A DISSERTATION ON

OPTIMIZATION OF KASHMIRI GREEN TEA (NOON CHAI) EXTRACTION USING RESPONSE SURFACE METHADOLOGY

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
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IN PARTIAL FULFILMENT FOR
THE MASTER OF TECHNOLOGY
IN FOOD TECHNOLOGY

BY
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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Mariya Ali**, a student of **M.Tech. Food Technology** (III/VI), Integral University have completed my six months dissertation work entitled **"Optimization of Kashmiri Green Tea (Noon Chai) Extraction Using Response Surface Methodology"** successfully from **Integral University** under the able guidance of **Dr. Kaiser Younis.**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Name and Signature of Student with Date

Name and Signature of Course Coordinator with Date

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CERTIFICATE

Certificate that Ms. **Mariya Ali** (Enrollment Number 1200100877) has carried out the research work presented in this thesis entitled **"Optimization of Kashmiri Green Tea (Noon Chai) Extraction Using Response Surface Methodology"** for the award of **M.Tech. Food Technology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **M.Tech. Food Technology**

I wish her good luck and bright future.

Dr. Kaiser Younis
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CERTIFICATE BY INTERNAL ADVISOR

This is to certify that Mariya Ali**,** a student of **M.Tech. Food Technology** (III Year/ VI Semester), Integral University has completed her six months dissertation work entitled **"Optimization of Kashmiri Green Tea (Noon Chai) Extraction Using Response Surface Methodology"** successfully. She has completed this work from Integral University under the guidance of Dr. Kaiser Younis, Assistant Professor, Department of Bioengineering. The dissertation was a compulsory part of her **M.Tech. Food Technology.**

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Dr. Kaiser Younis
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TO WHOM IT MAY CONCERN

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MARIYA ALI

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ACRONYMS

CCD	Central Composite Design
ROS	Reactive Oxygen Species
RSM	Response Surface Methodology
EGC	Epigallocatechin
GTE	Green Tea Extract
BTE	Black Tea Extract
DPPH	Diphenyl-2-picrylhydrazyl
RO	Reverse Osmosis
ANOVA	Analysis of Variance
FD	Freeze Drying
OD	Oven Drying

Abstract

The preparation of Kashmiri Noon tea has not been examined scientifically to optimize extraction techniques and make instant Kashmiri tea powder by using freeze dried method and oven dried method. In the present study, the Noon tea was procured and the extraction was optimized using response surface methodology. The effect of brewing conditions (temperature, aeration and sodium bicarbonate) on the absorbance and Colour of Noon Chai were studied. Tea was extracted in water at 100ºC for different time levels. The extracted was then aerated for different time periods. Central composite design (CCD) with 20 runs was employed using Design Expert 13.0.5.0 software with the quadratic design model. The best conditions obtained were 50 min of time and 300 mg of alkali concentration. The l, a, b value of freeze dried and oven dried sample were observed with the sensory evaluation of instant tea powder through a train panellist using hedonic scale.

Keywords: Kashmiri Noon Tea, Response surface methodology, Phytochemicals, Instant Tea Powder, Physiochemical properties.

CHAPTER 1
INTRODUCTION

The most popular beverage eaten worldwide is "tea," which was first produced in China and is now a recognised food item. Tea is grown in over 30 countries, with India being the world's second-largest producer after China. It is estimated that 18 to 20 billion cups of tea are consumed every day; for example, the United Kingdom's estimated average consumption is 1 L per person per day (Prasanth et al., 2019). All types of tea are made from the young, fragile leaves of the perennial, evergreen Camellia sinensis (L.) (family Theaceae), a leafy plant that thrives in warm, humid locations with abundant rainfall. Freshly harvested leaves are processed to create green, white, black (fermented), and oolong (semi-fermented) tea. Each kind differs from the others in terms of appearance, flavour, and preparation (Khan & Mukhtar, 2013). Black and green tea are two of the most popular forms of tea drank worldwide. To create various varieties of tea, several processing methods are used on the gathered leaves and shoots. Black tea makes up around 76–78% of all tea produced and consumed globally, followed by green tea (20–22%) and oolong tea (2%). Its aroma and flavour make it a prized culinary item. As a result, tea has attracted a lot of interest among health beverages in the food industry (Farooq & Sehgal; Nookabkaew et al., 2006) (Cabrera et al., 2006)

