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Research Article

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FT-IR study of green tea leaves and their diseases of Arunachal Pradesh, North East, India

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Abstract The fundamental aspect of green tea, its classification of disease and economic values has been briefly discussed. There are several diseases of green tea leaves; it is an experience by farmer of Arunachal Pradesh due to their climatic and topographic factors. With the help of FTIR study the effects of diseases on the qualities of Tea were studied. The FTIR analysis explores the vibration of functional groups present in macromolecules and shows the molecular structural changes through shifts in wave number. The decreased in wave number of protein at amide A observed in group II demonstrates the disorder of hydrogen bonding and pectin and lignin in infected groups. This change may reflect the overall changes in structure and synthesis of protein, pectin and lignin in tea during diseases. Control group may have prevented the changes of protein in tea by increasing the frequency through reducing the structural modification. The decreased protein content in tea was identified by the decreased in band area values of amide I and amide II bands. Amide I consists of functional vibrations of many secondary structures and it indicates that the amide I band, pectin and lignin frequency shifted to lower values in diseases infected tea groups.

Keywords Tea, diseases in Tea leaves, FTIR.

Introduction

Tea is an aromatic beverage commonly prepared by pouring hot or boiling water over cured leaves of the tea plant, After water, tea is the most widely consumed beverage in the world. It has a cooling, slightly bitter, and astringent flavour that many people enjoy. Tea likely originated in China during the Shang Dynasty as a medicinal drink [1]. In the time of the Shang dynasty (1766–1050 BC), the tea was consumed in Yunnan Province because of its medicinal properties. Tea was first introduced to Portuguese priests and merchants in China during the 16th century. Drinking tea became popular in Britain during the 17th century. The British introduced tea to India, in order to compete with the Chinese monopoly on tea. Tea has historically been promoted for having a variety of positive health benefits, and recent human studies suggest that green tea may help reduce the risk of cardiovascular disease and some forms of cancer, promote oral health, reduce blood pressure, help with weight control, improve antibacterial and anti-viruses activity, provide protection from solar ultraviolet light increase bone mineral density, and have "anti-fibrotic properties, and neuro protective power [2]. Additional research is needed to "fully understand its contributions to human health, and advise its regular consumption in Western diets. "Consumption of tea (especially green) is potentially beneficial to health and longevity given its antioxidant, flavanols, flavonoids, polyphenols, and catechins content. Tea catechins have known anti-inflammatory and neuro-protective activities, help to regulate food intake, and have an affinity for cannabinoid receptors, which may suppress pain, nausea, and provide calming effects [3]. Consumption of green tea is associated with a lower risk of diseases that cause

functional disability, such as “stroke, cognitive impairment, and osteoporosis” in the elderly. Tea contains L-theanine, and its consumption is strongly associated with a calm but alert and focused, relatively productive (alpha wave dominant), mental state in humans. This mental state is also common to meditative practice. The phrase "herbal tea" usually refers to infusions of fruit or herbs made without the tea plant, such as rosehip tea, chamomile tea, or rooibos tea. Alternative phrases for this are tisane or herbal infusion, both bearing an implied contrast with "tea" as it is construed here.

Materials and methods

Sampling and analysis

Study area: A green tea leaves were collected from “RONI TEA ESTATE”, Itanagar, Arunachal Pradesh North-east India. The Arunachal Pradesh recently started the cultivation of Tea, Rubber and other commercial plant in its valley, hills and plateau. Though the state has promising topography and climatic condition for good harvesting, but some disease exist which ruin the quality, yield and economy of the farmers as well as state.

Collected leaves can be classified into five categories as follows

1) Control leaves: The leaves which was not affected by any types of disease, supposed to be the best quality content of particular area under inspection.

Figure 1: Control Tea leaves

2) Pest eaten leaves: A bunch of caterpillar or looper caterpillar feeding on green tea leaves is great concern for tea farmer. Being a plantation crop that too formed at the cost of tropical and subtropical forests of north-east India, tea ecosystem is under constant pressure from insect pests since time immemorial. With global warming resulting in climate change, tea growing environments are undergoing rapid changes and new challenges and pest outbreaks are arising every now and then [4].

