 280 nm using a UV-2487 dual wavelength absorbance detector (Waters).

The chromatograms of green tea extracts / conserves are presented in the following sections.

3.2.11.1. Preparations of calibration curves

Known concentrations (1-10 μg/μl) of individual catechins were subjected to HPLC analysis under the above conditions. The calibrations curves are prepared for individual catechins viz. (+)-C, (-)-EGC, (-)-EGCG, EC, (+)-GCG, (-)-ECG, (+)-GC and by drawing graph concentration vs peak area.

3.2.12. Preparation of products using tea polyphenol conserve

3.2.12.1. Tea and conserve:

The polyphenol-rich conserve was isolated from the unused green tea leaves using the methods described earlier in this chapter. The basic material such as tea leaves were procured from a tea plantation in Nilgiris, Wynad area (South India).

3.2.12.2. Preparation of nutraceutical ice-cream

Ingredients

Fresh milk, skim milk powder, liquid glucose, GMS [glycerol mono stearate] were obtained from the local commercial source.

Procedure

Fresh milk 1L was concentrated by evaporation to 400mL by heating in stainless steal vessel. Skimmed milk powder (35g), liquid glucose (30g), emulsifier (GMS-20mg), powdered sugar (100g) and milk cream (100g) were added to concentrated milk by continuous mixing. This mix was pasteurized (80-100^{o}C, 10-30min). The tea catechin conserve was suitably solubilised

and was added to give homogenous product. After pasteurization it was homogenized and whipping was carried out using a food processor at an interval of 2-3hr for 5 times and subjected to freezing during the time (12-14hr). The ice-cream was poured into plastic cups and subjected to freezing. The frozen ice-cream subjected to sensory evaluation.

Sensory analysis of nutraceutical ice-cream

Sensory analysis was carried out as per the standard conditions in a booth room maintained at a temperature of 20±2°C under fluorescent lighting equivalent to day light. Samples were preserved in porcelain plates with 3-digit number. As it is a consumer acceptance study samples were presented to the panelists together. 20 – 25 members participated in the sensory evaluation (Chambers & Wolf, 1996). Care was taken to avoid interferences and biases from other sources.

Two ice-cream samples namely, control and ice-cream enriched with tea antioxidant were evaluated for consumer acceptance. Ice-cream was served in coded glass plates. The product was evaluated using Hedonic test which indicates the degree of liking for the sample as judged by the respondents. The 7-point scale ranged from ‘Like Very Much’ (LVM) to ‘Dislike Very Much’ (DVM) with ‘Neither Like Nor Dislike’ (NLND) as the mid point.

3.2.12.3. Preparation of nutraceutical cookies

Ingredients

Maida, sugar powder, Ghee were procured from the local commercial source.

Procedure

Tea polyphenol conserve emulsion was prepared using suitable solvent and emulsifier by mixing thoroughly and disolventized using rotavapour at < 50^0C. The tea polyphenol conserve emulsion was dissolved in water (5 mL) and was used for product preparation.

Tea polyphenol conserve emulsion (5mL) was added to mix of maida (250g) and powdered sugar (100g) in a cleaned aluminium tray and ghee (150g) was added. All the ingredients were mixed / kneaded thoroughly so that dough was prepared. It was divided in to small balls (9-12g) to form cookies. The ghee was applied on to the surface of the baking tray. Formed raw cookies were placed in tray and baked at 90-100^{o}C for 90 min. The prepared cookies were subjected for sensory analysis.

Sensory analysis of nutraceutical cookies

Sensory analysis was carried out as per the standard conditions in a booth room maintained at a temperature of 20±2°C under fluorescent lighting equivalent to day light. Samples were preserved in porcelain plates with 3-digit number. As it is a consumer acceptance study samples were presented to the panelists together. 20 – 25 members participated in the sensory evaluation (Chambers & Wolf, 1996). Care was taken to avoid interferences and biases from other sources.

Cookies were served in coded glass plates. The product was evaluated using hedonic test which indicates the degree of liking for the sample as judged by the respondents. The 7-point scale ranged from ‘Like Very Much’ (LVM) to ‘Dislike Very Much’ (DVM) with ‘Neither Like Nor Dislike’ (NLND) as the mid point.

3.3. RESULTS AND DISCUSSION

Value addition to unused green tea leaves (coarse / pruned tea leaves)

3.3.1. Preparation of Green tea and quality evaluation

Normal, coarse and pruned fresh tea leaves (low grade) were procured and subjected to enzyme inactivation using cross flow dryer (80-120^{o}C, 4 – 8h), (Fig 3.1.) and continuous infra red dryer (70-120^{o}C, 0.5-1.5h) at different temperature and time intervals (Fig 3.2.).

The chemical parameters (*viz.,* caffeine, total polyphenols and moisture) of the inactivated and powdered tea leaves were analysed and sensory qualities were examined. The chemical parameters are found to be in the following range (Table 3.1.)- Caffeine (1.30-3.20%); Total polyphenols (11.5-15.5%).

Sensory characteristics were found to be similar to that of the commercial green tea samples (Table 3.2.). Flavour score of the brews varied from 5.50-8.25 on ten-point scale (commercial samples 6.00-7.00). Taste and mouth feel score of the brews varied from 5.50-7.00 on ten-point scale (commercial samples 6.00). Colour of the brews varied from 5.00-8.00 on ten-point scale (commercial samples 7.00-8.00).

Figure 3.1. Cross Flow Dryer

Figure 3.2. Infra Red Dryer

Table 3.1. Polyphenol and caffeine contents of green tea from coarse / pruned (low grade)/ unused tea leaves

Sample	Inactivation conditions		Sensory scores (ten point scale)			Remarks
	Dryer	Temp (^oC)	Colour	Flavour	Taste	
Coarse	CFD	110	7.75	7.5	7.0	Less astringency
	CFD	95	7.5	8.25	7.0	Less astringency
	IRD	100	8.0	7.0	7.0	--
Low grade	CFD	110	5.0	5.5	5.5	--
	IRD	100	6.0	7.0	7.0	--
Green tea*	--	--	8.0	6.0	6.0	Smoky flavour
Green tea*	--	--	8.0	7.0	6.0	Smoky flavour

* Commercial Samples – Nilgiris (Market)

Table 3.2. Sensory evaluation of the Green teas from Coarse/pruned (low grade) / unused tea leaves

Sample	Inactivation conditions		Polyphenols (%)	Caffeine (%)
	Dryer#	Temp (^oC)		
Coarse	CFD	110	11.95±0.02	1.36±0.01
Coarse	CFD	95	11.48±0.01	1.68±0.03
Coarse	IRD	100	14.57±0.03	1.33±0.01
Low grade	CFD	110	14.26±0.01	3.16±0.04
Low grade	IRD	100	15.13±0.03	2.97±0.02
Green tea*	--	--	14.10±0.01	2.51±0.01
Green tea*	--	--	15.20±0.04	2.82±0.02

CFD – Cross Flow Dryer; IRD – Continuous Infrared Dryer, *Commercial samples
Values are mean ± SD (n=3) (--) = no activity

3.3.1.1. Chemical composition volatiles from green teas from Coarse/pruned / unused tea leaves

Forty four compounds have been identified from the volatiles of green teas (Table 3.3, Fig 3.3.- 3.48.) by comparing retention times of the GC peaks with those of reference compounds run under identical conditions and by comparison of retention indices with literature data (Adams, 2001; Davies, 1990; Jennings & Shibamoto, 1980), and fragmentation patterns in mass spectra were matched with those of the NIST62-LIB library and published mass spectra (Adams, 2001; Ten Noever de Bravw, *et al,* 1988). The constituents were quantified using internal standard method. Ethyl caproate was used as internal standard. The broad classification of the compounds identified includes ten terpenoids, three aromatic compounds, eight alcohols, seven aldehydes, four acids, eight esters and four compounds derived from carotenoids. Ethyl hex-(2*E*)-enoate and dihydroactinidiolide are exclusively present in the green teas derived from coarse leaves.

In general, coarse green teas irrespective of the method of processing contained more number of the volatile constituents, whereas the normal green teas irrespective of the method of processing contained less number of volatile constituents eluting before linalool. This can be attributed to the normal leaf quality, which contributes less volatiles from the group of constituents (Gr. I) which are undesirable, which is the prevalent practice in the industry as well. It is also evident from the results that normal as well as a commercial sample of green tea contained almost half the number of identified volatile constituents (Table 3.3) in the present investigation as compared to the coarse green teas contained. Dihydroactinidolide and ethyl hexenoate were found to be important markers and both were present in

coarse green teas, whereas both were not found in the normal green teas including the commercial one. It can be concluded that the commercial green tea (Nilgiris green tea) is also prepared from the normal tea leaves. This can be very well used in spotting the normal / coarse green tea or the admixture of the coarse green tea leaves with the normal green tea leaves. Other important marker volatiles found were the both heptadienals [(E,Z)-2, 4 / (E,E)-2,4]. Coarse green tea contained both the heptadienals, whereas normal green teas did not contain any of the identified heptadienals in the present investigation. *cis*-3-hexenyl-n-hexanoate, ∝-ionone, *cis*-geranylacetone and β-ionone-5,6-epoxide were not found in a normal green tea. This can be attributed to the method of processing (CFD) and higher temperature (110°C) used.

Table 3.3. Chemical composition of volatiles from Green tea (μg/100g)

Sl. No.	RT	KI	Compound	Quantity (μg)					
				Coarse –CFD (95)	Coarse –CFD (110)	Coarse-IRD	Normal -CFD (110)	Normal -IRD	Nilgiris -GT Market #
1	4.2		Sec-hexyl alcohol	-	-	340	-	-	-
2	4.5	755	*cis*-2-penten-1-ol	524	634	540	-	-	-
3	5.0	775	Hexanal	179	353	280	-	-	-
4	6.8		*trans*-2-hexenal	-	254	-	-	-	-
5	7.4	841	*trans*-3-Hexenol	1625	214	-	-	-	-
6	7.9	854	*trans*-2-Hexenol	530	-	-	228	-	-
7	14.3		6-methyl-5-heptene-2-one	-	268	-	-	-	-
8	14.5	967	(*E,Z*)-2,4-Heptadienal	458	1590	360	-	-	250
9	15.2	980	(*E,E*)-2,4-Heptadienal	815	1951	600	-	-	367
10	15.6	985	Ethyl caproate (Int. std.)	250	250	250	250	250	250
11	16.8	1001	Phenyl acetaldehyde	1339	330	470	1110	569	-
12	17.0	1004	Benzyl alcohol	905	692	660	382	-	-
13	17.7	1015	Limonene	214	-	-	-	-	267
14	18.5	1027	Ethyl hex-(2E)-enoate	1167	179	250	-	-	-
15	20.4	1055	*cis*-Linalool oxide	554	362	420	213	250	300
16	21.4	1069	*trans*-linalooloxide	815	188	430	375	486	567
17	22.6	1085	Linalool	1875	839	2160	2118	2472	5033
18	27.3	1151	cis-Linalyl oxide (pyranoid)	345	-	-	199	250	400
19	27.9	1160	Methyl salicylate	292	-	-	-	-	-
20	28.4	1166	α-Terpineol	286	241	600	397	569	967
21	29.4	1179	Octanoic acid	268	268	330	-	250	0
22	33.3	1239	*cis*-Geraniol	821	326	830	647	833	1700
23	34.4	1257	Unidentified	214	214	320	-	-	-
24	35.1	1268	(*E,Z*)2,4-decadienal	411	728	420	199	-	-
25	35.5	1274	Nonanoic acid	179	330	-	-	194	317
26	36.3	1286	(*E,E*)2,4-decadienal	1155	2241	1070	206	236	450
27	40.1	1362	cis-3-Hexenyl-n-hexanoate	321	339	-	-	0	567
28	41.7	1395	α-ionone	512	522	460	-	194	367
29	42.8	1424	cis-geranylacetone	1208	1411	1010	-	208	733
30	43.8	1451	β-Ionone-5,6-epoxide	381	429	420	-	222	250
31	44.0	1455	β-Ionone	655	625	710	213	361	467
32	44.5	1468	2(4H)-Benzofuranone / Dihydroactinidiolide	488	504	530	0	0	367
33	45.3	1490	Di-tert-butyl phenol	381	183	890	713	292	583
34	45.6	1497	Pentadecane	345	232	310	257	236	483
35	46.2	1513	Vanillin acetate*	393	330	590	338	236	283
36	46.9	1535	3-*Z*-Hexenyl benzoate	190	0	0	0	0	0
37	47.2	1543	Nerolidol	851	1080	1210	346	319	633
38	47.7		Dodecanoic acid	-	-	1320	-	-	-
39	47.9		Geranyl butanoate	-	-	320	-	-	-
40	57.9		Myristicacid methylester	-	-	660	-	-	1850
41	58.0	1914	Butyl octyl phthalate	1137	0	440	831	1458	683
42	59.00	1956	Palmitic acid	9190	8357	31510	19551	37208	40700
43	62.6	2100	Phytol *	8869	4830	23450	22397	52806	46250
44	62.9	2114	Methyl linolenate *	4577	3491	15540	10154	25097	33600

* Identified tentatively, Market sample # (Nilgiris-Green Tea)

Values expressed are means of three replications

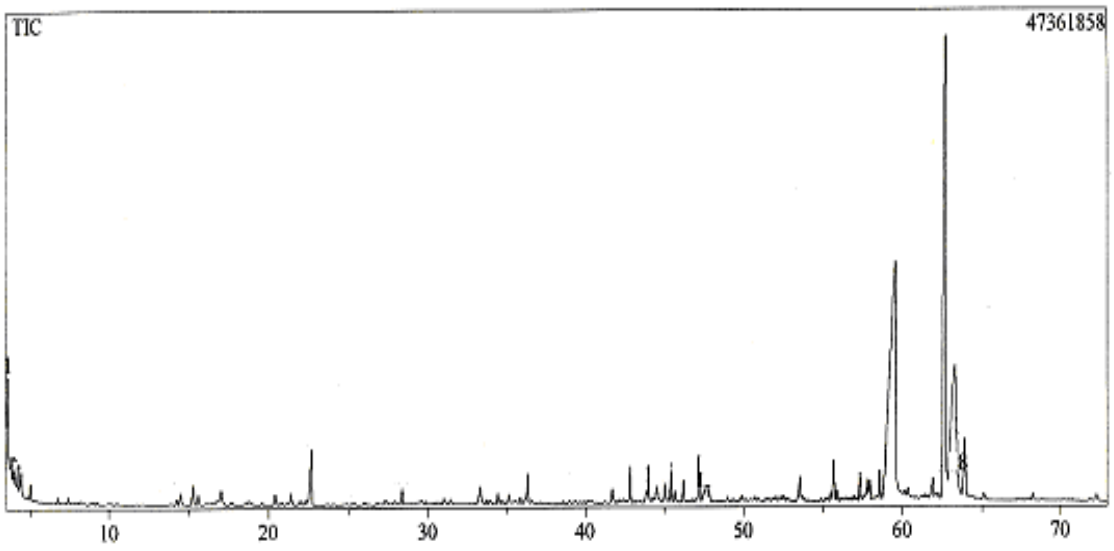


Figure 3.3.Total ion chromatogram of coarse IRD green tea volatiles

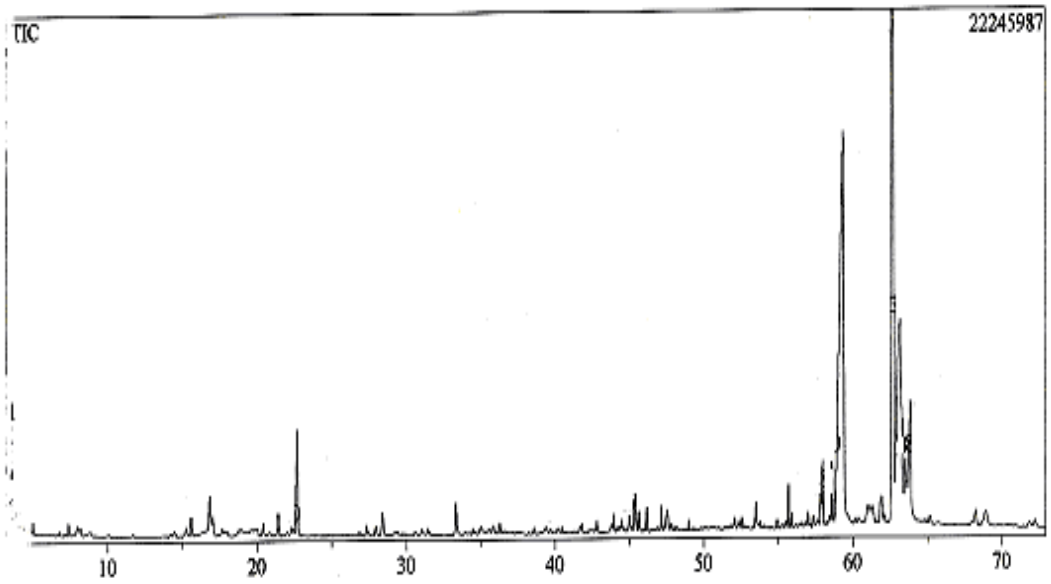


Figure 3.4.Total ion chromatogram of normal CFD green tea volatiles

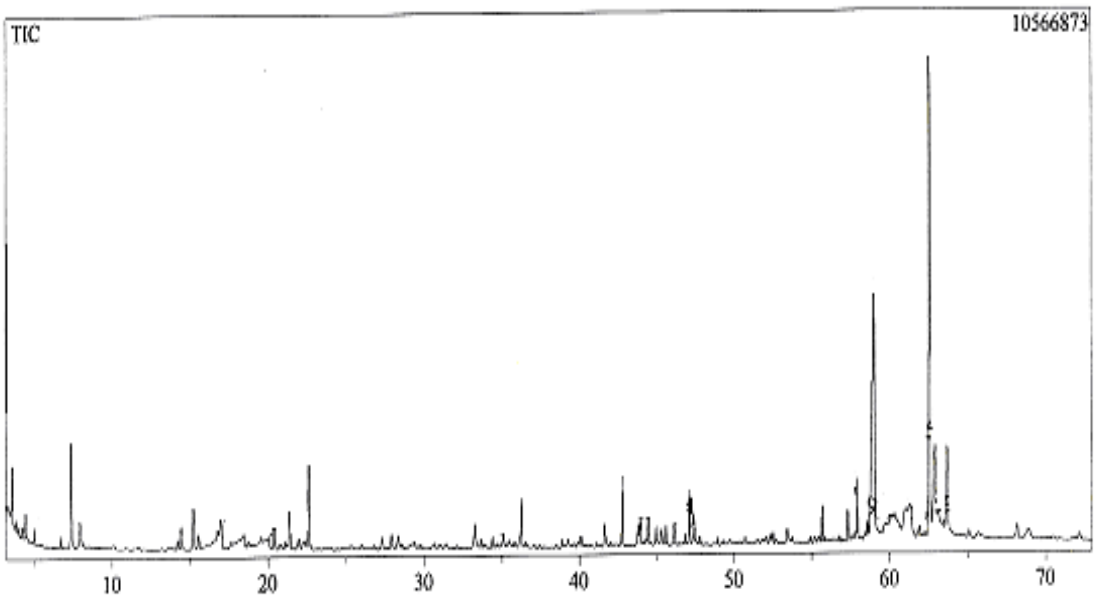


Figure 3.5.Total ion chromatogram of coarse CFD green tea volatiles

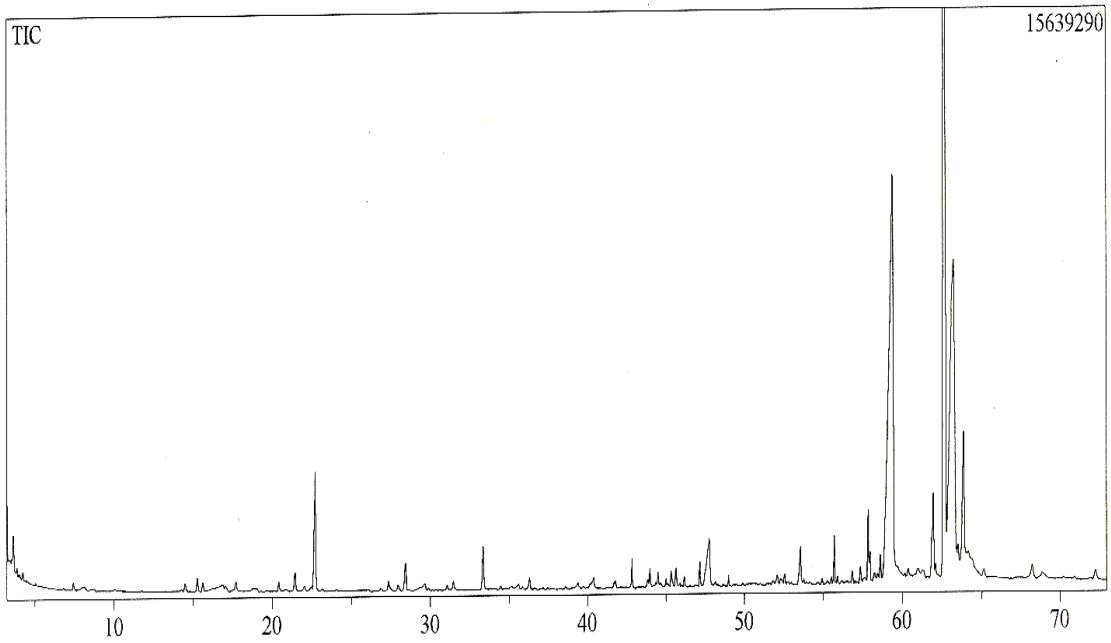


Figure 3.6.Total ion chromatogram of commercial sample from Nilgiris (Market) green tea volatiles