Globally, consumers of black tea outnumber those of green tea by a large margin. However, due to numerous studies showing a wide range of health benefits from regular consumption of green tea, especially in the West, including a decreased risk of cardiovascular disease and certain types of cancer, inflammatory bowel, liver, and neurodegenerative diseases, diabetes, and even weight loss, green tea consumption has recently increased (Astill et al., 2001). The high catechin content of this food is thought to provide health advantages since catechins are powerful antioxidants that can treat diseases brought on by reactive oxygen species (ROS) (Fang et al., 2008). Green teas are unfermented teas that are drank after being boiled briefly in hot water to extract the polyphenols.

In several regions of the world, the extraction process for green teas has undergone some changes. Salted green tea is brewed with water and sodium bicarbonate in the Himalayan area of India, particularly in Kashmir, for 15 to 60 minutes in order to extract the tea's solids, especially its polyphenols. The final product is concentrated and must be prepared

by dilution with water and the addition of milk since the extract so produced is concentrated (Cabrera et al., 2006). A hot cup of tea is served. It is also known as Pink Tea because of its pink colour. However, the colour is creamy to yellowish if sodium bicarbonate is not added during the extraction process (Khan & Mukhtar, 2013).

In rural areas of Kashmir where agriculture is the key occupation, Noon Chai use is slightly more common. It is frequently consumed throughout working hours in these locations in addition to the morning and afternoon. In the winter, people drink more chai in the afternoon, especially in rural areas where it provides warmth and indoor entertainment. According to Kashmiri mythology, Noon Chai is cooling in the summer and fights the cold in the winter. It is said to have diaphoretic qualities, maybe because of sodium bicarbonate concentration (Wani et al., 2013)

Green tea or a Kashmiri chai blend work well for making pink tea since they have greater polyphenol contents than other types of tea. White tea, on the other hand, contains a relatively low level of polyphenols. In fact, adding baking soda makes a white tea infusion, which first appears pale-yellow, light brown. Although there is a visible shift in colouring, it is not nearly sufficient to produce beautiful pink tea.(Parvez et al., 2022) The secret to achieving excellent pink tea is accurate amount of baking soda measurement. Less soda will not render tea pink, whereas too much sodium bicarbonate will turn it black.

Tea catechins, in particular, which are powerful antioxidant and antibacterial substances with favourable effects on human health, are found in high concentrations in tea. The antioxidant capacity and total phenolic content of newly produced green tea extract were studied by Molan and his coworkers (Fang et al., 2008). They came to the conclusion that key variables affecting the nutritional value of this beverage included the brewing conditions, including extraction temperature, extraction time, and the ratio of tea leaves to extraction water.

The use of the response surface methodology (RSM) is a useful statistical strategy for assessing the impact of many variables and their interactions. It is also useful for determining the combinations of these elements that will result in the best reaction (Bae et al., 2015). The optimization of extraction parameters including extraction duration, temperature, and solid-to-solvent ratio has frequently been employed. The best conditions for extracting tea polyphenols, epigallocatechin gallate, and theanine from summer green tea have been identified using response surface methodology (RSM). According to studies

on Kashmiri Noon Tea, producing the beverage concentrate in this way takes time and requires brewing expertise (Kilmer, 2010).

Presently, there is demand in the market for instant extracts of Kashmiri Noon Tea that are ready for usage. The Kashmiri Noon Tea's optimal extraction conditions, which provide a high antioxidant potential and the highest extraction of polyphenols, have not yet been the subject of a comprehensive research. As a result, the study's aim is to examine various extraction parameters and improve the extraction conditions for Kashmiri Noon Tea beverages with better yield, high antioxidant, and polyphenol content.

Objectives:

1. Optimization of Kashmiri Noon Chai Extract
2. Drying of optimized extract by freeze drying and oven drying
3. Physicochemical Properties of the dried extracts

CHAPTER 2
REVIEW OF LITERATURE

The Camellia sinensis plant's leaves are used to make tea. Tea was first cultivated in China and is now grown in over 30 nations. It is the most popular beverage worldwide, second only to water (Higdon et al., 2003) . Tea, which has its origins in China, has been more popular over the past 2000 years. At first, it was solely drunk by Chinese monks, but as its usage grew to other nations, including Great Britain, it effectively spread to Western nations. Tea is now consumed regularly by individuals as a daily beverage and as a treatment for a variety of ailments. There are several types of tea.