Figure 2: Pest eaten Tea leaves

3) Folded leaves: The folding of leaves are due to mites especially the top two to three leaves and the bud. Affected leaves become rough and brittle and corky lines and downward curling.

Figure 3: Folded Tea leaves

4) Yellow leaves: Deficiency of magnesium changes the green color of tea into yellowish. The deficiency of magnesium is caused by unavailability of those particular elements, in that particular place.

Figure 4: Yellow Tea leaves

5) White color leaves: White in colors of the green tea leaves is symptom of Leaves scorch or simply scorch. Leaves scorch is noninfectious condition caused by unfavorable environment. There is no chemical control of leaves scorch, so the most effective defense is good management.

Leaves scorch is often called as disease, but it is not caused by fungus, bacteria or virus, nor does it result from insect.

FTIR spectroscopy is a vibrational spectroscopic technique that can be used to optically probe the molecular changes associated with biological samples. Infrared spectroscopy utilizes the mid-IR region of the light to obtain an IR spectrum. It involves absorption measurements of different IR frequencies by a sample positioned in the path of an IR beam. The method is employed to find more conservative ways of analysis to measure characteristics within biological samples that would allow accurate and precise assignment of the functional groups, bonding types, and molecular conformations. Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition. FTIR peaks are relatively narrow and in many cases can be associated with the vibration of a particular chemical bond (or a single functional group) in the molecule.

Sample preparation: Thirty leaves were collected from control (uninfected) and four types of disease affected tea plants. Each group has 6 leaves sample. After washing thoroughly with tap water and finally with distilled water, the

samples were oven dried at 60 °C and ground into fine powder by agate mortar. Two milligrams of the sample were mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The spectra of all powdered and palletized samples were recorded under identical condition in the spectral range 4000-650 cm^{-1}.

Results and Discussion

FT-IR spectroscopy is a physical method applied to study the changes at the molecular level in various biological samples. Tea contains a large number of potentially bioactive chemicals, including flavonoids, amino acids, vitamins, caffeine and several polysaccharides, and a variety of health effects have been proposed and investigated. The band observed at the ~3415 cm^{-1} was assigned to Amide A ; mainly presence of N-H stretching of proteins due to little contribution of hydrogen molecules [5]. The peak at ~2956 $cm^{-1}$and ~2932 cm^{-1} shows CH_3 asymmetric and CH_3 symmetric stretching respectively, depicting mainly lipids [6]. The peak corresponding to wave number ~2852 cm^{-1} has given CH_2 asymmetric stretching; which are mainly due to lipids [6].

The amide I was assigned to the both band observed at ~1657 cm^{-1} [7] and ~1640 cm^{-1} due to C=O stretching of protein and C=O stretching of protein (random coil) [8] respectively. Similarly the wavenumber ~1630 cm^{-1} was represented by Amide II, which is because of N-H bending and C-N stretching of proteins [9].

At wave number ~1612 cm^{-1}, ~1321 cm^{-1} and ~1244 cm^{-1} due to presence of lipids, protein and lipids, and nucleic acid with the little contribution of phospholipids, the peaks are assign with CH_2 symmetric stretching [8], COO- symmetric stretching [5] and PO_2^- asymmetric stretching respectively [10].

The observed band corresponds to wave numbers ~1142 cm^{-1}, ~1074 cm^{-1}, ~1038 cm^{-1} and ~664 cm^{-1} were assigned with CO-O-C asymmetric stretching due to glycogen and nucleic acids, PO_2^- symmetric due to nucleic acids [5], CN stretching due to nucleic acids [11] and CH_2 bending due to carbohydrate, protein and lipids respectively [10].

The wave number versus absorbance plots gives the peak values, peak area and assignment of functional groups in green tea leaves of control and various disease affected in the wave number range of 4000-650 cm^{-1} as shown in Figure 6.The graph for control leaves are represented by black line, likewise yellow color leaves by pink color, folded leaves by green color, white color by red color and pest eaten leaves by violet.