2-Hexanol

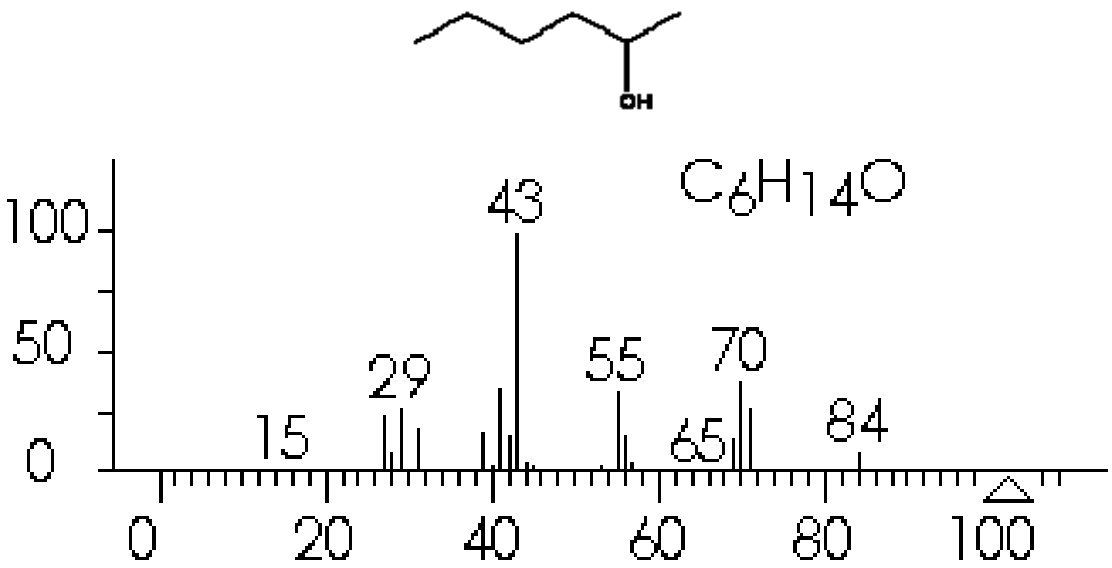


Fig.3.7. Mass spectrum of *Sec*-hexyl alcohol

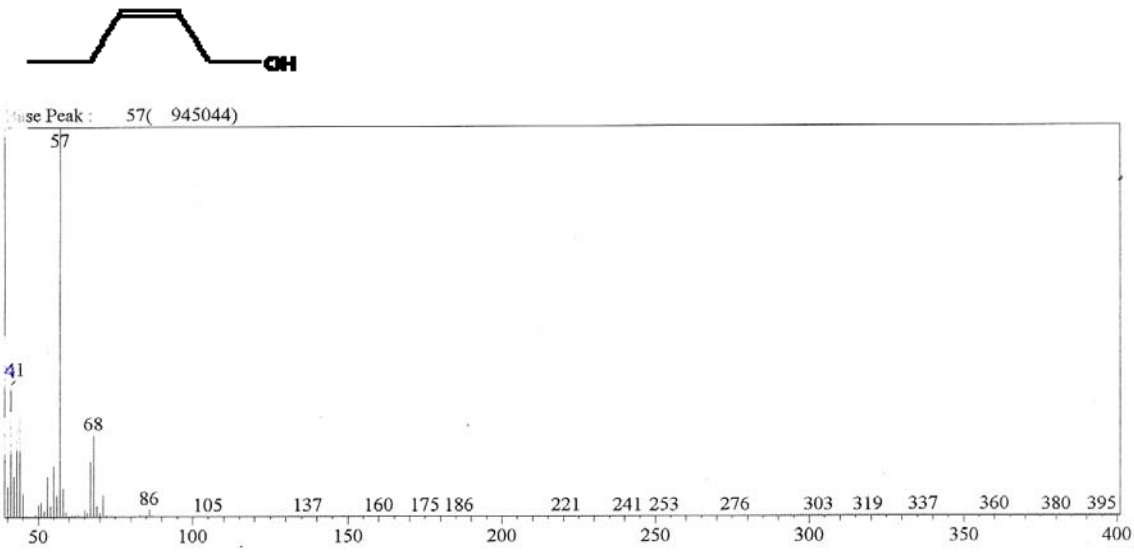


Fig.3.8. Mass spectrum of *cis*-2-pentenol

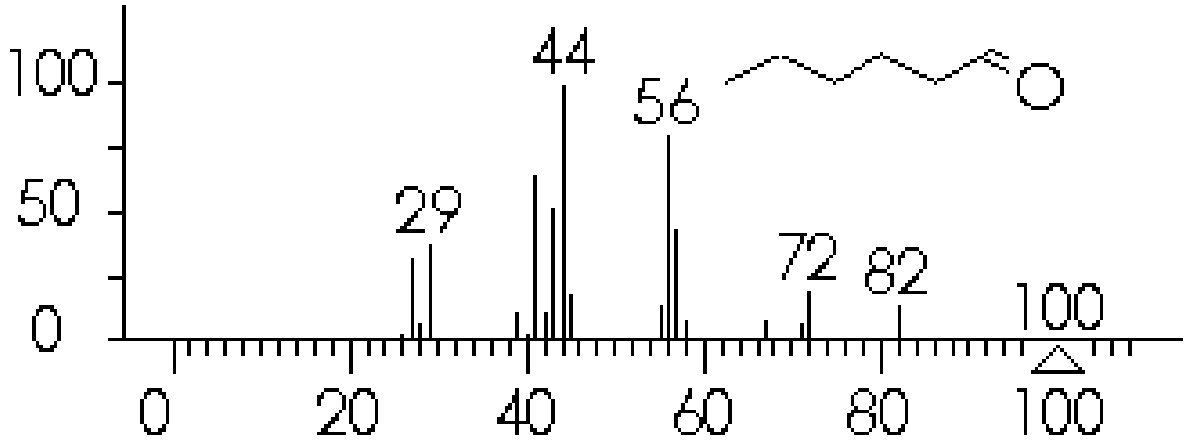


Fig.3.9. Mass spectrum of Hexanal

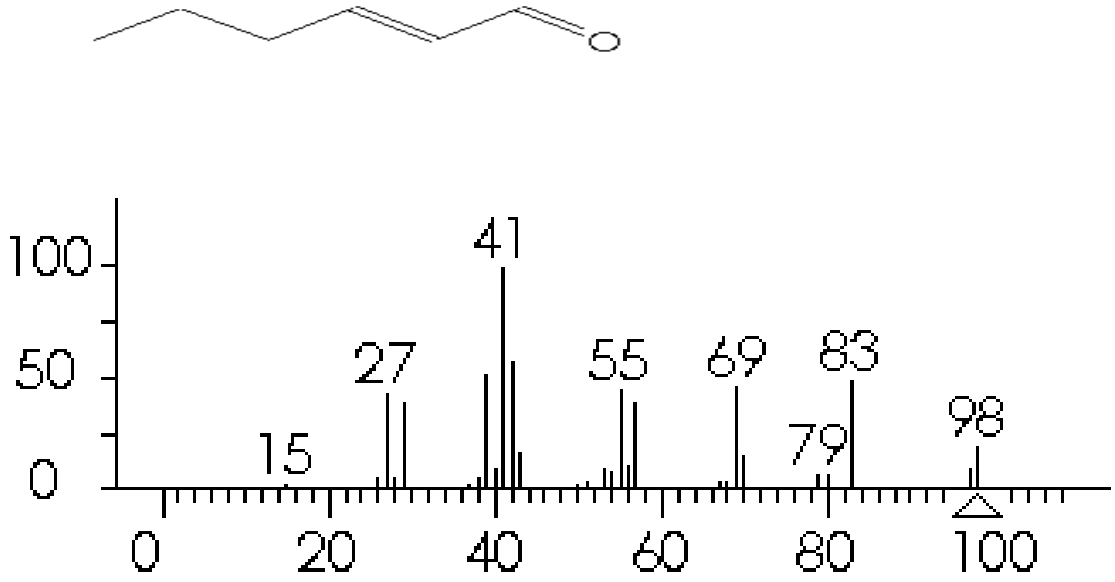


Fig.3.10. Mass spectrum of *trans*-2-hexenal

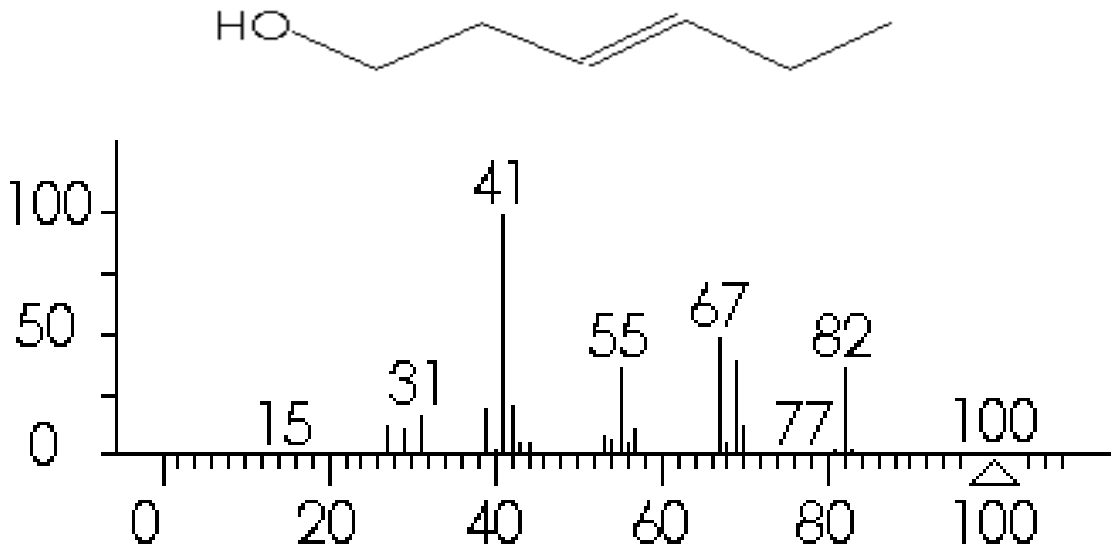


Fig.3.11. Mass spectrum of *trans*-3-Hexenol

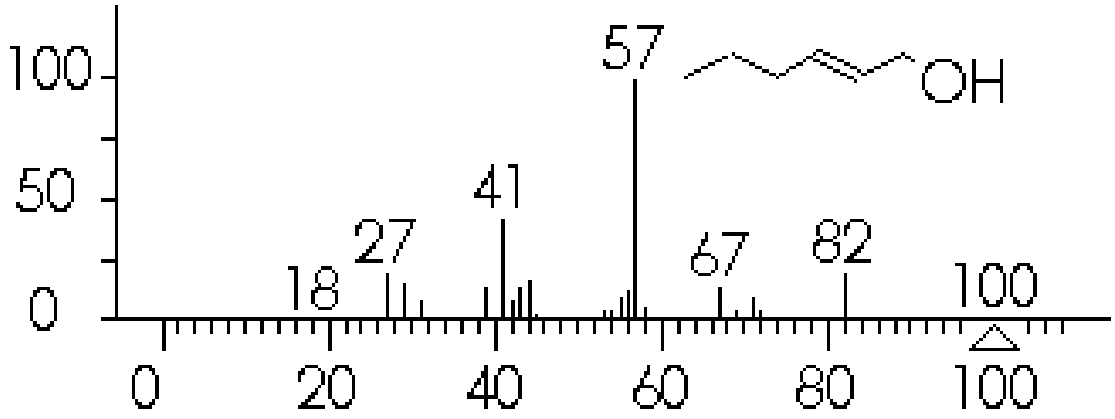


Fig.3.12. Mass spectrum of *trans*-2-hexenol

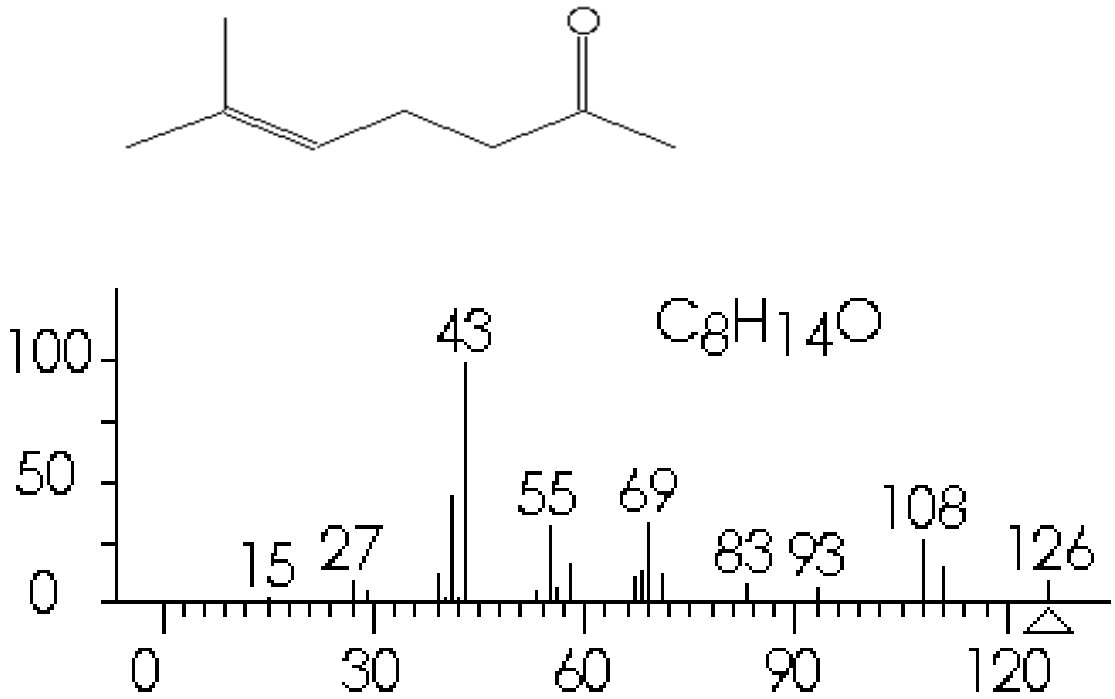


Fig.3.13. Mass spectrum of 6-methyl-5-heptene-2-one

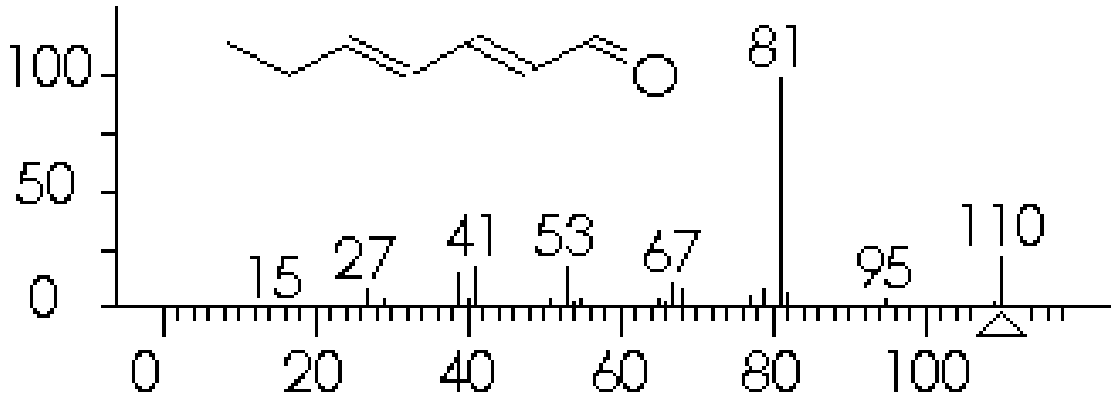


Fig.3.14. Mass spectrum of (E,E) 2,4-heptadienal

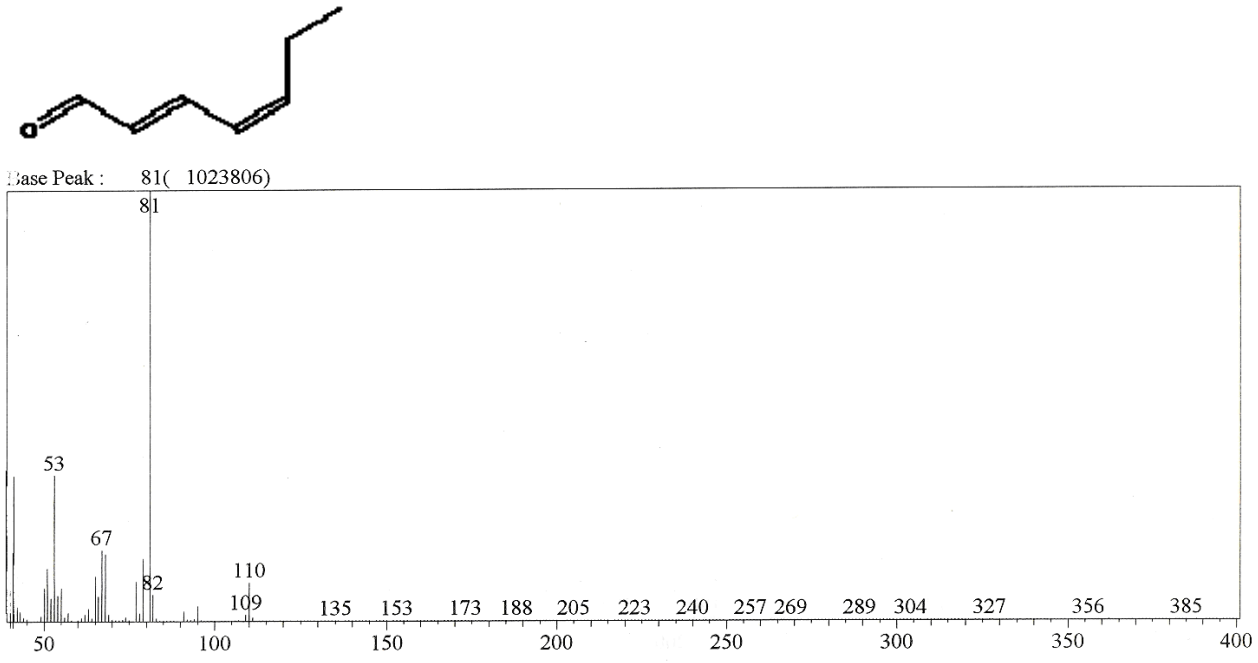