In the global scenario, Indian tea India is the world's largest producer of black tea in addition to being its biggest consumer. India now generates 23% of the world's total tea production and uses roughly 11% of the tea consumed globally. This demonstrates that around 80% of the tea produced is consumed domestically. Due to fierce competition from Sri Lanka, Kenya, and China over the past 20 years, India's export ranking has dropped from first to fourth. India's population, income, and tea consumption are all rising, which is causing a decline in tea exports (Wagh, 2014).

Table 1**,** Scenario of Indian Tea

Particulars	**World**	**India**	**Rank**
Tea Area (Million hectors)	3.96	0.59	2nd
Production of Tea (Million Kg.)	4169	976	2nd
Yield of Tea (Kg/Hector)	1144	1670	1st
Tea Exportation (Million Kg)	1739	195	4th
Tea Consumption (Million Kg)	3983	840	2nd

Each kind stands out due to its distinctive look, flavour, and preparation. Black tea accounts for 80% of all tea consumption worldwide and is the most popular beverage in Europe, North America, and North Africa (apart from Morocco), whereas green tea is preferred in Asia, and oolong tea is popular in China and Taiwan (Wu & Wei, 2009). Black (fermented),

green (non-fermented), and oolong teas can be classified according on processing or harvested leaf

development (semifermented). In accordance with the various drying and fermenting procedures that affect the chemical composition of tea, these primary tea kinds differ in how tea is manufactured and processed (Khan & Mukhtar, 2013).

To prepare black tea, the tea leaves must first oxidise by being exposed to air. The flavour of the leaves is enhanced during the oxidation process, which turns them in a dark brown colour. After that, the leaves can either be left alone or roasted, dried, and crushed. Emerging research indicates that black tea may have similar health-promoting properties to green tea, which has been the subject of the most research on health benefits, including cancer chemoprevention and chemotherapeutic actions (Tejero et al., 2014). Young tea leaves are used to make green tea, which is then graded, withered, steamed or pan fired, dried, and marketed for consumption devoid of fermentation. To stop the tea leaves from fermenting due to natural enzyme activity, pan firing is necessary. Before being smoked, flamed, or steamed to create black tea, tea leaves are left to ferment for a number of hours. Oolong tea is made by partially oxidising the leaf, a step between making green and black teas (D'Ulivo, 2018).

Due to its numerous health advantages and unique flavour, green tea is a preferred beverage around the globe, but it is especially popular in most Asian nations like China and Japan. Non-fermented green tea has more polyphenols than black and oolong teas since it is not fermented. Epigallocatechin (EGC), pigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), and epicatechin (EC) are the distinctive polyphenolic chemicals found in green tea (EC). Tea also contains flavonols including kaempferol, quercetin and glycosides as well as its myricitin. The polyphenolic favonol component catechins and its gallate derivatives are what give green tea its unique properties. Contrarily, green tea's caffeine content has both beneficial and detrimental impacts on health (Liu et al., 2018). Numerous psychostimulant effects, such as motor stimulation and reinforcing effects, have been associated to caffeine use in studies (Malik et al., 2009). On the other hand, excessive intake is considered to lead to agitation, anxiety, arrhythmia, a lower rate of conception, and many other symptoms (Wani et al., 2013). In order to obtain the most health advantages from green tea extracts, the cafeine level must be reduced.

Green tea has been utilised in traditional Chinese medicine as a beverage to enhance human health since ancient times. In addition to other physiological processes including body weight regulation, bone mineral density enhancement, and sun UV protection, green tea has been proven to have antibacterial, antimutagenic, anti-inflammatory, antidiabetic, and hypocholesterolemic effects (Cabrera et al., 2006). A green tea infusion has three flavours that make up its overall flavour: astringency, bitterness, and umami The first two flavours are its main flavours (Yu et al., 2014).