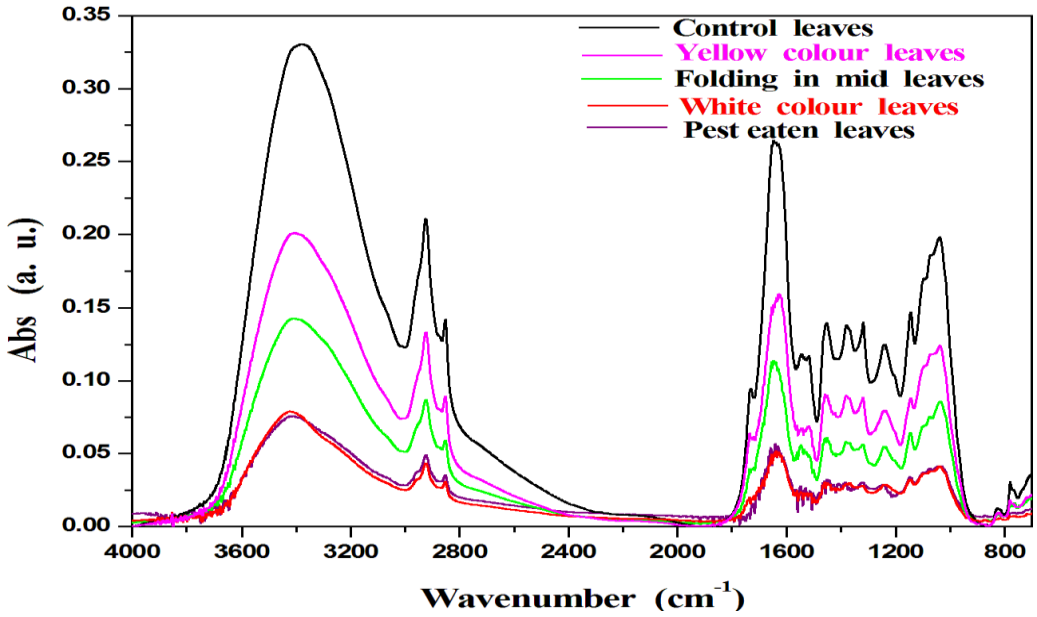

Figure 6: Representatives FT-IR spectrum of control, yellow color, folding in mid, white color and pest eaten Tea leaves in the range of 4000-650 cm^{-1}

Table 1: FT-IR spectra vibrational Assignments for control, pest eaten, folded, yellow and white tea leaves.

Control	Pest eaten	Folded leaves	Yellow leaves	White leaves	Peak assignment
3415	3377	3408	3404	3422	Amide A: mainly N-H stretching of proteins due to little contribution of hydrogen molecules
2956	----	----	----	----	CH_3 asymmetric stretching mainly lipids
2923	2924	2924	2924	2923	CH_3 symmetric stretching mainly lipids
2852	2852	2852	2852	2852	CH_2 asymmetric stretching mainly lipids
-----	1723	------	1729	------	Carbonyl C=O stretching due to lipids
1657	------	-----	----	---	Amide I : C=O stretching of protein
1640	1647	1647	-------	1637	Amide I : C=O stretching of protein (random coil)
1630	----	-----	1624	-----	Amide II : N-H bending and C-N stretching of proteins
1612	-------	------	------	------	CH_2 symmetric stretching mainly lipids
------	1545	1545	----	-----	CH_3 Asymmetric bending mainly proteins
------	1510	------	-----	----	CH_3 symmetric bending mainly proteins
------	1452	1451	1457	1458	asymmetric deformation (bend) of CH_3 and CH_2 from proteins lipids, lignin
------	1379	1379	1379	1364	COO- symmetric stretching mainly from pectin
1321	1317	------	1318	-------	COO^- symmetric stretching due to protein and lipids
1244	1240	1240	1240	1239	C-C, C-O stretching from carbohydrates and lignin
1142	1146	1147	1146	1147	C-O-C stretching of polysaccharides
1074	--------	------	-----	------	PO_2^- symmetric stretching mainly due to nucleic acid
1038	1038	1037	1037	1038	C-N stretching due to nucleic Acids
------	825	-------	825	-----	C-H bending due to lipids
-------	781	-------	775	-----	C-H out of plane bending due to lipids
664	-----	660	669	669	CH_2 bending due to carbohydrate, protein and lipids