Fig.3.15. Mass spectrum of (E,Z) 2,4-heptadienal

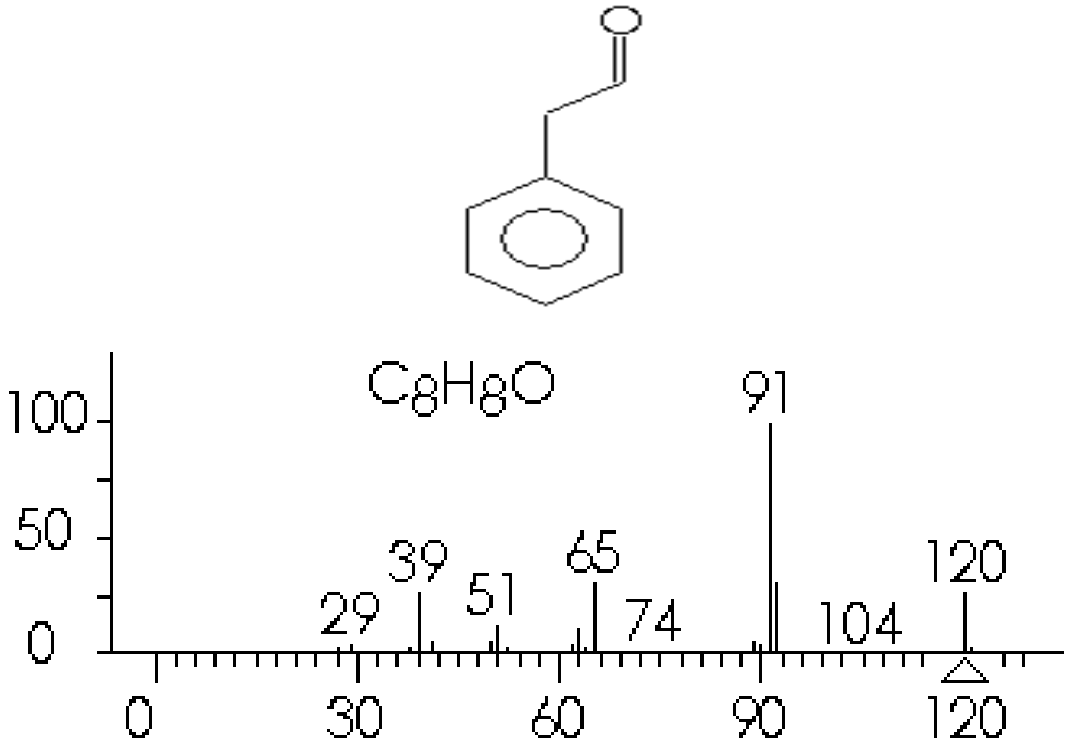


Fig.3.16. Mass spectrum of Phenyl acetaldehyde

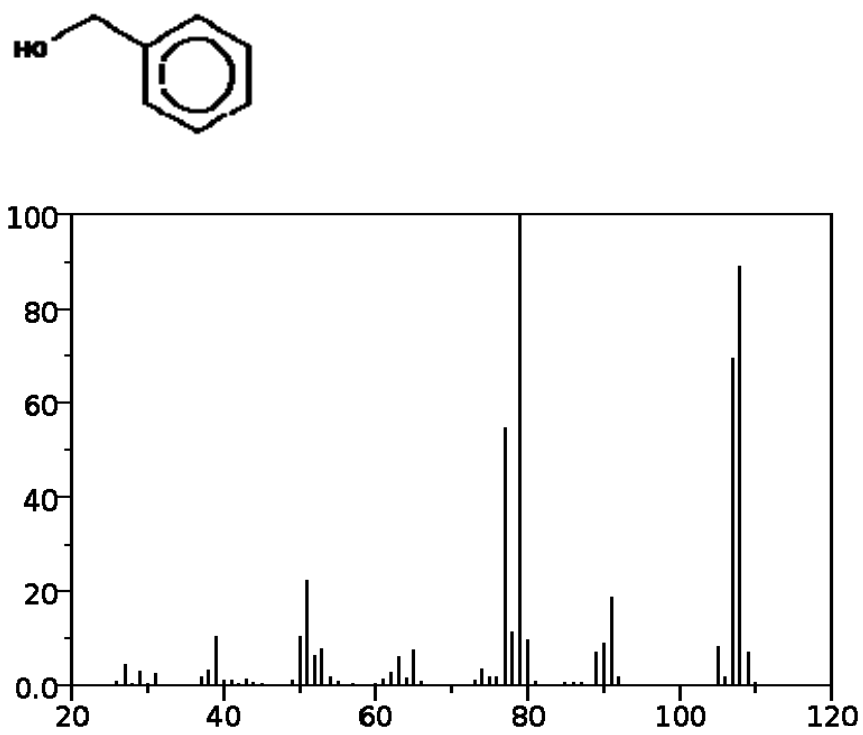


Fig.3.17. Mass spectrum of Benzyl alcohol

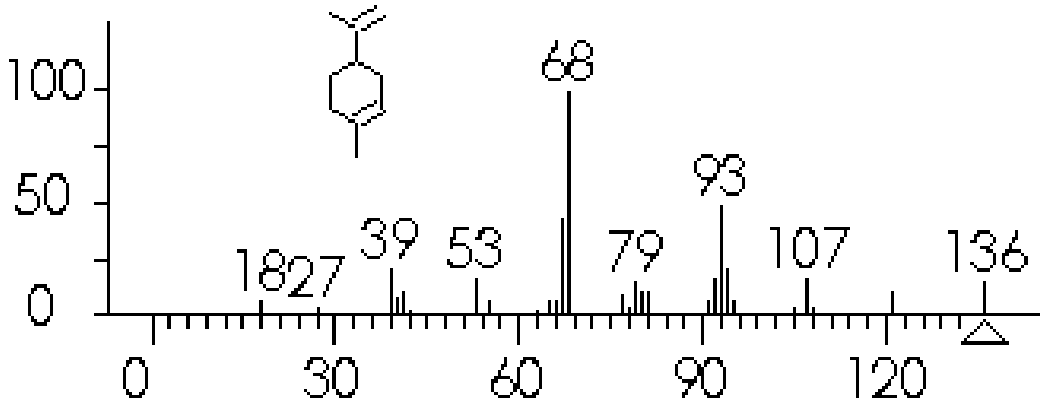


Fig.3.l8. Mass spectrum of Limonene

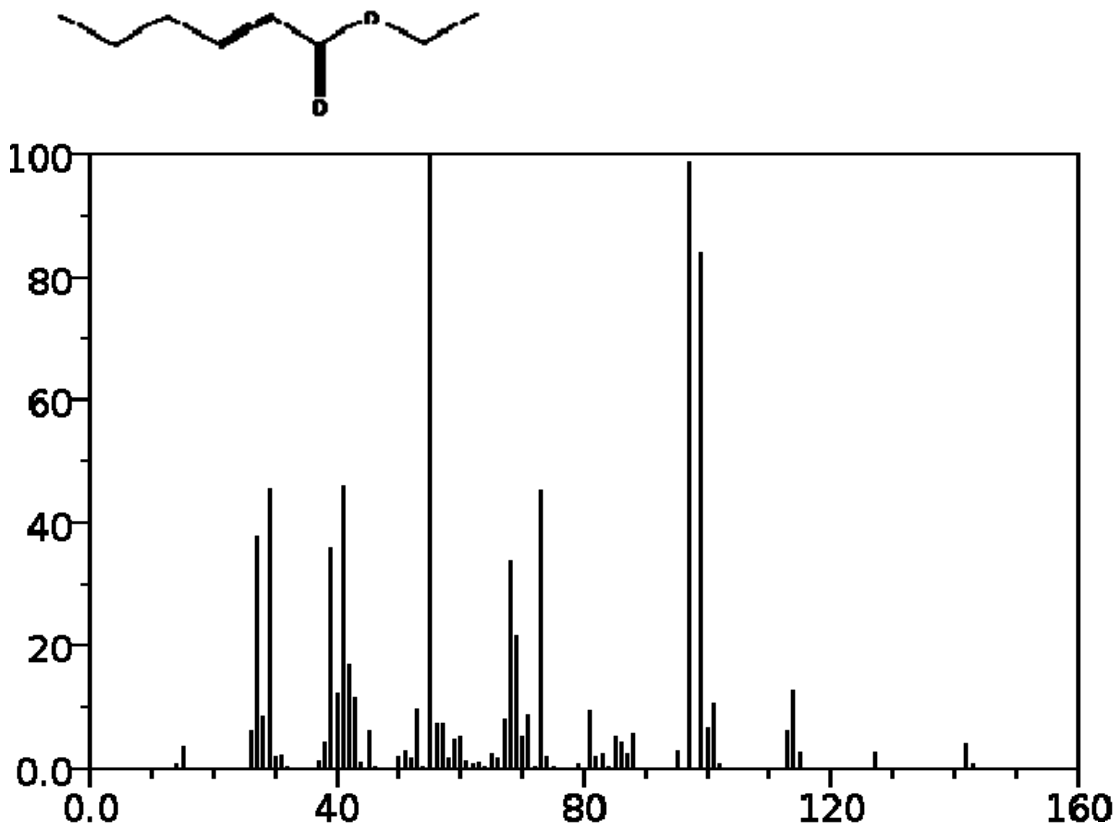


Fig.3.19. Mass spectrum of Ethyl hex-2-enoate

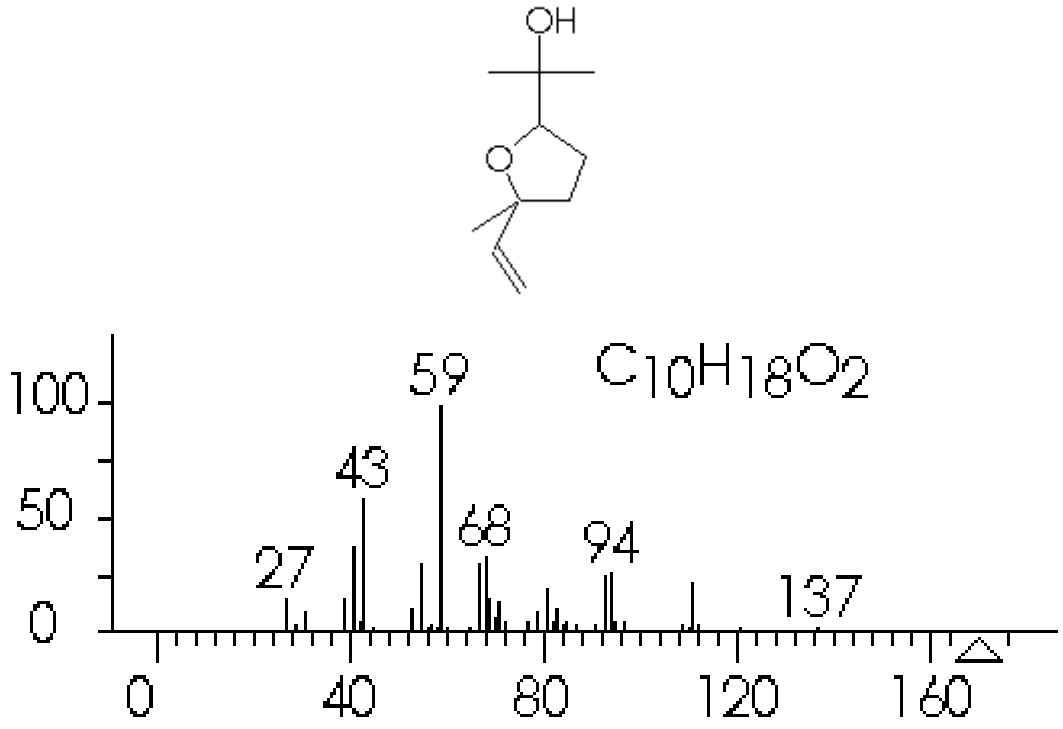


Fig.3.20. Mass spectrum of *cis*- linalool oxide

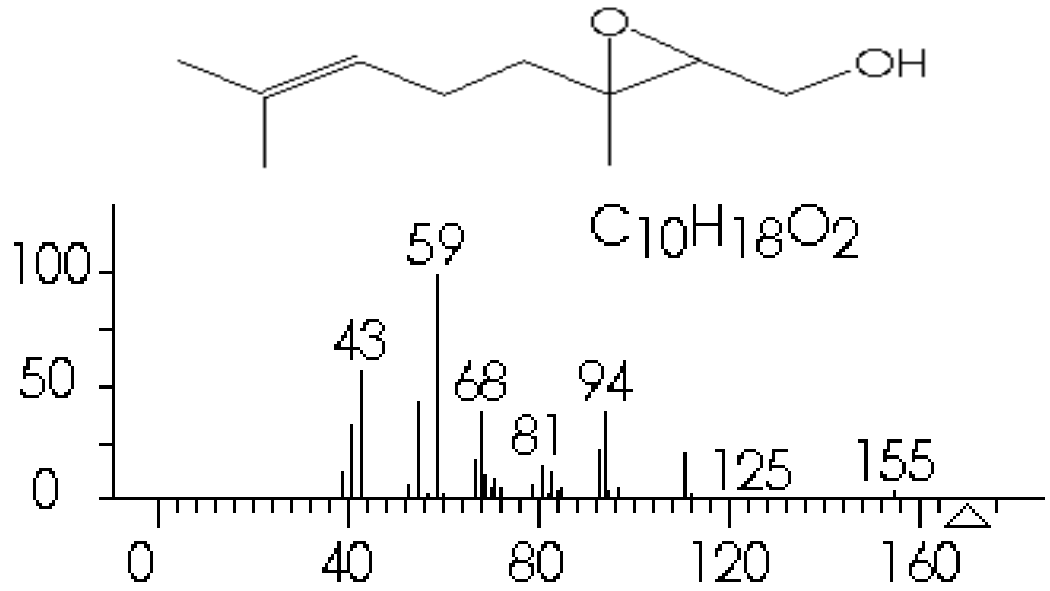


Fig.3.21. Mass spectrum of *trans* - linalool oxide

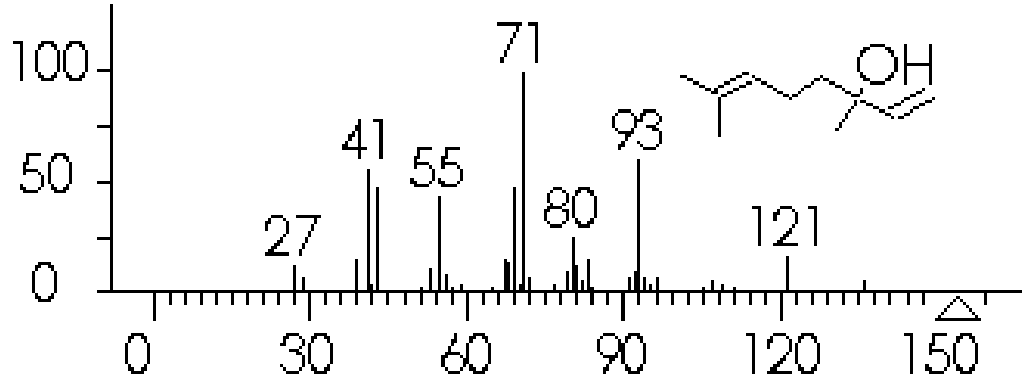


Fig.3.22. Mass spectrum of Linalool

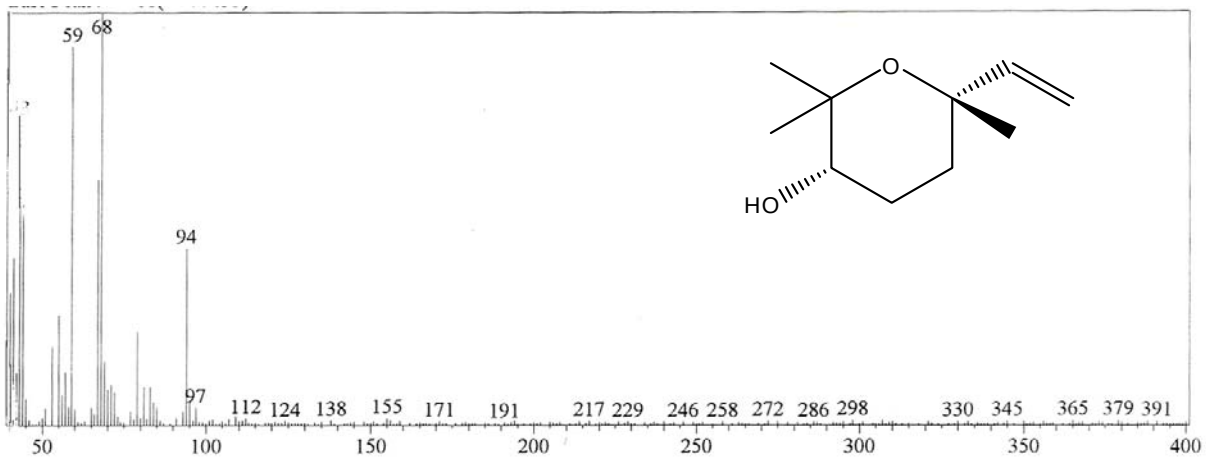


Fig.3.23. Mass spectrum of *cis*- Linalyl oxide (pyranoid)