The least oxidised and fermented of these teas, green tea, has the highest concentration of polyphenols. Many health-promoting properties of green tea, such as its anticancer, anti-inflammatory, antibacterial, antiviral, anti-obesity, and antioxidant properties, have been identified in recent years (Pastoriza et al., 2017). The phytochemical composition of green tea, which consists of a complex mixture of polyphenolics, methyl xanthine, free amino acids, vitamins, and other minor components that individually or synergistically affect in vitro and in vivo biological activities, is thought to be responsible for these functions (Khan & Mukhtar, 2013).

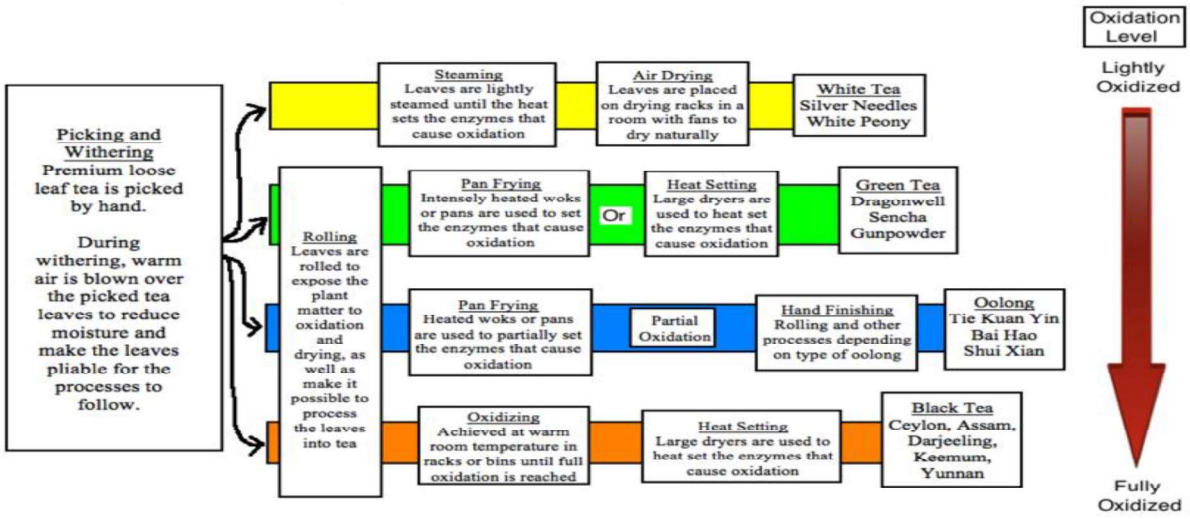


Figure 1: Schematic diagram of the conventional manufacture process of green, black, oolong and white tea (Source Net)

Chromatographic Methods Used to Determine the Chemical Composition of Tea:

Chromatographic procedures are essential for tea analysis because they separate distinct components on specific chromatographic columns depending on how they interact with the stationary phase and mobile phase of the column. After being divided, each component is

measured using specialised detectors at the column exit (including mass spectrometer, electrochemical detection, diode array and absorbance detector).

Numerous sample categories, including gases, high-molecular weight chemicals, cation and anion mixes, have been identified and quantified using chromatographic techniques. Unsurprisingly, the two main techniques used to examine the components of tea are chromatography and capillary electrophoresis (see Table 1).

Table 2, Chromatographic techniques were utilised to analyse tea's chemical composition

No.	Methods	Notes: Specific Characteristics and Applications	Reference
1	Gas chromatography (GC)	Volatile components which determine the aroma of tea. Low volatile components in the form of derivatives, particularly in the form of trimethylsilyl derivatives.	(Yashin et al., 2015)
2	SPME-GC	Vitamin K, volatile flavour compounds,	(Yashin et al., 2015)
3	GC-ECD	Pesticides, organochlorides, aroma precursors	(Xiao et al., 2020)
4	Gas chromatography with mass spectrometry (GC-MS)	Used for identification of a range of compounds, including Pesticides, Essential oils, volatile compounds, metabolic fingerprinting, acrylamides	(Pongsuwan et al., 2007)
5	Paper chromatography	Two-way chromatography with visual spectrophotometry.	(Singh et al., 1999)
6	Thin layer chromatography	Separation of catechins on cellulose plates; chemically modified phases spread on plates.	(Ligor et al., 2008)