3600-2400 cm^{-1} region: It is evident from Figure 7 absorption intensity are dominated by Amide A, CH_3 and CH_2 groups contained mainly lipids and proteins with little contribution hydrogen molecules. In this region control group appeared at 3415 cm^{-1}, pest eaten appeared at 3377 cm^{-1}, folded appeared at 3408 cm^{-1}, yellow colored appeared at 3404 cm^{-1} and white colored appeared at 3422 cm^{-1} due to Amide A in the green tea leaves mainly N-H stretching of proteins due to little contribution of hydrogen molecules. Only control leaves appeared at 2956 cm^{-1} in this region due to CH_3 asymmetric stretching mainly lipids. This means that wave number decreased in folded, pest eaten, yellow color and white color tea leaves. Except at 2852 cm^{-1} control and disease affected appeared same due to CH_2 asymmetric stretching mainly lipids. Again in this region control and white color appeared at 2923cm^{-1} due to CH_3 symmetric stretching mainly lipids and pest eaten, folded and yellow color appeared at 2924 cm^{-1}.

The out-put graphs shown by Figure 6 have band areas and intensity of absorptions of control group is much higher than other groups as well as larger quantities of Amide A, amide I, amide II, pectin and lignin and all others functional groups where lipids and proteins are more responsible.

1800-800 cm^{-1} region: The absorption intensity are dominated by Carbonyl C= O, Amide I, Amide II,CH_3 ,CH_2, COO^-,PO_2^-, C-N and C-H groups contain mainly lipids, proteins , proteins random coil and nucleic acids as shown in Figure 8. The peak of pest eaten appeared at 1723 cm^{-1} and yellow leaves appeared at 1729 cm^{-1} but absent for control, folded and white leaves mainly Carbonyl C=O stretching due to lipids. In this region control appeared at 1640 cm^{-1}, pest eaten and folded leaves appear at 1647cm^{-1}, yellow color does not appear and white appear at 1637 cm^{-1} due to Amide I : C=O stretching of protein(random coil).