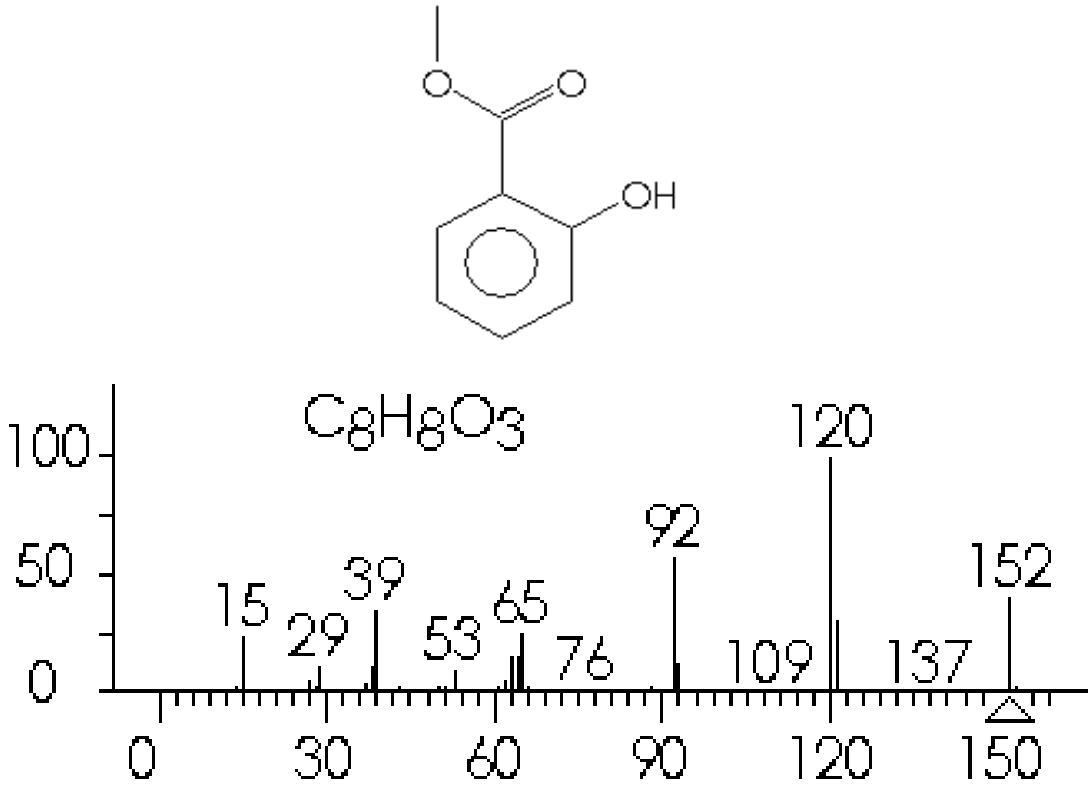


Fig.3.24. Mass spectrum of Methyl salicylate

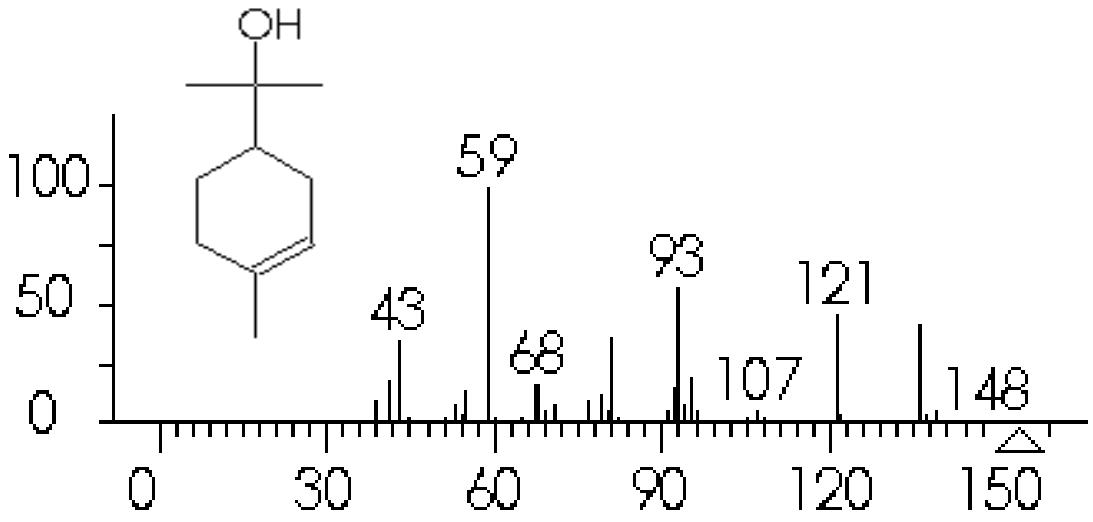


Fig.3.25. Mass spectrum of α-Terpineol

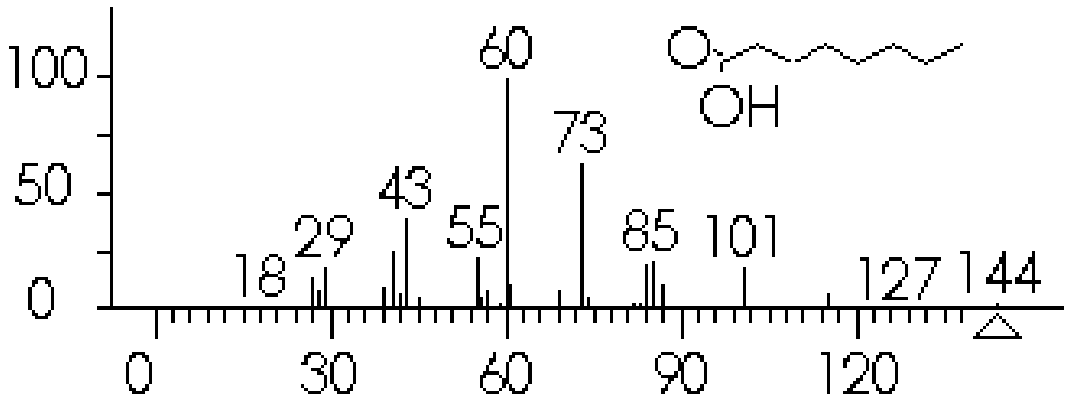


Fig.3.26. Mass spectrum of Octanoic acid

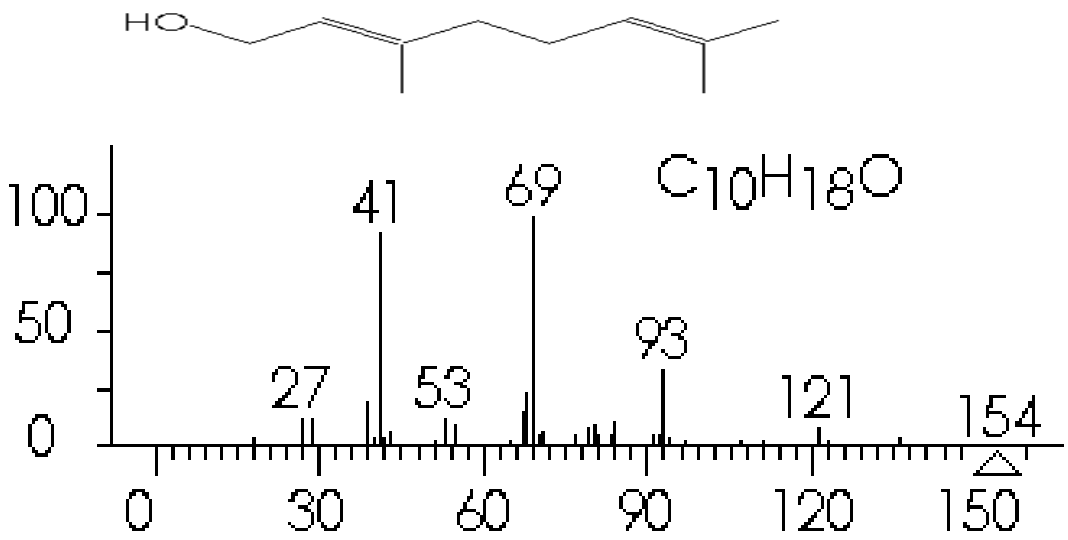


Fig.3.27. Mass spectrum of *cis*-Geraniol

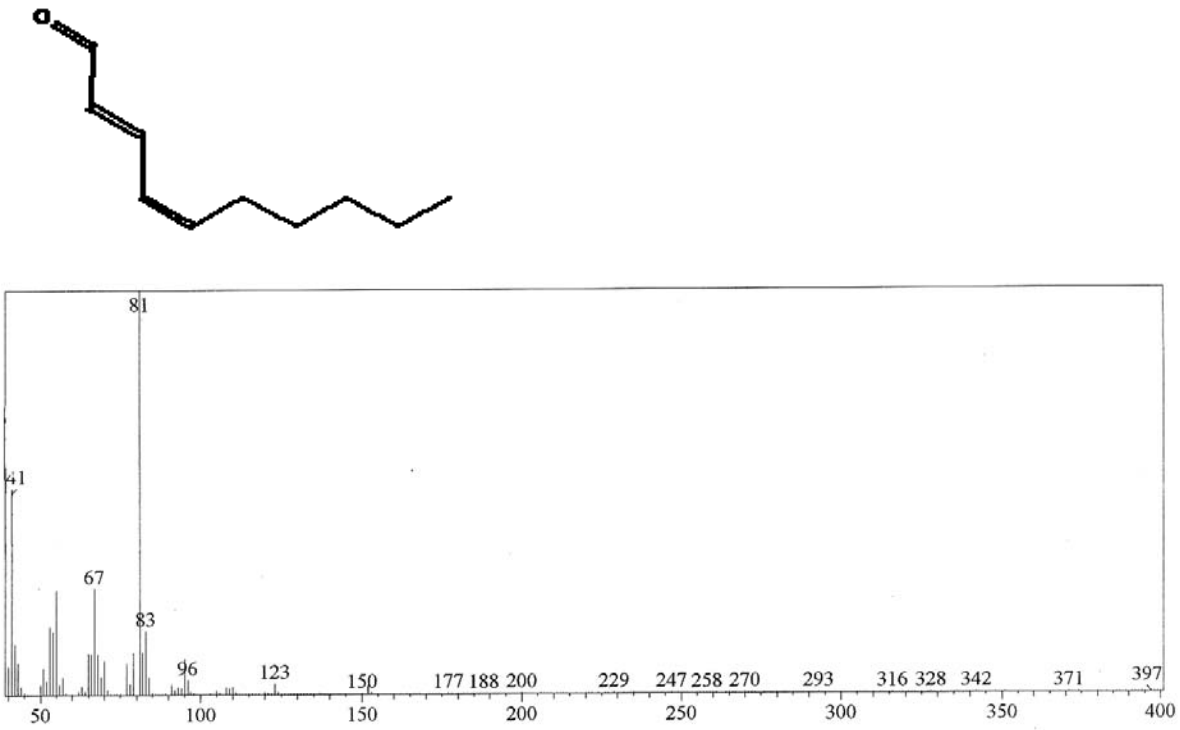


Fig.3.28. Mass spectrum of *(E,Z)*2,4-decadienal

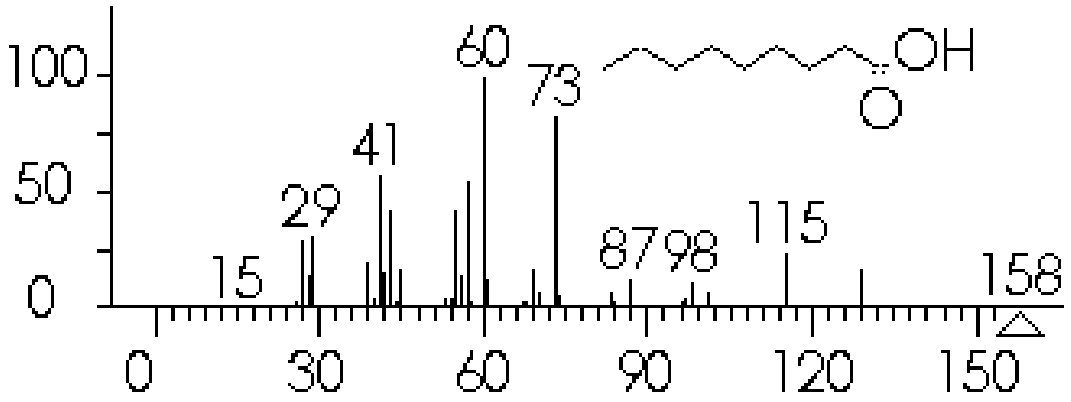


Fig.3.29. Mass spectrum of Nonanoic acid

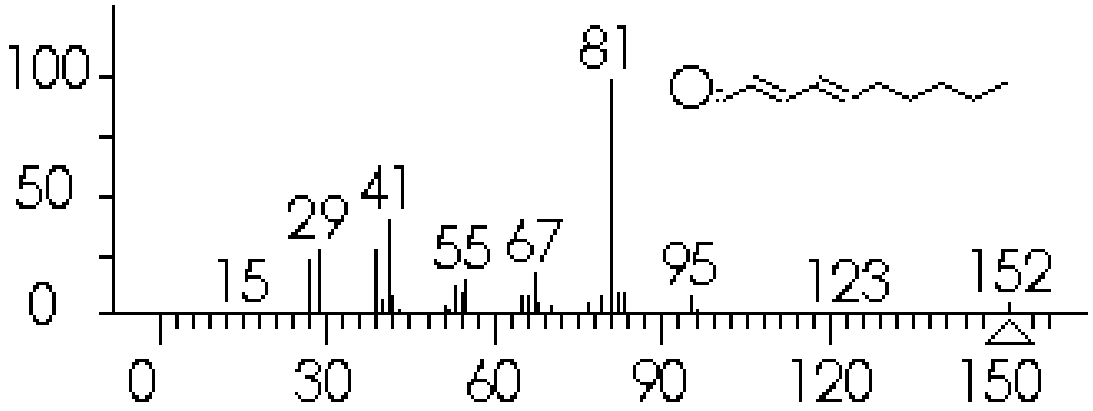


Fig.3.30. Mass spectrum of (*E,E*)2,4-decadienal

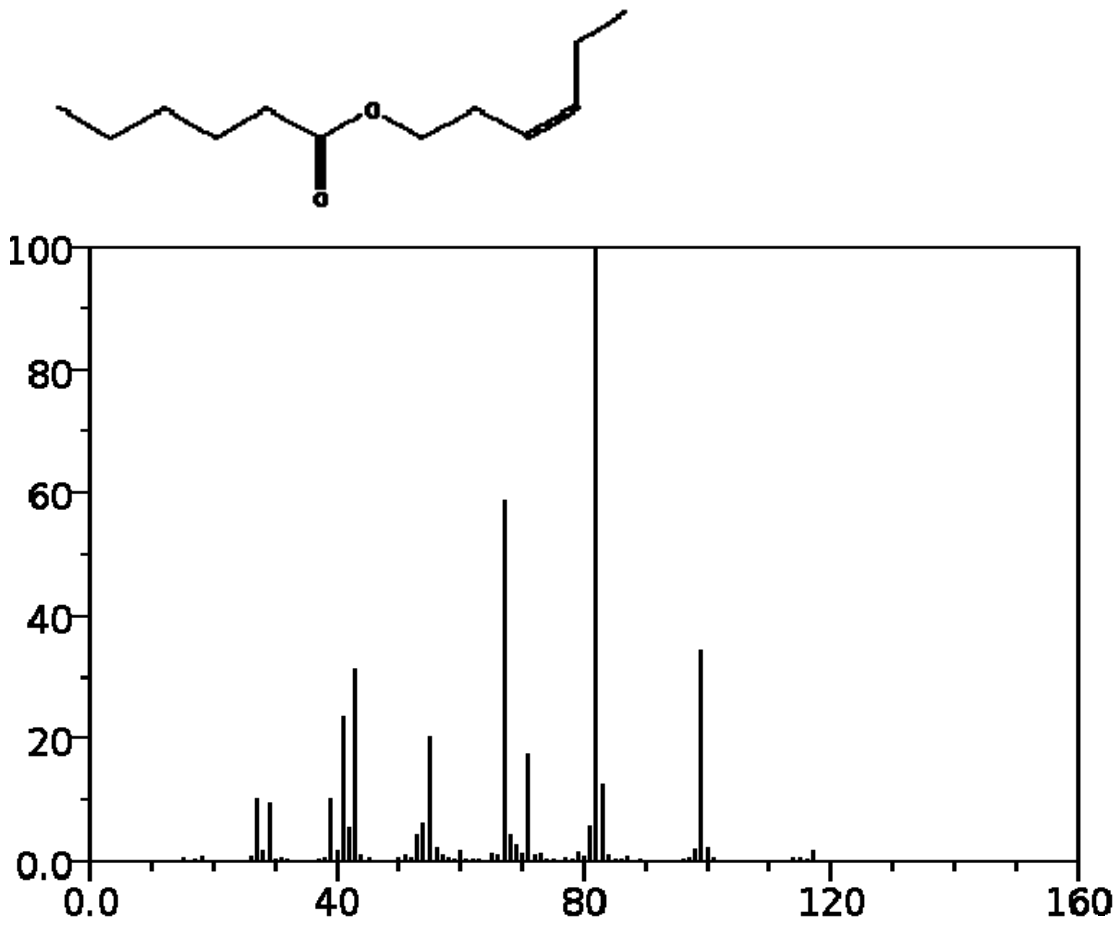


Fig.3.31. Mass spectrum of *cis*-3-Hexenyl-n-hexanoate

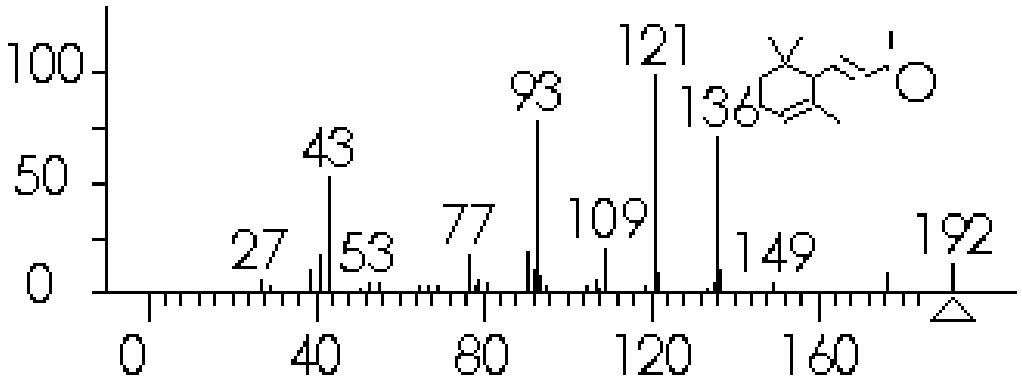


Fig.3.32. Mass spectrum of α-ionone

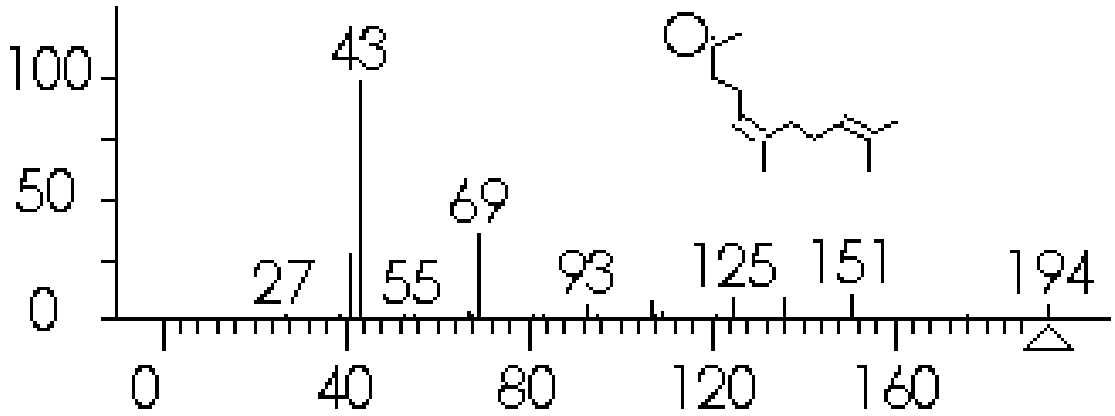


Fig.3.33. Mass spectrum of *cis*-geranylacetone

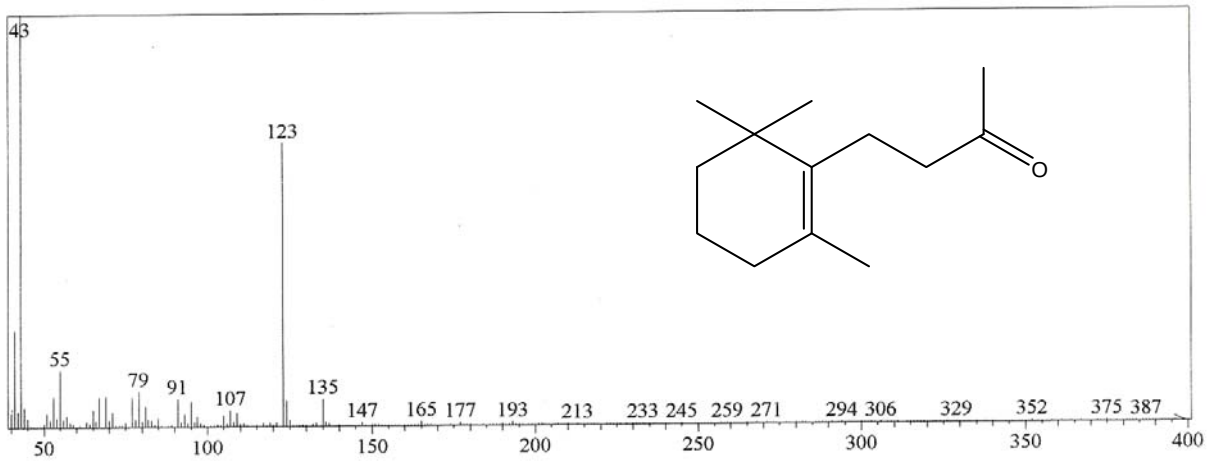


Fig.3.34. Mass spectrum of β-ionone-5, 6-epoxide

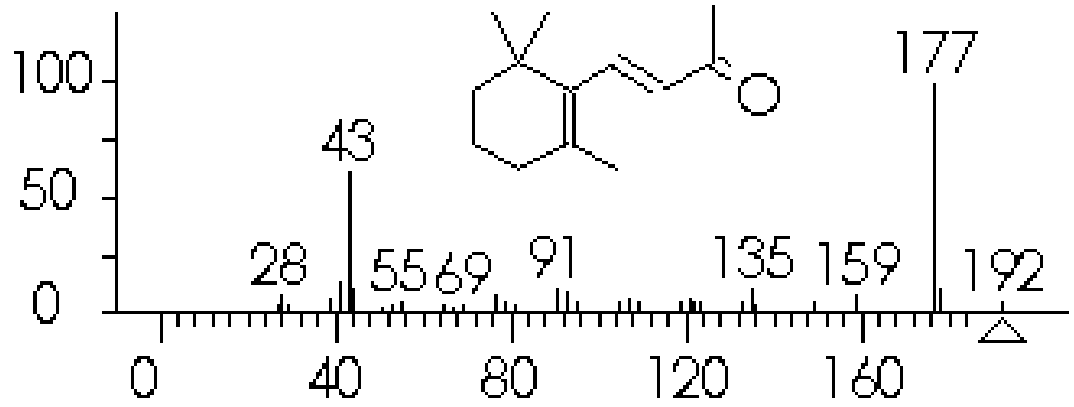


Fig.3.35. Mass spectrum of β-ionone

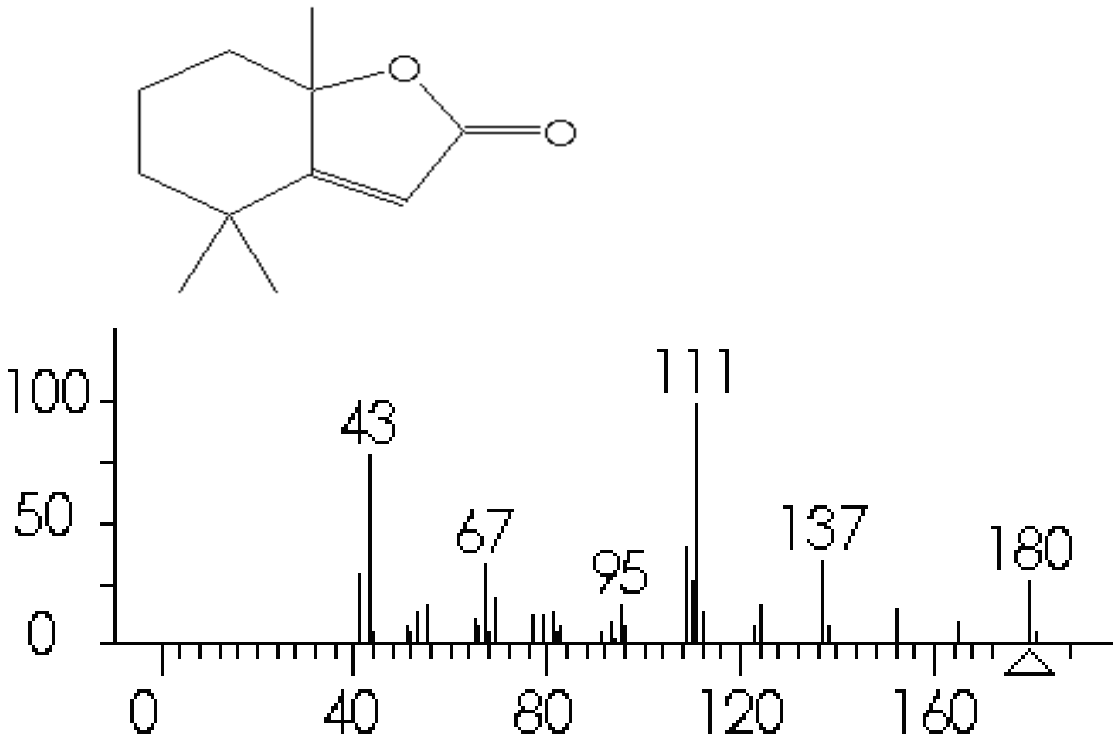


Fig.3.36. Mass spectrum of Dihydroactinidiolide

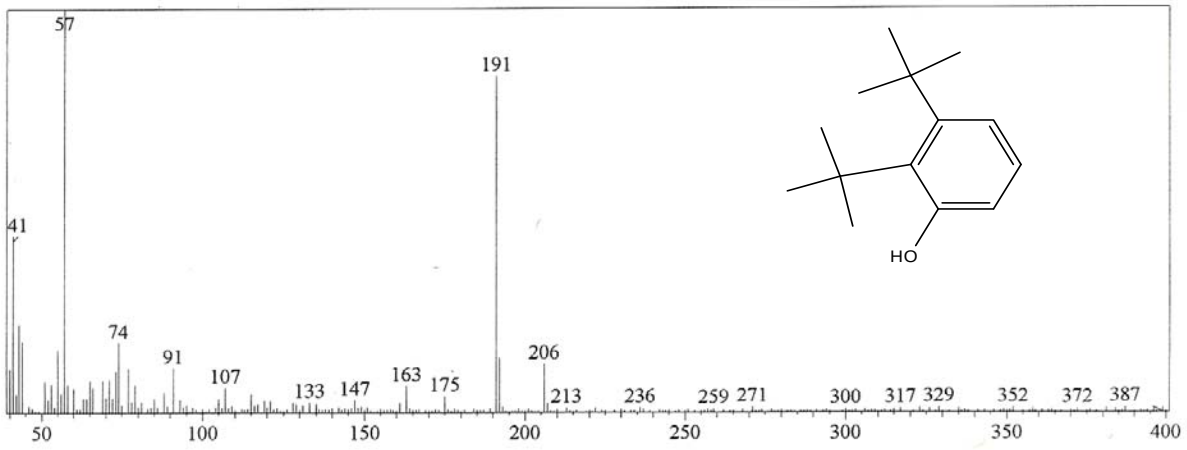


Fig.3.37. Mass spectrum of Di-tert-butyl phenol

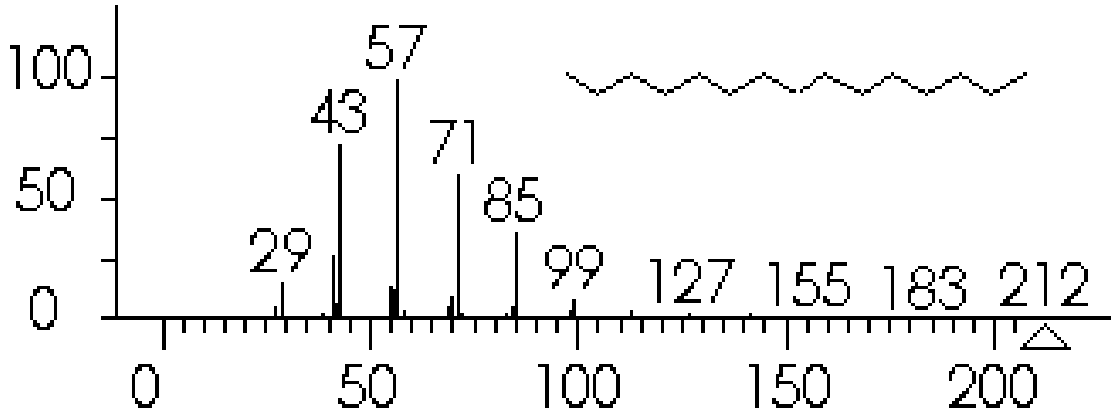


Fig.3.38. Mass spectrum of Pentadecane

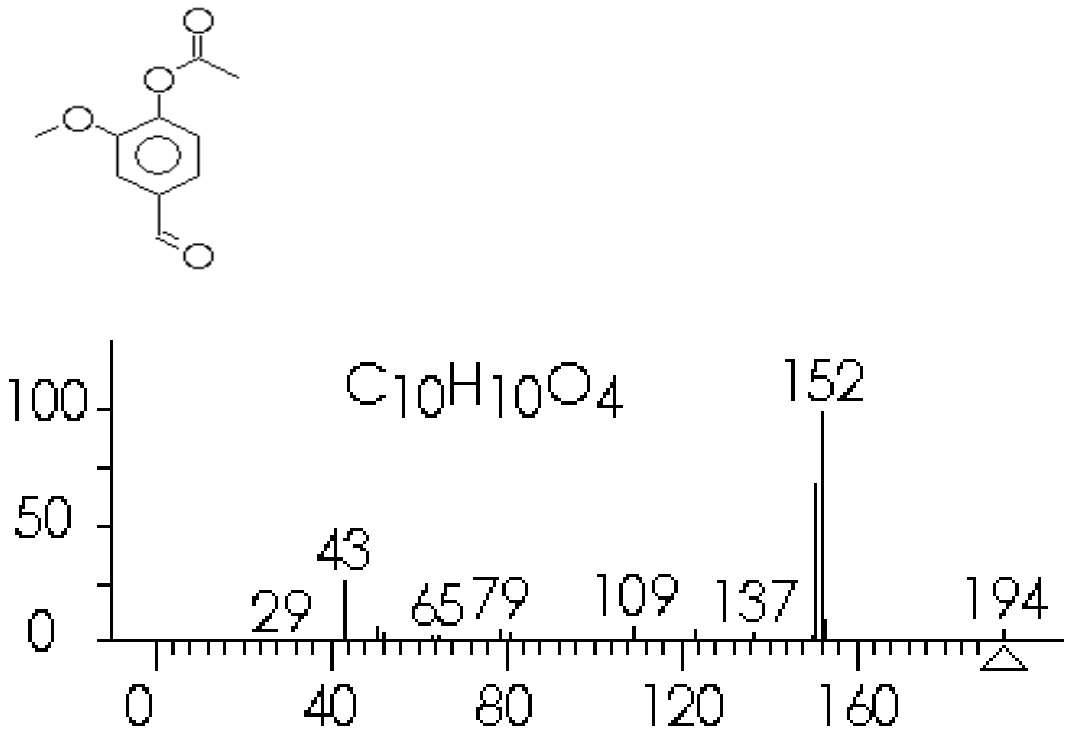


Fig.3.39. Mass spectrum of Vanillin acetate

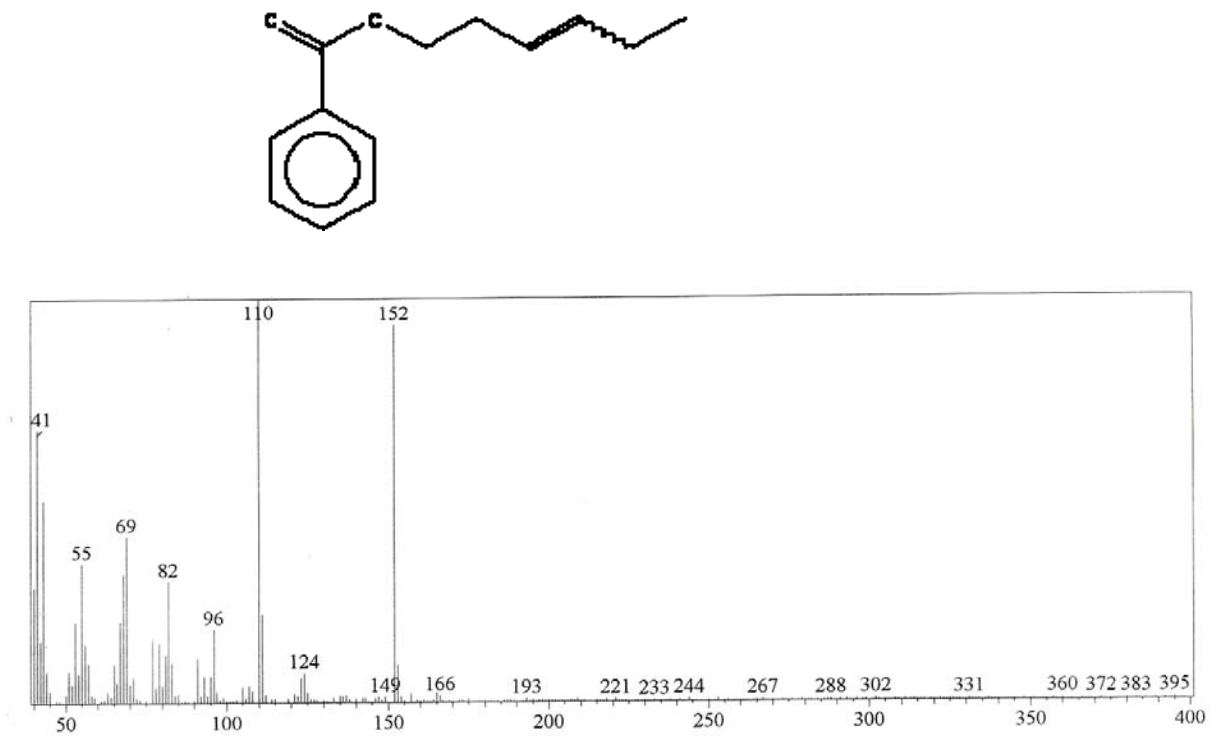


Fig.3.40. Mass spectrum of 3-Z-Hexenyl benzoate

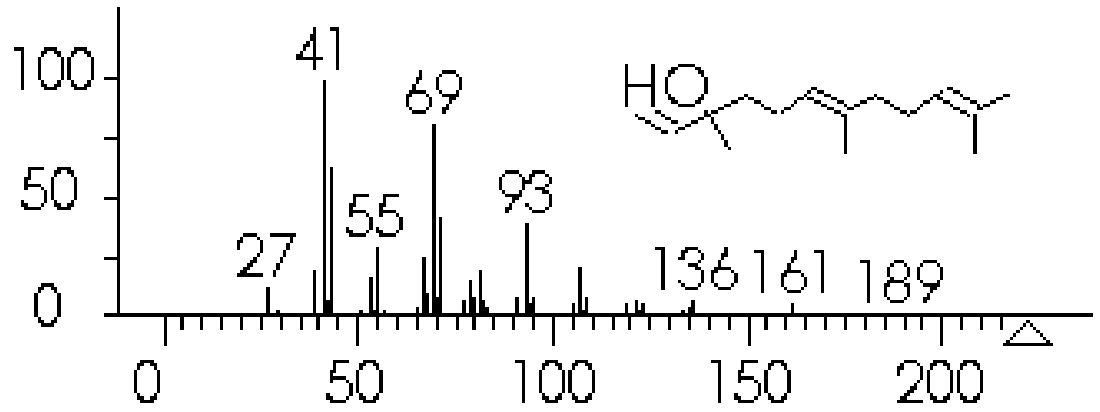


Fig.3.41. Mass spectrum of Nerolidol

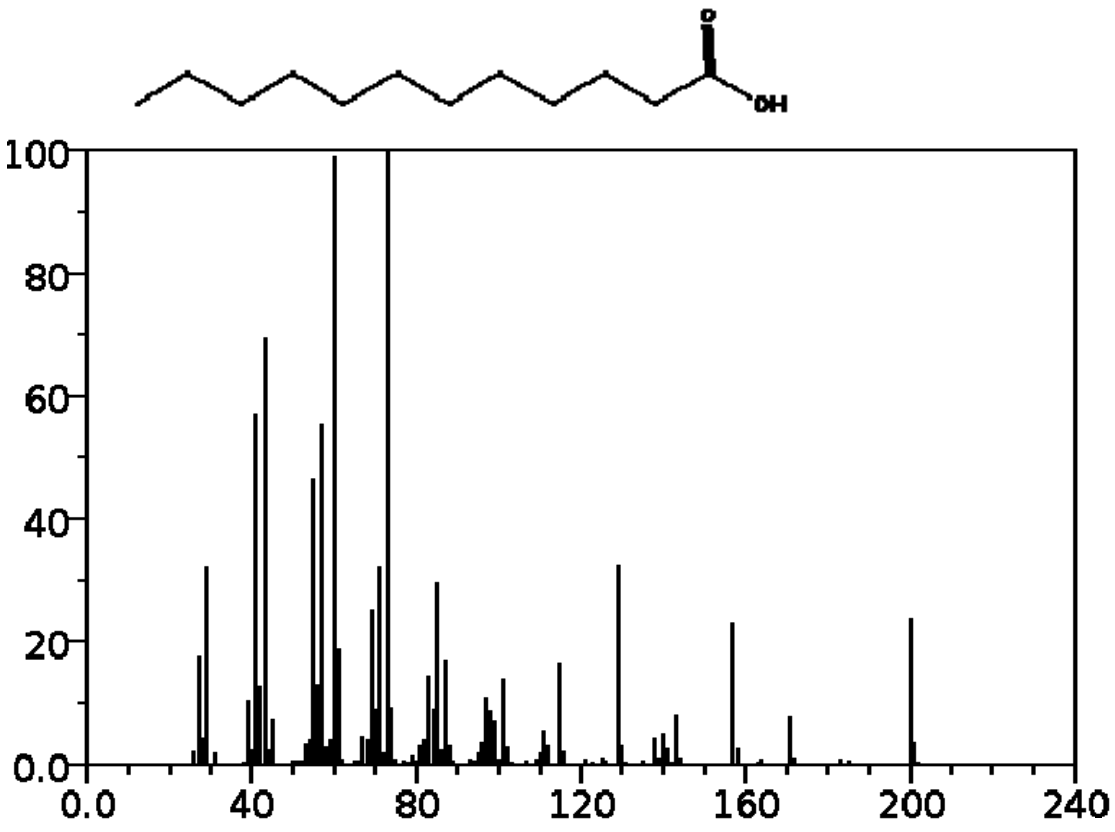


Fig.3.42. Mass spectrum of Dodecanoic acid

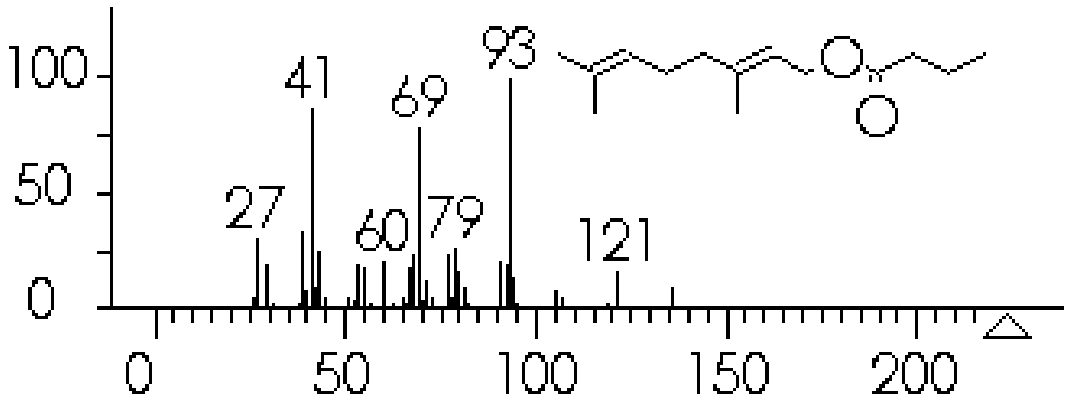


Fig.3.43. Mass spectrum of Geranyl butanoate

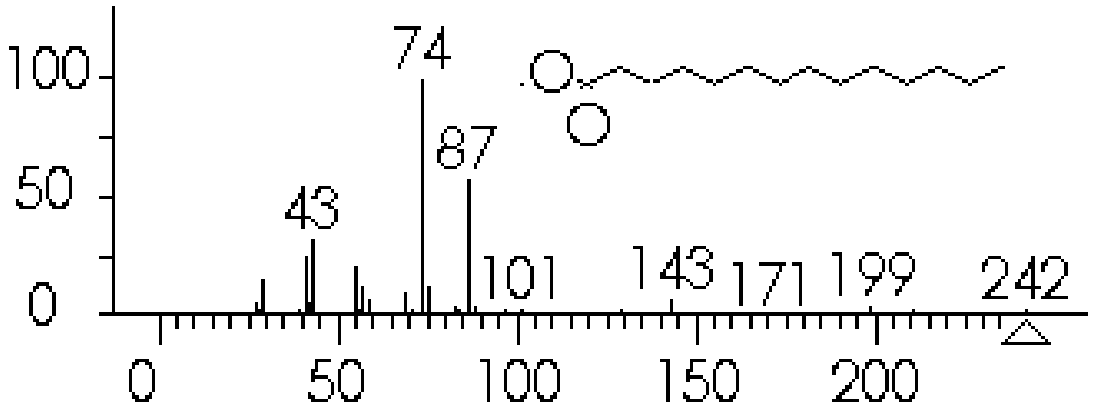


Fig.3.44. Mass spectrum of Myristic acid methyl ester

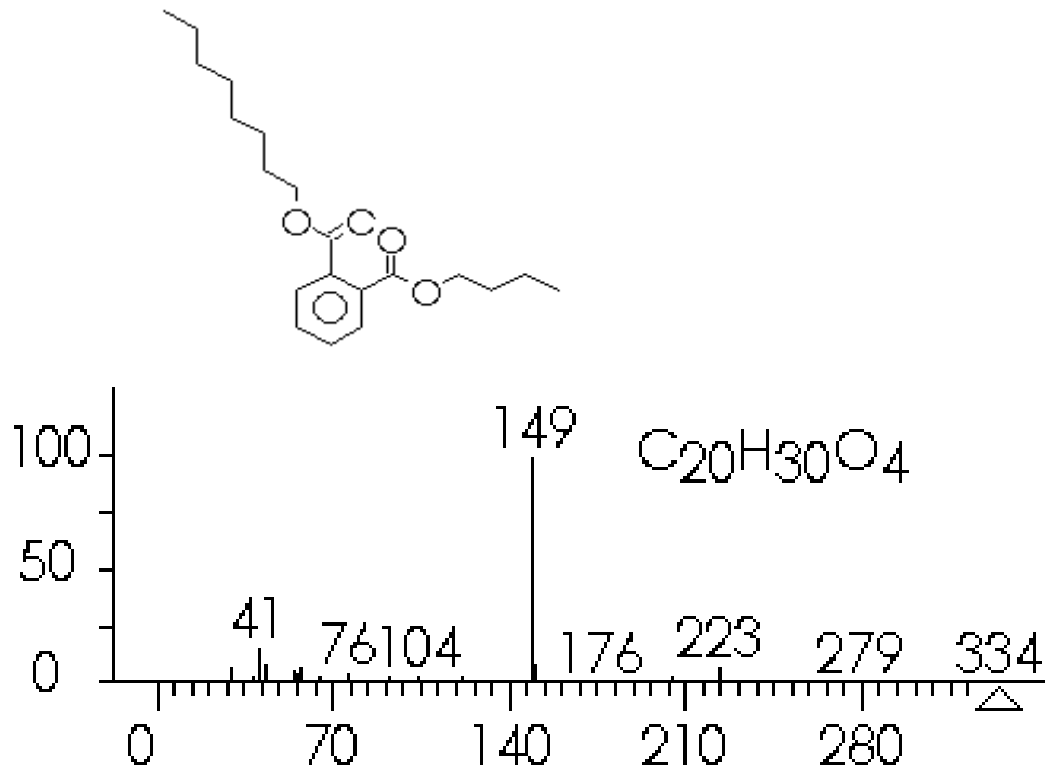


Fig.3.45. Mass spectrum of Butyl octyl phthalate

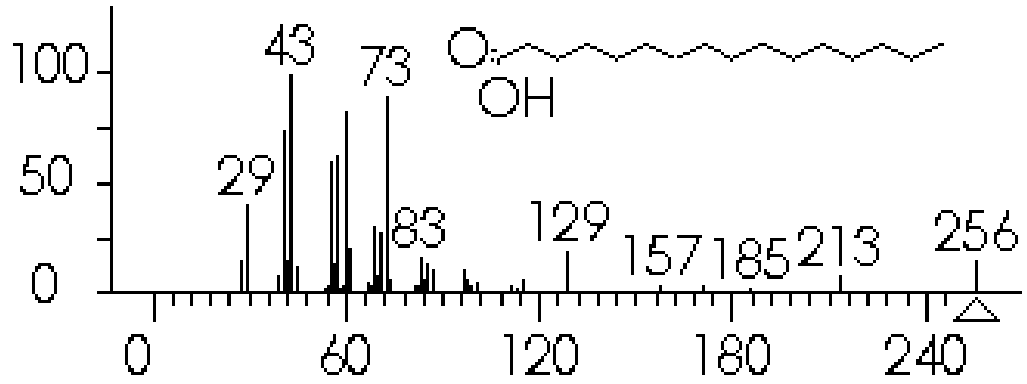


Fig.3.46. Mass spectrum of Palmitic acid

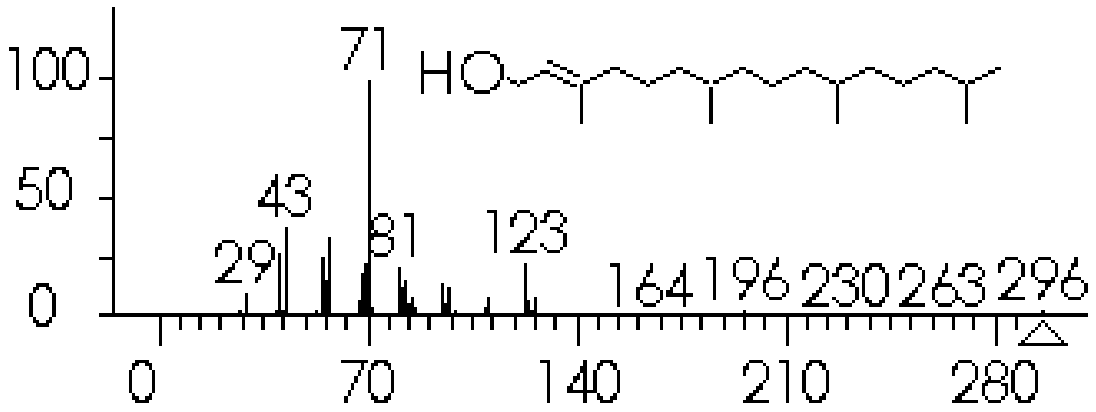


Fig.3.47. Mass spectrum of Phytol

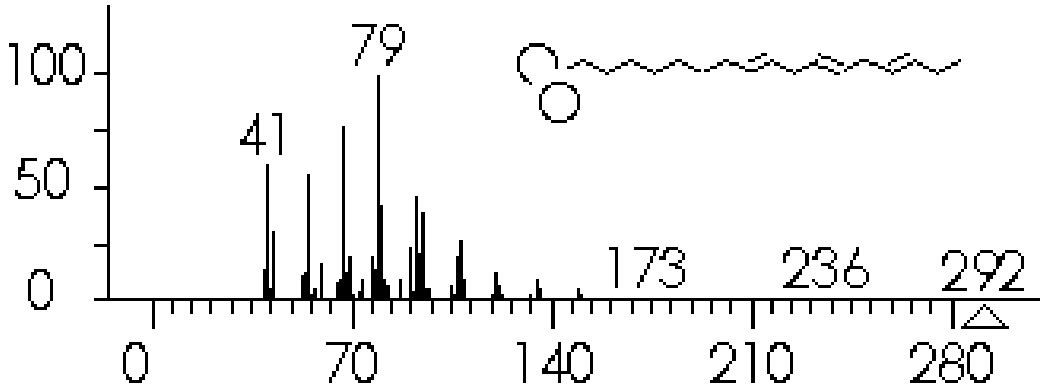


Fig.3.48. Mass spectrum of Methyl linolenate

3.3.2. Green tea extract

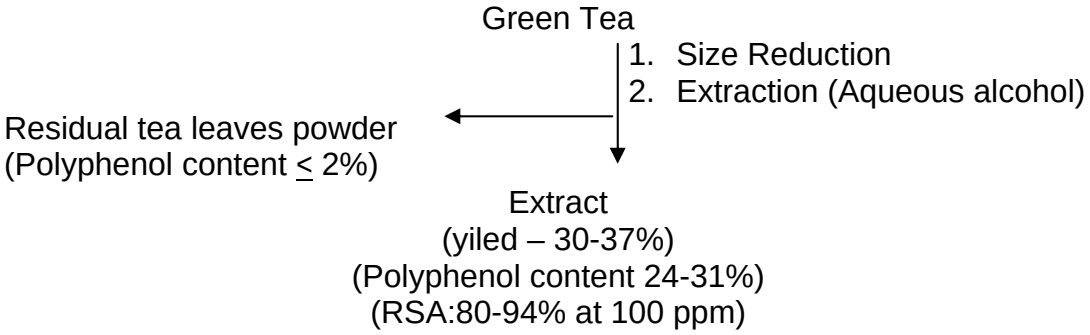
The extraction of green tea samples at lab scale, using different solvents (viz., Ethyl acetate, acetone, ethyl alcohol, methyl alcohol and their aqueous mixtures) was carried out. The solvents are removed from these extracts under vacuum and the yields are calculated on moisture free basis. These extracts were evaluated for their polyphenol content (Singleton and Rossi, 1965) and radical scavenging activity (Jayaprakasha *et al*., 2001). The radical scavenging activity (RSA) of these extractives at 50 and 100 ppm concentrations (which are found to be optimal for these extracts in the preliminary study varying from 10 – 1000 ppm concentrations) were evaluated using the DPPH model system (Table 3.4). The order of activity and extractability are as follows: Methanol > Ethanol> Acetone > Ethyl acetate. It is observed that the extractability as well as activity of the extract increased with polarity of the solvent used. This may be due to the increase in extraction of the active components along with the polarity of the solvent. Hence, alcohols are found to be the best solvents to obtain maximum extractives with high activity. The aqueous alcoholic mixtures showed higher activity and extractability than the respective single solvents. Both the alcohols were found to be better and the aqueous alcohol were used for further processing (Scheme 3.1).