7	High performance liquid chromatography (HPLC)	HPLC-UV Determination of polyphenols, monosaccharides, flavonoids, caffeine	(Wright et al., 2001)
8	HPLC-ECD	Selective determination of polyphenols, including catechins	Sano et al., 2001;
9	HPLC-FD	Determination of (+)-catechin in tea	(Kayali-Sayadi, 1998)
10	HPLC-MS	Identification of theaflavins, etc.	(Martínez-Domínguez et al., 2015)
11	High-speed countercurrent chromatography	Separation of black tea theaflavins. Catechins, theflavins	(Yashin et al., 2015)
12	Chiral chromatography	Separation of catechin optical isomers.	(Gotti et al., 2009)
13	Ion chromatography	Separation of cations and anions of dibasic acids.	(Ding et al., 1997)
14	Size-exclusion (molecular-sieve) chromatography	Separation of high-molecular weight compounds.	(Ødegård & Lund, 1997)

Abbreviations: UV-ultraviolet detector, ECD-electrochemical detector, FD-fluorometric detector, CL-chemiluminescence, MS-mass spectrometry.

Composition of tea

In addition to polyphenols, alkaloids (including caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile chemicals, fluoride, minerals and trace elements, and other unidentified substances, tea also contains a variety of additional chemical components. The most intriguing category of these tea leaf constituents is made up of polyphenols, which both in vitro and in vivo have strong antioxidant activity.

Table 3, Primary chemical compounds present in tea (analysed by Chromatographic Methods) (Yashin et al., 2015)

No.	Compound name	Notes: Main representative elements
1	Catechins	Flavanols: 12 catechins are indentified, including 8 occurring in significant quantity, i.e., (+)-catechin, (-)- epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-gallocatechin gallate, (-)-epigallocatechin gallate
2	Oxyaromatic acids	Gallic, caffeic, quinine, chlorogenic, n-coumaric acids
3	Flavonols Quercetin	Kaempferol, myricetin
4	Theaflavins Theaflavin,	theaflavin-3-O-gallate, theaflavin-3'-O-gallate, theaflavin-3-3'-O-gallate
5	Teagallins	Teagallin
6	Thearubigins	High-molecular weight polymers of catechin gallates with molecular weight from 1000 to 40000 Da
7	Pigments	Carotenoids and chlorophyll
8	Alkaloids Caffeine	theophylline, theobromine
9	Sugars	Glucose, fructose, saccharose
10	Amino acids	Isoleucine, leucine, methionine, threonine, phenylalanine, glutamine, asparagine, alanine, serine, proline, histidine, glutamic acid, aspartic acid, theanine
11	Vitamins	C, α-, β-, γ-, δ-tocopherols, riboflavin
12	Dibasic acids	Succinic, malic, tartaric, citric, quinic, aspartic, glutamic, oxalic acids
13	Cations	K+, Na+,Ca2+, Mg2+, NH4+ Al3
14	Metals	Fe, Zn, Cu, Ni, Al