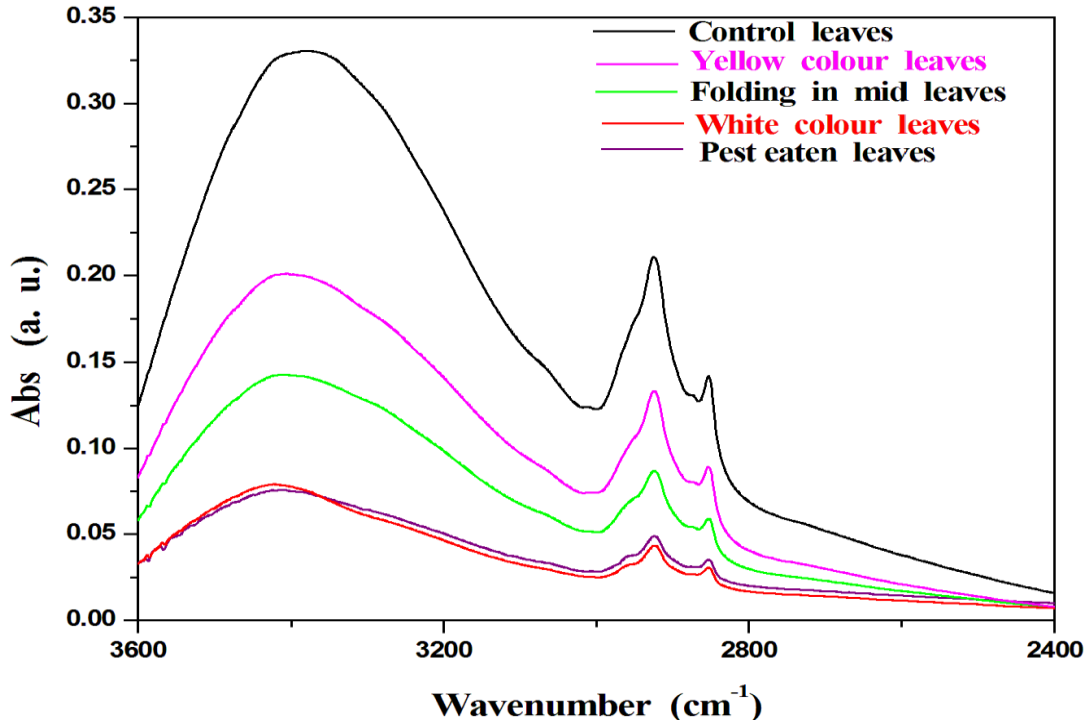

Figure 7: Selected FT-IR spectra in the range of 3600-2400 cm^{-1}

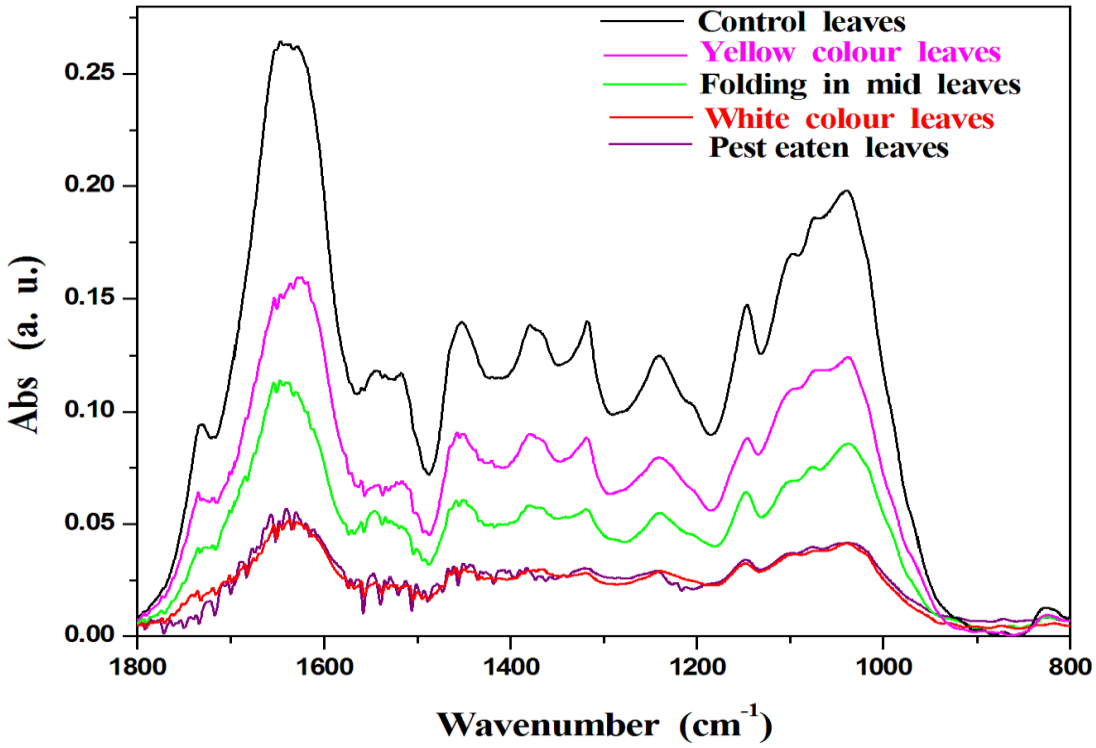

Figure 8: Selected FT-IR spectra in the range of 1800-800 cm^{-1}

Only control leaves appeared at 1657 cm^{-1},1612 cm^{-1} and 1074 cm^{-1} in this region due to Amide I : C=O stretching of protein, CH_2 symmetric stretching mainly lipids and PO_2^- symmetric stretching mainly due to nucleic acid. In some region control disappeared but others, such as pest eaten and folded leaves appeared at 1545 cm^{-1} due to CH_3