Table 3.4. The yields and RSA of normal green tea extractives using different solvents

Solvents	Yield (%)	Radical scavenging activity (%)	
		50 ppm	100 ppm
Ethyl acetate	23.9±0.4	64±0.8	91±0.4
Acetone	27.7±0.4	67±0.5	91±0.5
Ethyl alcohol	32.7±0.5	76±0.7	92±0.6
Methyl alcohol	34.8±0.6	82±0.9	92±0.5
Ethyl acetate+ water	24.5±0.4	70±1.1	91±0.5
Acetone + water	29.2±0.5	75±1.0	91±1.2
Ethyl alcohol + water	36.3±0.6	82±0.5	92±0.7
Methyl alcohol + water	36.7±0.7	86±0.9	93±0.6
BHA	---	83±0.5	92±0.6

Values are mean ± SD (n=3), (---) = no activity

Scheme 3.1. Preparation of Green tea extract



The extractions for the further work on the normal and coarse tealeaves were carried out using methanol water mixture (Fig 3.45). The green teas from normal and coarse leaves were subjected to solvent extraction, followed by solvent removal. The miscella was subjected to lyophilization for removal of aqueous portion. The yields are computed on moisture free basis (Table 3.5). The extractives were evaluated for their polyphenol content and radical scavenging activities and the results are presented in Table 3.5. It was found that the yields of the extractives from green teas of coarse leaves are relatively low on the expected lines.

However, the radical scavenging activities of the extractives of green teas from coarse leaves are marginally low at different concentrations. This observation indicated that the green teas from coarse leaves could be used for the preparation of radical scavenging conserves, by separating / enriching the active components using suitable technique.

Table 3.5. The yields, polyphenol contents and RSA of normal / coarse green tea extractives prepared under optimized conditions

Tea sample	Yield (%)	Polyphenol content (%)*	Radical scavenging activity (%) at ppm concentrations			
			25	50	100	200
Normal	36.7±0.7	31.0±0.6	50±0.7	83±0.9	93±0.8	92±0.3
Coarse	32.5±0.5	20.6±0.8	45±1.0	74±0.2	92±0.4	91±0.8
BHA	---	----	82±0.8	92±0.4	93±0.7	93±0.4

* as gallic acid equivalents, Values are mean ± SD (n=3), (---) = no activity

3.3.3. Fractionation of the green tea extract

The extractives were subjected to liquid-liquid extraction using water and low molecular weight ester to fractionate the catechins into the solvent fraction (Fig 3.46). These extracts were analyzed for total polyphenol content and evaluated for radical scavenging activity (Table 3.6). The polyphenol content of the solvent extracts was found to be 30±2.3% as gallic acid equivalents for coarse leaves, while, polyphenol content of the solvent extract of normal leaves was found to be 31±2.4% as gallic acid equivalents. The total polyphenol content in the aqueous portion of these extracts was 23±2.1% as gallic acid equivalents for normal leaves, while that for coarse leaves extracts was found to be 18±3.0% as gallic acid equivalents. The