15	Lignans and triterpenoid saponins	Mixture of many compounds

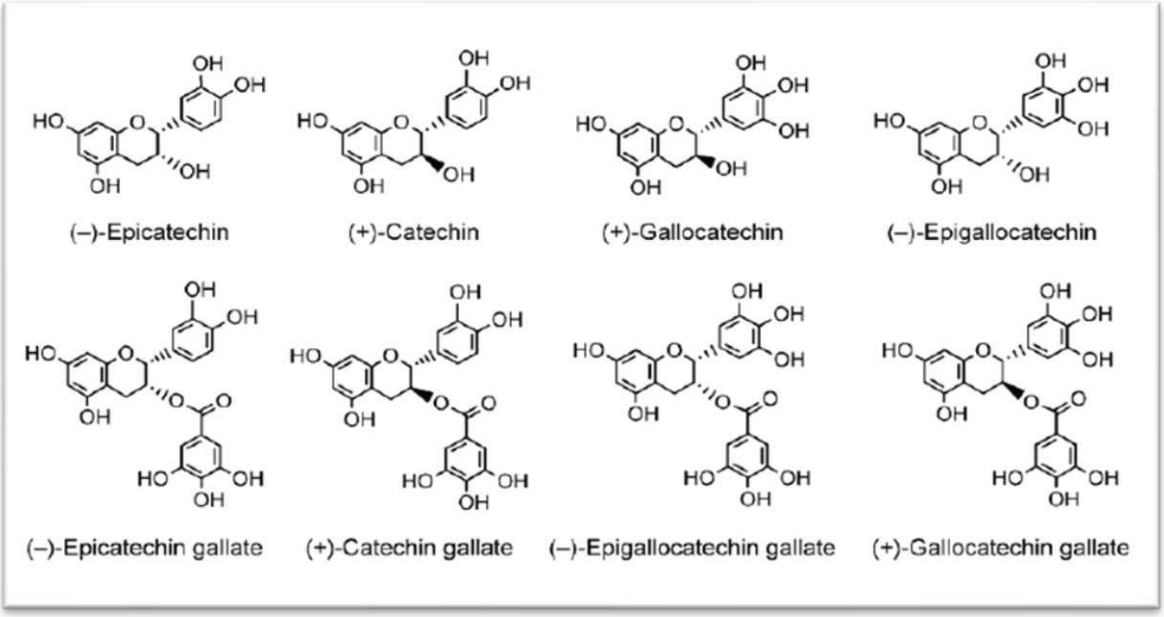


Figure 2, Eight major catechins found in tea (Yashin et al., 2015)

Black tea mostly includes tannins, and green tea primarily contains catechins (Yashin et al., 2015). Notably, green tea is regarded as the most important dietary source of catechins, outpacing apples, red grapes, wine, and chocolate. Depending on the age of the leaf, the amount of caffeine varies between 2 and 5 percent, with younger leaves having a greater quantity (Malik et al., 2009). The European Food Safety Authority (EFSA) estimates that there are 126 mg of catechins in every 100 mL of green tea. However, the Food and Drug Administration (FDA) estimates that there will be 71 mg of epigallocatechin gallate in every 100 mL of green tea. Black tea has 200 mg of flavonoids for every 100 m.

Table 4, Classes of compounds contained in aroma of black tea (Wu & Wei, 2009)

No.	Compound class	Number of identified compounds
1.	Acids	71
2.	Ketones	57
3.	Aldehydes	55

4.	Esters	55
5.	Alcohols	46
6.	Hydrocarbons	37
7.	Pyridines	23
8.	Pyrazines	22
9.	Phenols	19
10.	Amines and nitrogen-containing compounds	18
11.	Lactones	16
12.	Pyrroles	10
13.	Furans	9
14.	Thiazoles	7
15.	Sulfides and sulfur-containing compounds	5
16.	Oxazoles	2
17.	Thiophenes	1
18.	Other compounds	14

Total: 467 compounds.

The substances listed below determine the characteristic scent of green tea:

coumarone, indole, coumarin, coumarone, 6-methyl-ionone, and D-nerolidol. The freshness of the green tea scent is produced by aliphatic alcohols, aldehydes, 3-hexene acid, and methyl jasmonate. The depth of fragrance is provided by 3,5,5-trimethyl-2(5H)-furanone and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4)-benzofuranone. The flowery and fruity undertones of the scent are enhanced by linalool, 2,6-dimethyl-1,3,7-octatriene-6-ol, benzeneacetaldehyde, and 3-hexanyl hexanoate. A burned and somewhat sweet odour is produced by 1-ethyl-1H-pyrrole-2-carboxaldehyde, 3-ethyl-4-methyl-1H-pyrrole-2,5-dione, 3-ethyl-3-methyl-2,5-pyrrolidinedione, coumarone, and coumarin. Capillary column gas chromatography in temperature-programming mode with flame ionisation or mass spectrometry detection is often employed to identify the volatile elements (Prasanth et al., 2019).

Chemical and health associated benefits

Since ancient times, tea has been regarded as a medicine and a healthy beverage, but recently, it has gained a lot of attention since tea polyphenols are potent antioxidants. It has been demonstrated that oxidative stress plays a role in the aetiology of several illnesses, including cancer (4, 5). In addition, certain epidemiological studies have linked tea drinking to a reduced risk of a number of malignancies, including lung, oesophageal, stomach, and oral cancers. In light of this, tea seems to be a potent chemo preventive agent for harmful substances and carcinogens (Embola et al., 2002). Due to its numerous physiological advantages in addition to its nutritional value, tea is regarded as a functional food. Its antioxidant properties make it a key mediator in free radical mediation, which is of significant use in healthcare.