Asymmetric bending mainly proteins. Only pest eaten appeared at 1510 cm^{-1} due to CH_3 symmetric bending mainly proteins. Again control is absent but pest eaten appeared at 1452 cm^{-1} & 1379 cm^{-1}, folded leaves appeared at 1451 cm^{-1} & 1379 cm^{-1}, yellow leaves appeared at 1457 cm^{-1} & 1379 cm^{-1} and white color at 1457 cm^{-1} & 1364 cm^{-1} due to CH_2 bending mainly lipids and COO^-asymmetric stretching mainly fatty acids and amino acids. The control leaves appeared at 1321 cm^{-1}, pest eaten appeared at 1317 cm^{-1} and yellow leaves appeared at 1318 cm^{-1} was mainly COO^- symmetric stretching due to protein and lipids. The control leaves appeared at 1244 cm^{-1}, pest eaten and others appeared at 1240 cm^{-1} due to PO_2^- asymmetric stretching mainly nucleic acid with the little contribution from phospholipids. The control leaves appeared at 1142 cm^{-1}, pest eaten and yellow leaves at 1146 cm^{-1}, and folded and white leaves appeared at 1147 cm^{-1} were mainly CO-O-C asymmetric stretching due to glycogen and nucleic acids. With slight change control, pest eaten and white leaves appeared at 1038 cm^{-1} and folded leaves and yellow leaves appeared at 1037 cm^{-1} mainly C-N stretching due to nucleic Acids. The control, folded, and white color absent but pest eaten and yellow leaves appeared at 825 cm^{-1} mainly C-H bending due to lipids.

Discussion

According to the peak assignments for wave number of observed bands of disease affected leaves were compared with control leaves were presented in Table 1. Wave number at assignments of amide A, COO^- symmetric and PO_2^- asymmetric of various disease affected leaves were lower than the control leaves (amide A-3415 cm^{-1},COO^- symmetric-1321 cm^{-1} & PO_2^- asymmetric-1244 cm^{-1}) as proteins, nucleic acid and lipids contents were decreased. On control sample CH_3 asymmetric stretching (2956 cm^{-1}), Amide I (1657 cm^{-1}),CH_2 symmetric stretching(1612 cm^{-1}) and PO_2^- symmetric (1074 cm^{-1}) were appeared but not appeared in diseased leaves. Some assignment like, CH_3 symmetric (2923 cm^{-1}), CH_2 asymmetric(2852 cm^{-1}) and C-N stretching (1038 cm^{-1}) has either wave number similar or else shown wave number very near to each other. But in some assignments like carbonyl C=O stretching, CH_3 asymmetric bending, CH_3 symmetric, CH_2 bending, COO^- asymmetric stretching, C-H bending, and C-H out of plane were not present in control leaves but due to amino acid, and fatty acid content, appeared at uncontrolled leaves.

Conclusion

FTIR spectroscopy is a physical method applied to the study of cellular changes and toxicological study at the molecular level in various biological samples. The FTIR analysis explores the vibration of functional groups present in macromolecules and shows the molecular structural changes through shifts in wave number. In this study, the decreased in wave number of protein at amide A observed in group II demonstrates the disorder of hydrogen bonding and alteration of pectin and lignin in the under the infected groups. This change may reflect the overall changes in structure and synthesis of protein, pectin and lignin in tea during diseases .From the above results it is evident that, chemical composition is greatly influence by tea diseases which are prominently active in Arunachal Pradesh. The chemical constituents of green tea leave such as lipids, protein, carbohydrate, Amino acid, nucleic acid and fatty acid are venerable which account for the reduced in qualities of tea due to above mention diseases. Thus, prevailing diseases are great challenge to the tea consumers of Arunachal Pradesh and in future taking great consciousness about these diseases.

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