yields of the solvent extracts are found to be 15±0.8% for coarse leaves and for normal leaves the yield of solvent extract was found to be 17±0.8%. The yield of the aqueous extract is 17±0.9 % for coarse leaves and for normal leaves the yield of solvent extract was found to be 19 ±1.0 %. However, the radical scavenging activity of the solvent extracts from both normal and coarse leaves was found to be same (92±1% at 15 ppm). The RSA of the aqueous extracts was found to be low. Hence, it may be concluded that the solvent used separated the compounds responsible for the radical scavenging activity.

Antioxidant reacts with DPPH, which is nitrogen centered radical with a characteristic absorption at 517 nm and convert it to 1, 1-diphenyl-2-picryl hydrazine, due to its hydrogen donating ability at a very rapid rate (Yamaguchi *et al*, 1998). The degree of discoloration indicates the scavenging potentials of the antioxidant. The activity of the extracts is attributed to their hydrogen donating ability (Shimada *et al*, 1992). It is well known that free radicals cause auto-oxidation of unsaturated lipids in food (Kaur and Perkins, 1991). On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming stable end product, which does not initiate or propagate further oxidation of lipid (Sherwin, 1978). The data obtained reveal that the green tea extracts / conserve is free radical inhibitor and primary antioxidant that react with DPPH radical, which may be attributed to its hydrogen donating ability.

Table 3.6. Fractionation of Green tea extracts - Yields, polyphenol contents and RSA of solvent and aqeous extracts

Tea sample	Yield (%)		Polyphenol content (%)*		Radical scavenging activity (%) at ppm concentrations			
					Conserve (Solvent)		Conserve (Aqueous)	
	Conserve (Solvent)	Conserve (Aqueous)	Conserve (Solvent)	Conserve (Aqueous)	15	100	15	100
Normal	17 ±0.8	19±1.0	31±2.4	23±2.1	92±1	>94	45±1	92±2
Coarse	15 ±0.8	17 ±0.9	30±2.3	18±3.0	92±1	>94	40±1	85±2
BHA	---	---	---	---	82±1	>94	82±1	>94

* as gallic acid equivalents, Values are mean ± SD (n=3), (---) = no activity

Scheme 3.2. Preparation and fractionation of green tea extract

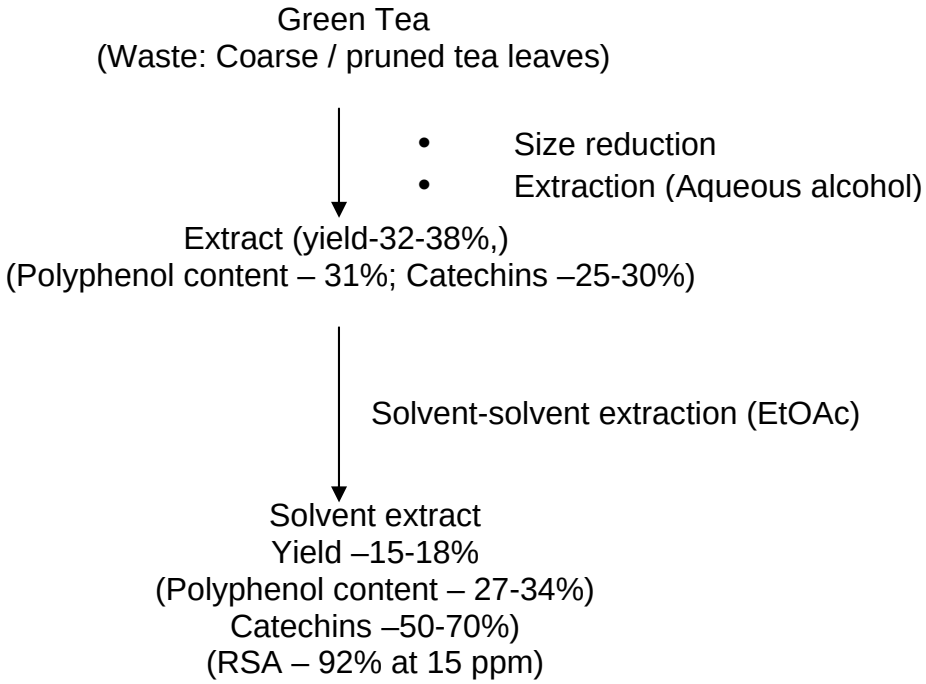


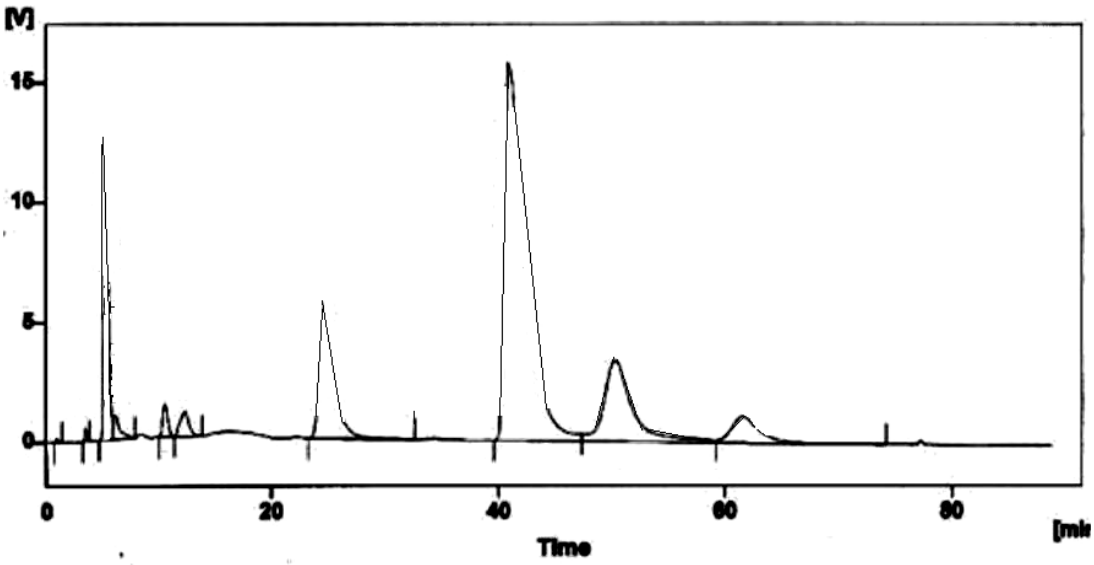
Figure 3.49. Green tea extraction

Figure 3.50. Fractionation of green tea extract

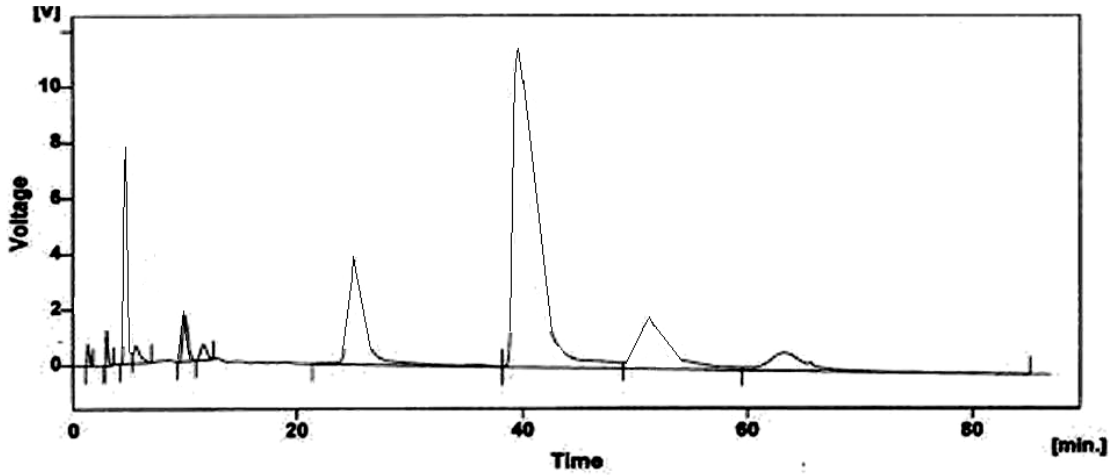
3.3.4. HPLC profiling of green tea extractives, chemical composition and quantification

To determine the chemical composition of the green tea extracts / conserves, the calibration curves for each of the catechins were prepared. The concentrations ranges used for the calibration curves were 5-50μg. The retention times of each of the catechin were noted. Under optimized conditions the green tea extracts, fractionated solvent portion and aqueous portion were subjected to HPLC (Terasawa *et al*, 2001). The profiles are presented in the fig.3.51.-3.53. The quantification was carried out using the external standard method. Solutions of each standard at various concentration levels were injected in to HPLC system and the peak areas were recorded. Thus the calibration curves and response factors were calculated under the same conditions as that of the sample. The total catechin content in the green tea extract based on the comparison of peak areas with that of authentic samples and from calibration curves was found to be in the range of 20–30 %. After fractionation the solvent extract is enriched with catechin and the total catechin content was found to be in the range of 55-85 % (Table 3.7.), while the HPLC profile of aqueous extracts showed only the presence of gallic acid and caffeine.

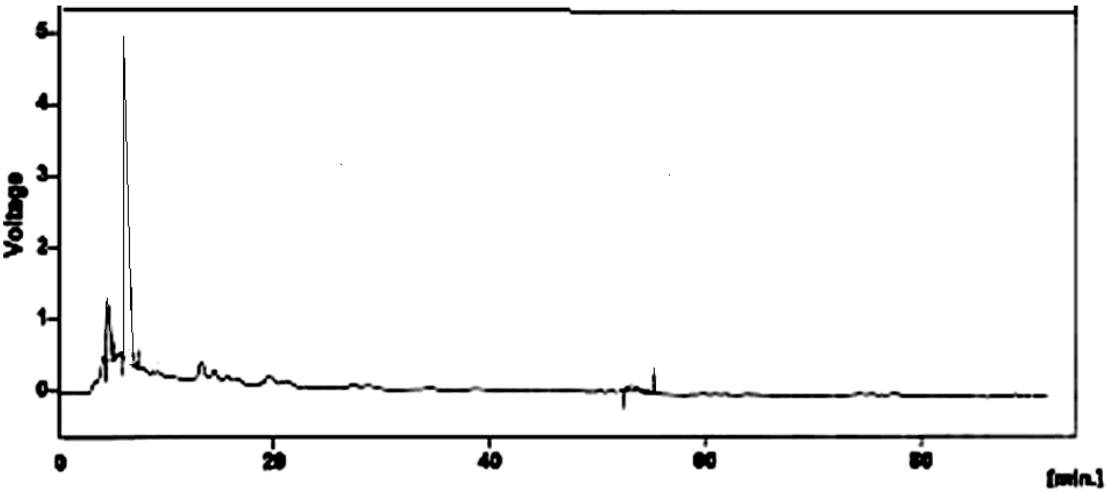
Coarse green tea leaves extract obtained from the solvent-solvent extraction method was analyzed for individual catechins, methyl xanthines and gallic acid by employing HPLC method, the content of the above compounds were calculated as microgram per milligram of dry weight.



3.51. HPLC profile of green tea extract



3.52 HPLC profile of solvent portion of tea extract



3.53 HPLC profile of aqueous portion of tea extract

Table 3.7. Catechin composition of radical scavenging (solvent) conserve

Compound	Retention time (min)	Quantity* μg/mg
Caffeine	4.7	104.8±10.4
Catechin	10.2	64.5±5.6
Epigallocatechingallate	25.0	177.4±20.1
Gallocatechingallate	39.6	371.0±42.1
Epicatechingallate	51.4	182.3±12.1

*Epicatechin and epigallocatechin were found in traces only
Values are mean ± SD (n=3)

In order to clarify the above finding, the radical scavenging activity of the pure gallic acid, alkaloid such as theophylline, theobromine and catechins such as catechin, epicatechins, epigallocatechin, gallocatechingallate, epicatechingallate, epigallocatechin gallate derivatives was carried out and the results are presented (Table 3.8). EGC showed the highest activity at 25 ppm concentration among the catechins. The EGCG and GCG show the almost identical activity. The activity of EGC, ECG, EGCG and GCG are nearly equal to BHA at 25 ppm concentrations. However, all the catechins showed close to 90% activity at 50 ppm concentration, except epicatechin.

In the DPPH test; the extracts were able to reduce the stable radical DPPH to the yellow coloured diphenyl picryl hydrazine. The method is based on the reduction of an alcoholic DPPH solution at 517nm in the presence of a hydrogen donating antioxidant (AH) due to the formation of the non-radical form (DPPH-H), according to the following reaction:

$$DPPH + AH \longrightarrow DPPH\text{-}H + A$$

Table 3.8: Radical scavenging activity of gallic acid, alkaloids and catechins

Standard	25 ppm	50 ppm
Gallic acid	90±1.0	92 ±0.9
Theophylline	-	-
Theobromine	9±0.3	1±0.2
Catechin	79±0.7	89 ±0.8
Epicatechin	61 ±0.8	83±0.8
Epicatechingallate	84 ±0.9	90±0.9
Epigallocatechingallate	86±0.8	89±0.7
Gallocatechingallate	85±0.7	89±0.8
Epigallocatechin	86±0.9	90±1.0
BHA	92±1.0	92±0.9

Butylated hydroxyanisole (BHA) was used as positive control.

Values are mean ± SD (n=3), (-) = no activity

The remaining DPPH, measured after a certain time, correspond inversely to the radical-radical interaction, the radical A can contribute to the formulation of stable molecules. This method is simple, rapid (20min) and sensitive. No expensive reagent or sophisticated instruments are required.

In the literature, the radical scavenging activity of the phenolic compound is described as being largely influenced by the number of hydroxyl groups on the aromatic ring. The higher the number of hydroxyl groups, the greater the radical scavenging activity .The results of this study are in perfect agreement with data. It has already been reported that more than 70% of the antioxidant activity in green tea extracts can be attributed to tea catechins and (-)-ECG and (-)-EGCG in the particular strongly contributed to the antioxidant activity of green tea (Salah *et al*, 1995).

Extracts from unused fresh green tea leaves have the potential for large-scale application as natural antioxidants. Extracts of the green tea are becoming increasingly important as functional ingredients in the diet and are being added to a range of foods and beverages (Zandi and Gordon, 1999).

3.3.5. Extraction of Green tea from Pruned tea leaves on pilot scale

Green tea obtained from drying of pruned tea leaves (30 Kg fresh leaves / batch) was ground in a hammer mill. The ground green tea was charged into extraction unit (Figure 3.54) and subjected to extraction using alcohol: water mixture. The obtained solution of the extract was subjected to concentration by reducing the volume and the extract was subjected to freeze-drying. The yield was in the range of 25-26%. The total poly phenol content of this extract was determined using the Folin-Ciocalteu's phenol reagent. The polyphenol content was found to be in the range of 35-37%. Radical scavenging Activity of the extract was determined using DPPH method. It showed an activity of 90-92% at 15 ppm concentration.

The Spent green tea leaves were subjected to soxhlet extraction using alcohol and water mixture, and the yield of residual extract was found to be 5.5%.

The solvent-solvent extraction of the green tea extract using ethyl acetate was carried out. The Green tea extract (original), ethyl acetate extract, aqueous extract were subjected to HPLC analysis, and were also evaluated for the polyphenol content and RSA activity. However, the solvent-solvent extraction on large scale was found to be time consuming and energy intensive step. In spite of these problems, the yield of the conserve was found to be less. Hence, the alternate methods were attempted.

Figure 3.54. Pilot scale extraction of green tea leaves powder

3.3.6. Improved method for the active conserve

One more batch of fresh green tea leaves (Coarse / pruned, 100 kg) were procured from the Nilgiris, Tamilnadu and processing was started within the 6-10 h of plucking. The initial moisture content of the leaves was found to be ~60-65% and the final moisture in prepared green tea was ~6-8%.

To minimize the processing cost and to control the epimerisation of catechins during processing, alternate methods were explored. Green tea sample from fresh batch was subjected to aqueous alcoholic extraction. The extract was concentrated to remove the alcohol to the extent possible. The obtained miscella was cooled to 10^{o}C and kept over night at that temperature. The separated solids were filtered (Figure 3.55) and dissolved in low molecular weight ester and the filtrate was also treated with the same ester. Solvent was removed from the combined portion and the yield was found to be 12±2%. Both aqueous and ester portions were subjected to HPLC analyses. Ester soluble portion was found to contain most of the catechins. The aqueous portion (filtrate) was freeze dried and the solid yield was ~12±2%. Total polyphenol content and Radical scavenging activity (RSA) of the aqueous portion and ester portions were evaluated. RSA of the aqueous portion was found to be in the range of 70-85% at 40-50 ppm concentration, while that of ester portion was in the range of 85-90% at 10-15 ppm concentrations. The total polyphenol content in the aqueous portion was found to be 23-25%, while that of in the ester portion are in the range of 27-32% (Borse *et al*, 2007).

Figure 3.55. Separation of solids from green tea extract

3.3.7. Economics of the process

Cost of production (Basis: 800kg leaves/shift; 3 shifts/day; 300day)

Total cost of production per annum = Rs. 1, 91, 80, 000/-

Tea catechin conserve = Rs.1332/kg

Total sales envisaged = Rs. 2, 53, 44, 000/-

Market Price and returns (Figure 3.56.)

Tea catechin conserve = Rs.1600/kg

Tea gallic acid conserve = Rs.160/kg

Break even capacity = 38%

Basis- 2 shifts / day

Return on investment = 23%

Payback period = 4.35 Y

Basis- 3 shifts / day

Return on investment = 41%

Payback period = 2.43 Y

Figure 3.56. Tea gallic acid conserve and catechin conserve

3.3.8. Applications of green tea catechin conserve

3.3.8.1. Introduction

This section of the chapter describes the process for the application of the green tea conserve in the preparation of nutracutical food products selectively ice-cream and cookies. The significance of the new process lies in the fact that the catechins isolated from the natural source possessed good antioxidant and as well as radical scavenging activity resulting in products having high neutracutical value to the consumers. The bioactive attributes and health benefits of green tea are discussed in greater detail in previous chapters.

3.3.8.2 Back ground for the products development

The prepared processed tea consists of two leaves and a bud which contains mainly polyphenols, alkaloids and being used as a most popular beverage which is having major health benefits.

India is the largest producer of tea. The tea leaves (except two leaves, a bud, third and fourth leaf) are not plucked for tea making and are pruned in a periodic cycle of 2-3 years. These pruned tea leaves have good amount of polyphenols claimed to have several health benefits. Keeping this in view, a process was developed as described earlier in this chapter. In this process these coarse / pruned tea leaves were dried (cross flow dryer / infrared dryer) and ground. Powdered green tea were subjected to extraction using aqueous polar solvent. The miscella after removal of the solvent were subjected to fractionation using different technique to obtain the radical scavenging conserve (e.g., solvent - solvent extraction (or) chilling and separation of the precipitate and extraction with solvent). The conserve thus obtained showed

very good (90-92% at 10 -15 ppm concentration) radical scavenging potential in model system. The yield and polyphenol content of radical scavenging conserve was in the range of 10-15% and 25-30% respectively. Tea catechin conserve was found to show high radical scavenging activity. Its application in food products could provide health benefits to the consumer. Two products (i.e., Ice-cream and cookies) containing the conserve are optimized with regard to its incorporation in the final product.

In a study by Wang *et al* (2007) comparison of the effect of green tea extract (GTE) on the quality of bread by instrumental analysis and sensory evaluation was carried out. The level of incorporation of green tea extract (GTE) was at 1.5 and 5.0 g/kg of flour. The threshold level of green tea extract (GTE) was at 5.0 g/kg of flour for astringency and sweetness, and 1.5 g/kg of flour for brightness, hardness and stickiness.

The product Ice-cream (i.e. Green tea nutraceutical ice cream), incorporating the catechin-rich conserve was optimized (Jagan Mohan Rao *et al*, 2008).

3.3.8.3 Catechin-rich nutraceutical ice-cream

Tea catechin conserve was found to show high radical scavenging activity. Its application in food products could provide health benefits to the consumer. Ice-cream (Figure 3.57) was prepared using the food ingredients such as milk, cream, sugar, milk powder along with the polyphenol conserve. The concentration of tea catechin conserve was tried in the range of 5-200 ppm. The tea catechin conserve was suitably solubilised to give homogenous product. The optimum range of tea catechin concentration was found to be in the range of 20-30 ppm.

Figure 3.57. Ice-cream enriched with antioxidants from tea conserve

Figure 3.58. Cookies enriched with antioxidants from tea conserve

3.3.8.4. Sensory evaluation of catechin rich nutraceutical ice-cream

The results of the sensory analysis as shown in following figure (3.59.) depicts ice-cream with tea antioxidant extract; 15% of the respondents rated under ‘LVM’, 45% of the respondents under ‘LM’ and another 40% under ‘LS'. However the ice-cream samples were acceptable as the scores were falling on ‘Like’ category.

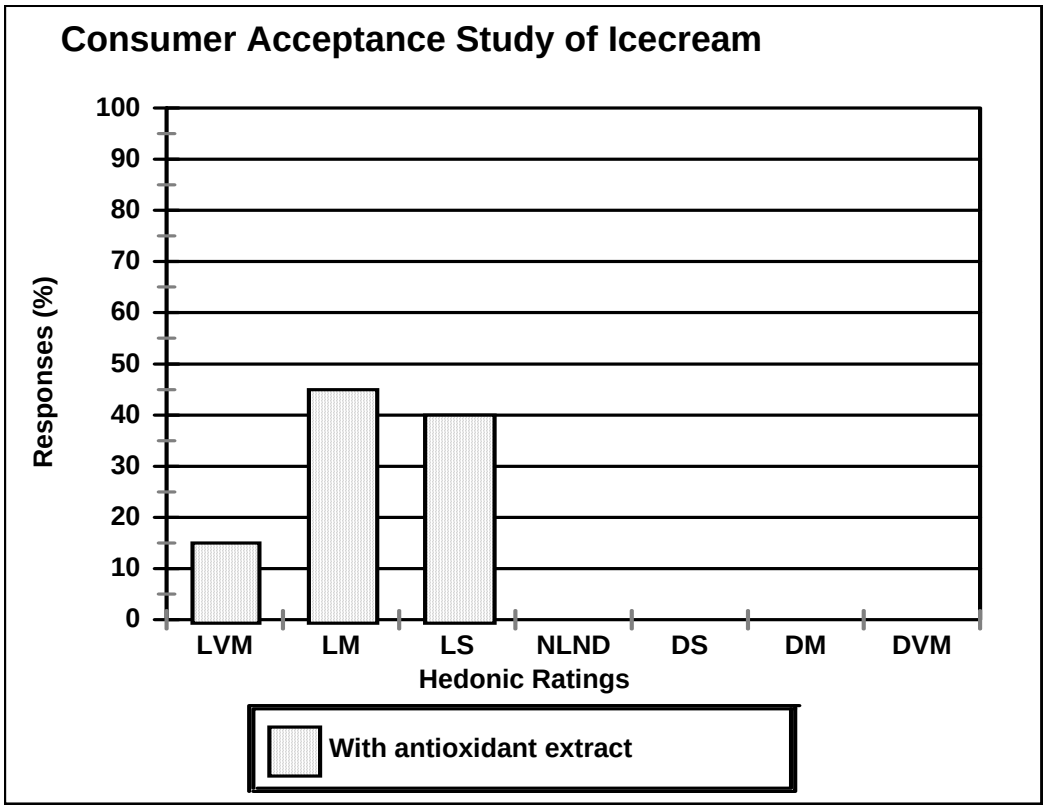


Figure 3.59. Sensory evaluation of Ice-cream enriched with tea conserve

LVM – Like Very Much
LM – Like Moderately
LS – Like Slightly
NLND – Neither Like Nor Dislike
DS – Dislike Slightly
DM – Dislike Moderately
DVM – Dislike Very Much

3.3.8.5. Catechin-rich nutraceutical cookies

The second product tried in this direction was cookies (Figure 3.58). The cookies were prepared using the required food ingredients such as maida, sugar, ghee along with green tea catechin conserve. The concentration of green tea catechin conserve was tried in the range of 10-400 ppm. The tea catechin conserve was suitably solubilised to give homogenous product. The optimum concentration was found to be in the range of 20-50 ppm by sensory evaluation.

3.3.8.6. Sensory evaluation of catechin rich nutraceutical cookies

The results of the sensory analysis indicated that 53% of the respondents rated cookies as 'Like Very Much', 32% of the respondents rated it as 'Like moderately' and 15% of the respondents rated it as 'Like Slightly' indicating the product is acceptable. As the results shown in the following figure (3.60.) are falling on the 'Like' category, the product is acceptable.

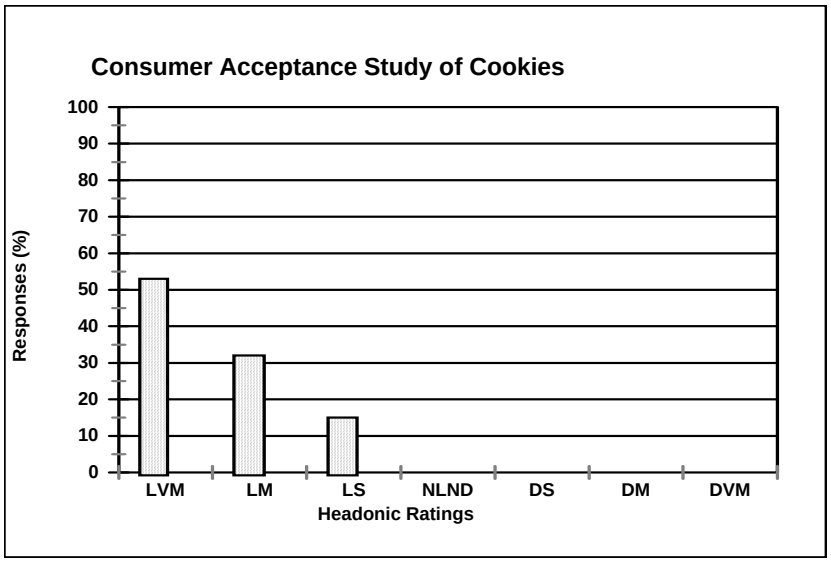


Figure 3.60. Sensory evaluation of Cookies enriched with tea conserve

LVM – Like Very Much
LM – Like Moderately
LS – Like Slightly
NLND – Neither Like Nor Dislike
DS – Dislike Slightly
DM – Dislike Moderately
DVM – Dislike Very Much

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CHAPTER 4
SUMMARY AND CONCLUSION

INTRODUCTION AND REVIEW OF LITERATURE

Sheng Nung the Chinese emperor (2737 B.C.) was the first to recognise the refreshing stimulant effect of tea. Tea is one of the important agro-industrial plantation crops of India. Tea is the beverage with which most of the Indians start their day. The recent research findings indicative of several health benefits have further popularized tea as a beverage. During the year 2007, India produced 945 million kg of tea from 38,705 gardens spread over an area of 4, 35, 057 ha. Out of this, domestic consumption accounts for 76 per cent and exports accounts for 24 per cent.

Tea plant belongs to the *Camellia* species of *Theaceae* family. The two basic varieties are recognised namely Chinese variety *Sinensis* and Assamese variety - *Assamica*. The commercially grown tea plant is highly heterogeneous. Tea flush contains polyphenols, amino acids, organic acids, polysaccharides, lipids, carotenoids, caffeine, chlorophylls, minerals and volatiles. The polyphenols which includes catechins constitute 25-30% of the fresh flush on dry weight basis. These are converted to theaflavins, thearubigins, theaflavic acids and bisflavonols during the manufacture of black teas and are responsible for colour, briskness, brighness and astrigency. Theaflavins are determined qualitatively and quantitatively whereas quantitative determination of thearubigins has been possible tentatively but their structures are yet to be explored completely. Caffeine is the major alkaloid present in tea and it is responsible for

stimulating action. Highly efficient HPLC method to determine soluble caffeine reported from this laboratory is used. Carbohydrates play an important role in formation of tea aroma. Lipid concentration increases with the maturity of the leaves and is responsible for the formation of C_6 volatiles during the manufacture of black tea. Three types of organic acids are present in tea viz., dicarboxylic acids, fatty acids and monocyclic acids. Monocyclic acids (e.g., Quinic and Shikimic) are the precursors of polyphenols. Chlorophyll a and b are reported to be present in the tea and are converted to pheophytins which are responsible for black colour of commercial tea. ß-carotene is the major compound among the carotenoids and degrades to character-impact volatile compounds such as theaspirone, ß-ionone and related compounds. Although K (Potassium) is the major mineral found the Cu (Copper) and Al (Aluminium), are important for the colour and taste of brewed teas. Theanine is the most abundant amino acid and accounts for 50% of the total amino acids and 1% of the dry weight of tea. Theanine is a constituent of the 'thearubigin' fraction while glutamic acid and ethylamine are its precursors. Amino acids and glucose interact with tea polyphenols during thermal processing and yield coloured moieties and Amadori products, which improve the flavour of tea.

Catechins theaflavins and thearubigins contribute to the bitterness, astringency, brightness and total colour of black tea infusion. Further thearubigins are responsible for body and richness of the tea brew. Theaflavin digallate is having lowest threshold value for the astringency. Caffeine contributes towards the bitter taste in tea. Characteristic umami or brothy taste

of black teas is due to the presence of amino acids. The ionone related aroma compounds such as theaspiranes found to have different odour properties. The aroma quality of tea with respect to theaspiranes is yet to be revealed completely.

Volatile compounds play a major role in determining the unique flavor of tea. Although >600 compounds are reported but the unique composition for character impact aroma of black tea is not yet established. The aroma quality of black teas with respect to the VFCs is measured by different ratios / indices viz., Mahanta ratio, Yamanishi-Botheju ratio, Terpene index, Wickremasinghe-Yamanishi ratio. Wickremasinghe-Yamanishi ratio is the ratio of sum of the peak areas of compounds eluting before linalool to the sum of the peak areas linalool plus all the compounds that elute after linalool. Smaller the ratio better is the quality. Mahanta ratio is the sum of the peak areas of terpenoids to non-terpenoids. Yamanishi-Botheju ratio is the ratio of peak area of linalool to E-2-hexenal. All the three ratios mentioned above have limitations for their applicability.

Another aroma quality indicator called flavour index (F.I.), the ratio of VFC II to VFC I is reported for kenyan clonal black teas and F.I. is positively correlated to tasters evaluations. This confirms that F.I. is a good aroma quality indicator for Kenyan black teas. However, it should only be used qualitatively since the olfactory perception limits of individual VFC are different. The FI for Indian black teas is yet to be explored and the limitation could be wide due to variation in weather. A new approach in terms of novel quality index for tea has been

innovated through this work. The sum of TF/TR ratios of tea and the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) added together is proposed for the first time as a new and novel quality index, hence forth referred to as Borse-Rao quality index, considered to be an overall quality indicator of tea as both the non-volatiles and volatiles are given due consideration in this quality index.

Tea is a good source of flavanoid antioxidants which has a role in prevention of cancer and coronary heart diseases. Tea is known to improve blood flow, eliminate alcoholic toxins, relieve joint pains and acts as a diuretic and improves resistance to diseases.

Flavonoids present in tea can effectively stabilize free electrons through several mechanisms viz., delocalisation of electrons, formation of intramolecular hydrogen bonds and rearrangement of their molecular structure. This may be the reason for their antioxidant property. The catechins ranked depending on their antioxidant potential as ECG > EGCG > EC > GA > GC > EGC > C. Theaflavins and thearubigens. These inhibited the formation of TBARS and are more effective than vitamin E, glutathione, vitamin C and synthetic phenolic antioxidants. Catechins are also found to be the scavengers of peroxynitrites which are capable of oxidising LDL.

Theaflavins and catechin gallates are more effective scavengers of aqueous and lypophilic stable radicals than many other flavonoids and many antioxidant vitamins. The inhibition mechanism of tea flavonoids is independent of metal ion chelation properties.

Tea flavonoids were found to reduce oxidative damage in animals from radiation, chemical oxidants, diet stress. Drinking of tea beverage was shown to reduce oxidative biomarkers in chronic smokers. Tea was found to reduce the metabolism of compounds to known carcinogens and enhance their detoxification. Thus, it is claimed to inhibit variety of cancers such as oesophageal, gastrointestinal, lung and skin cancers.

A cup of black tea was three and two times more effective than one serving of common vegetables and one serving of common fruits respectively.

PROFILING OF INDIAN BLACK TEAS

Indian teas especially Darjeeling, Assam and Nilgiris are valued world over for their superior aroma and taste.

In order to improve our scientific understanding on objective tea quality and thereby to help retain supremacy in the world tea trade, it was proposed to make an in depth study to generate fingerprint profile of teas grown in different regions of India which ultimately may result in a database with respect to volatile flavour compounds (VFC) as well as non volatile flavour compounds (NVFC) which are responsible for aroma, taste and quality of tea.

A study was carried on tea samples collected from nine regions spread over four seasons. The outcome of the research findings is given below:

Seasonal variation of TF/TR ratio over tea producing region/grade and quality

The TF content of a tea or the ratio TF/TR is considered to be a good quality indicator of tea. Accordingly seasonal variations of TF/TR ratios over the coded tea producing regions / grades in all the four seasons (s_1, s_2, s_3, s_4) were studied. The teas having TF/TR ratios up to 0.04, >0.04-0.08 and >0.08 can be considered to be a good, better and best quality indicator of tea quality respectively. Teas from the regions A to I are the better (TF/TR ratios >0.04-0.08) to best (TF/TR ratios >0.08) quality teas over all the four seasons except for the teas from region A, C, D (s_1), which fall under good quality category considering their TF/TR ratios (up to 0.04).

Also the teas from regions K to L (s_2), regions N,O (s_1), regions P,Q (s_3) and regions R,S (s_4) are the better (TF/TR ratios >0.04-0.08) quality teas except for the teas from region J (s_2), M(s_3) and T(s_4) teas, which fall under good quality category considering their TF/TR ratios (upto 0.04).

The teas from region/grade JKL (s_1, s_3), M toT (s_2), O,R,S,T (s_3), M,P (s_1), Q (s_4) are also falling under good quality category teas, considering their TF/TR ratios (upto 0.04).

Seasonal variation of sum of Yamanishi-Botheju and Mahantha ratio over tea producing region/grade and tea quality

The VFC (Volatile Flavour Compounds) content of a tea or the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) is considered to be a good quality indicator of tea. Accordingly seasonal variations of or the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) over the tea producing

regions / grades in all the four seasons (s_1, s_2, s_3, s_4) were studied. Accordingly, based on the sum of the VFC ratios (i.e.Yamanishi-Botheju ratio and Mahanta ratio) the teas can be categorized as a good (upto 1), better (>1-4) and best (>4) quality indicator of tea respectively.

The teas from regions A to J (all seasons) have better (>1-4) to best (>4) quality as indicated by sum of the VFC ratios (*i.e.*Yamanishi-Botheju ratio and Mahanta ratio) except for teas from regions A,E,F,H,I,J,(s_2), K to T (s_2), B,D,F,H,I,J(s_1), B,I,J(s_3) which are good (up to 1) quality teas, as indicated by sum of the two VFC ratios. Also, the teas from regions M (s_3), O (s_1), P (s_3), R,S,T (s_4) are good (up to 1) quality teas as indicated by sum of the two VFC ratios.

Seasonal variation of Borse-Rao quality index over tea producing region/grade and tea quality

The sum of TF/TR ratios of tea and the sum of the VFC ratios (Yamanishi-Botheju ratio and Mahanta ratio) added together is proposed for the first time as a new and novel quality index, henceforth referred to as Borse-Rao quality index, considered to be an overall quality indicator of tea as both the non-volatiles / volatiles are given due consideration in this quality index. Accordingly seasonal variations of the Borse-Rao quality index over the coded tea producing regions / grades in all the four seasons (s_1, s_2, s_3, s_4) were studied. Based on the the Borse-Rao quality index teas can be categorized as a good (up to 1), better (1-4) and best (>4) quality tea respectively.

The teas from regions/grade having Borse-Rao quality index more than four are C (s_2, s_4), D (s_3, s_4), G (s_1, s_2, s_3), H (s_3) and I (s_4) are the best (>4) quality teas.

The teas from regions having Borse-Rao quality index ranging from 1 to 4 are A (s_1, s_2, s_3, s_4), B (s_2, s_3, s_4), C (s_1, s_3), E(s_3, s_4), F(s_3, s_4), H (s_2, s_4), I (s_3, s_4), J(s_2), M (s_3) and RS (s_4) and indicate that these are better (1-4) quality teas.

The rest of the teas from regions having Borse-Rao quality index upto one are good quality teas which are B,D,F,H (s_1), E,F (s_2), I (s_1, s_2), J,K,L (s_3), K-T (s_2) and P-T (s_3).

FUNCTIONAL INGREDIENTS FROM UNUSED GREEN TEA LEAVES: BIOACTIVITY AND APPLICATIONS

Despite several reports on the radical scavenging activity of green tea from two leaves and a bud, the radical scavenging activity of green tea from coarse and pruned leaves in particular is not studied. The pruned and coarse tea leaves are tea plantation waste, India is one of the largest producers of tea. Therefore, testing of its radical scavenging properties is of interest primarily in order to find new promising sources for natural antioxidants. In the present work preparation of the green teas from the pruned or coarse tea leaves and optimization of extraction conditions to obtain catechin rich radical scavenging conserve was carried out with the following two objectives.

1. To isolate active conserves from coarse and pruned green tea leaves.
2. To use the active conserves for food applications

Normal, coarse and pruned fresh tea leaves (low grade) were procured and subjected to enzyme inactivation using cross flow dryer (80-120^{o}C, 4 – 8h), and continuous infra red dryer (70-120^{o}C, 0.5-1.5h) at different temperature and time intervals. The chemical parameters are found to be in the following range, Caffeine (1.30-3.20%); Total polyphenols (11.5-15.5%). Sensory characteristics were found to be similar to that of the commercial green tea samples.

Forty four compounds have been identified from the volatiles of green teas by comparison of retention indices as well as mass spectra individual compounds with that of literature data and also comparing retention times of the GC peaks with those of reference compounds run under identical conditions, wherever possible. The broad classification of the compounds identified includes ten terpenoids, three aromatic compounds, eight alcohols, seven aldehydes, four acids, eight esters and four compounds derived from carotenoids. Ethyl hex-(2*E*)-enoate and dihydroactinidiolide are exclusively present in the green teas derived from coarse leaves.

In general, coarse green teas irrespective of the method of processing contained more number of the volatile constituents, whereas the normal green teas irrespective of the method of processing contained less number of volatile constituents eluting before linalool. This can be attributed to the normal leaf quality, which contributes less volatiles from the group of constituents (Gr. I) which are undesirable and is the prevalent practice in the industry as well. It is also evident from the results that normal as well as a commercial sample of

green tea contained almost half the number of identified volatile constituents in the present investigation as compared to the coarse green teas contained. Dihydroactinidolide and ethyl hexenoate were found to be important markers and both were present in coarse green teas, whereas both were not found in the normal green teas including the commercial one. It can be concluded that the commercial green tea (Nilgiris green tea) is also prepared from the normal tea leaves. This can be very well used in spotting the normal / coarse green tea or the admixture of the coarse green tea leaves with the normal green tea leaves. Other important marker volatiles found were the both heptadienals [(E,Z)-2, 4 / (E,E)-2,4]. Coarse green tea contained both the heptadienals, whereas normal green teas did not contain any of the identified heptadienals in the present investigation. *cis*-3-hexenyl-n-hexanoate, ∝-ionone, *cis*-geranylacetone and β-ionone-5,6-epoxide were not found in a normal green tea. This can be attributed to the method of processing (CFD) and higher temperature (110°C) used.

Green tea extract

The extraction of green tea samples at lab scale, using different solvents (viz., Ethyl acetate, acetone, ethyl alcohol, methyl alcohol and their aqueous mixtures) was carried out.

The radical scavenging activity (RSA) of these extractives at 50 and 100 ppm concentrations were evaluated using the DPPH model system. The order of activity and extractability are as follows: Methanol > Ethanol> Acetone > Ethyl acetate. The aqueous alcoholic mixtures showed higher activity and polyphenol extractability than the respective single solvents.

It was found that the yields of the extractives from green teas of coarse leaves are relatively low on the expected lines and also the radical scavenging activities of the extractives of green teas from coarse leaves are marginally low at different concentrations. This observation indicated that the green teas from coarse leaves could be used for the preparation of radical scavenging conserves, by separating / enriching the active components using suitable technique.

Fractionation of the green tea extract

The extractives were subjected to liquid-liquid extraction using water and low molecular weight ester to fractionate the catechins into the solvent fraction. The yields of the solvent extracts are found to be 15±0.8% for coarse leaves and for normal leaves the yield of solvent extract is found to be 17±0.8%. The yield of the aqueous extract is 17±0.9 % for coarse leaves and for normal leaves the yield of solvent extract is found to be 19 ±1.0 %. These extracts were analyzed for total polyphenol content and evaluated for radical scavenging activity. The polyphenol content of the solvent extracts found to be 30±2.3% as gallic acid equivalents for coarse leaves, while, polyphenol content of the solvent extract of normal leaves is found to be 31±2.4% as gallic acid equivalents. The total polyphenol content in the aqueous portion of these extracts is 23±2.1% as gallic acid equivalents for normal leaves, while that for coarse leaves extracts is found to be 18±3.0% as gallic acid equivalents. However, the radical scavenging activity of the solvent extracts from both normal and coarse leaves is found to be same (92±1% at 15 ppm). The RSA of the aqueous extracts is found to be

lower. Hence, it may be concluded that the solvent selectively separated the compounds responsible for the radical scavenging activity.

The data obtained reveal that the green tea extracts / conserve is free radical inhibitor and primary antioxidant that react with DPPH radical, which may be attributed to its hydrogen donating ability.

HPLC profiling of green tea extractives, chemical composition and quantification

The total catechin content in the green tea extract based on the comparison of peak areas of each peak with that of authentic samples and from calibration curves was found to be in the range of 20–30 %. After fractionation the solvent extract is enriched with catechins and the total catechin content is found to be in the range of 55-85 %, while the HPLC profile of aqueous extracts showed only the presence of gallic acid and caffeine.

Extracts from unused fresh green tea leaves have the potential for large-scale application as natural antioxidants. Extracts of the green tea are becoming increasingly important as functional ingredients in the diet and are being added to a range of foods and beverages.

Improved method for the active conserve

To minimize the processing cost and to control the epimerisation of catechins during processing, alternate methods were explored. Green tea sample from fresh batch was subjected to aqueous alcoholic extraction. The extract was concentrated to remove the alcohol to the extent possible. The obtained miscella was cooled to 10^{o}C and kept over night at that temperature.

The separated solids were filtered and dissolved in low molecular weight ester and the filtrate was also treated with the same ester. Solvent was removed from the combined portion and the yield was found to be 12±2%. Both aqueous and ester portions were subjected to HPLC analyses. Ester soluble portion was found to contain most of the catechins. The aqueous portion (filtrate) was freeze dried and the solid yield was ~12±2%. Total polyphenol content and Radical scavenging activity (RSA) of the aqueous portion and ester portions were evaluated. RSA of the aqueous portion was found to be in the range of 70-85% at 40-50 ppm concentration, while that of ester portion was in the range of 85-90% at 10-15 ppm concentrations. The total polyphenol content in the aqueous portion was found to be 23-25%, while that of in the ester portion are in the range of 27-32%.

Economics of the process for catechin conserve

Cost of production (Basis: 800kg leaves/shift; 3 shifts/day; 300day)

Total cost of production per annum = Rs. 1, 91, 80, 000/-

Tea catechin conserve = Rs.1332/kg,Total sales envisaged = Rs. 2, 53, 44, 000/-

Market Price and returns

Tea catechin conserve = Rs.1600/kg, Tea gallic acid conserve = Rs.160/kg

Break even capacity = 38%

Basis- 2 shifts / day

Return on investment = 23%, Payback period = 4.35 Y

Basis- 3 shifts / day

Return on investment = 41%, Payback period = 2.43 Y

Catechin-rich nutraceutical ice-cream

Ice-cream was prepared using the other ingredients along with the tea polyphenol conserve. The concentration of tea catechin conserve was tried in the range of 5-200 ppm. The optimum range of tea catechin concentration was found to be in the range of 20-30 ppm.

The results of the sensory analysis for ice-cream with tea antioxidant extract show that 15% of the respondents rated under ‘Like Very Much (LVM)’, 45% of the respondents under ‘Like moderately (LM)’ and another 40% under ‘Like Slightly (LS)'. However the ice-cream samples are acceptable as the scores are falling on ‘Like’ category.

Catechin-rich nutraceutical cookies

The cookies were prepared using the required food ingredients along with green tea catechin conserve. The concentration of green tea catechin conserve was tried in the range of 10-400 ppm. The optimum concentration was found to be in the range of 20-50 ppm by sensory evaluation.

The results of the sensory analysis indicated that 53% of the respondents rated cookies as ‘LVM’, 32% of the respondents rated it as ‘LM’ and 15% of the respondents rated it as ‘LS’ indicating the product is acceptable. As the results are falling on the ‘Like’ category, the product is acceptable.

CONCLUSIONS

India is a long time leading tea producer except for the recent overtake by the China. India can boast of world class teas called trinitea - the Darjeeling, Assam and Nilgiris tea.

Black tea is the most consumed form of tea having health promoting attributes. But more focused research is needed to the understand the Science behind it's health benefits to realize health and wellness.

The profile of Indian black teas in terms of a bio-chemical fingerprint is carried out in present study which will not only help in understanding the intrinsic quality objectively but also help in tracing the origin of the teas based on the markers identified.

For the first time a novel approach has been evolved to mark teas on the basis of TF/TR ratio, flavour indices and a novel tea quality index (Borse-Rao quality index) is proposed which takes both volatiles and non-volatiles into account. However, further more research is needed to simplify the tea quality evaluation.

Processes for utilization of pruned/coarse green tea leaves (a plantation waste) have been worked out. A novel process for green tea preparation has been standardized and patented.

Processes for isolation, fractionation and enrichment (50-70 % catechin) and separation of radical scavenging conserve (10-15 ppm, 90-94% RSA) from pruned/coarse green tea leaves have been standardized and patented.

Application of this catechin-rich radical scavenging conserve in nutraceutical ice-cream and cookies was worked out and patented.

Tea being India’s strength, further more research work is needed in this direction to tap the potential of health benefits of tea and its high value products.

Further there is need for developing more economical and efficient processes.