Numerous chronic illnesses, including malignancies, cardiovascular disorders, and neurological diseases, have been linked to the pathophysiology of oxidative damage to biomolecules. There is a lot of interest in the possible health advantages of drinking tea because of the known in vitro antioxidant effects of the catechins and other polyphenolic chemicals in tea. The associations between tea intake and the prevalence of cardiovascular illnesses and cancer in humans have been the subject of several epidemiologic research.

The information they can offer on tea use and the risk of developing chronic diseases is constrained since many of the older epidemiologic studies listed below were created to investigate the impact of coffee, caffeine, or other lifestyle variables on chronic illness endpoints.

It is also known to increase human heart and central nervous system activity. Depending on the fermentation method, age, and size of the tea leaves, various minerals, such as fluoride, manganese, chromium, selenium, calcium, magnesium, and zinc, are found in tea leaves in varying amounts. According to Obanda, Owuor, and Mang'oka (2004), fermentation is the enzymatic oxidation of tea polyphenols, specifically colourless flavanols, by endogenous polyphenol oxidases and peroxidases, which results in theaflavins and thearubigins, which give black and oolong teas their distinctive flavour and colour. The traditional rollers or machines (CTC: crush-tear-curl) are used to rupture the withered tea leaves, speeding up the oxidation process by releasing oxidases that can more easily react with oxygen. Before rolling and drying in unfermented teas, endogenous enzymes are inactivated by steaming or heating using a variety of techniques (pan frying,

roasting, baking). This prevents fermentation of the withered leaves. White tea, on the other hand, is the least processed because all that it goes through is sun withering and drying. Additionally, white teas are distinguished by the usage of just buds—which are still coated in fine white hair—and one or two very immature leaves (Balentine, 1992)

Tea's cancer preventive impacts in human

There are now 1000 research articles describing tea's capacity to prevent cancer in the scientific literature that can be located on PubMed. Catechins and theaflavins, which are present in tea, may lower the incidence of a number of malignancies in humans, according to many studies. Several studies have demonstrated a negative correlation between tea drinking and the emergence of certain cancer types (Adhami et al., 2003). Table 3 displays the documented effects of tea on human skin, prostate, lung, and breast cancer.

Table 5, Tea and human health (Khan & Mukhtar, 2013)

S.No.	Type of cancer	Beneficial effect of tea
1	Skin Cancer	It was suggested that tea concentration, brewing time and beverage temperature have major influences on the potential protective effects of hot black tea in relation to squamous cell carcinoma of the skin. A population-based case-control study was conducted to evaluate the relationships between citrus peel use and black tea intake and squamous cell carcinoma of the skin. In a case-control study conducted in Italy, a significant inverse association between vitamin A intake and cutaneous malignant melanoma risk was found. The effect of Polyphenon E ointment was investigated for efficacy and safety in the treatment of anogenital warts in immunocompetent men and women. (Nguyen et al., 2012)

2	Lung Cancer	Various studies have demonstrated the relationship between tea consumption and threat of lung cancer. Tea drinking was associated with reduced risk of lung cancer in male cigarette smokers in a case control study in Uruguay (Mendilaharsu et al., 1998). In a case control study, a protective effect of frequent, daily or several times/week black tea drinking appeared among non-smoking women. The maximum tolerated dose of green tea extract (GTE) in patients with advanced lung cancer was determined by Laurie et al. The maximum tolerated dose of GTE was found to be 3 g/m2/day without grade 3 or4 toxicity. A case-control study was conducted on 241 lung cancer patients in Taiwan and the effects of smoking, green tea consumption, IGF1, IGF2, and IGFBP3polymorphisms were evaluated on lung cancer risk. It was found that lung cancer cases had a higher proportion of smoking, green tea consumption of <1 cup/day, exposure to cooking fumes and family history of lung cancer than controls. There was higher risk of lung cancer in smokers who never drank green tea, as compared to smokers who drank green tea >1cup/day.
3		According to epidemiological studies, there is no clear correlation between drinking green tea and the risk of developing breast cancer. When ingesting more than 5 cups of green tea per day, stage I and stage II breast cancer patients demonstrated a decreased recurrence rate and a longer disease-free time compared to those consuming fewer than 4 cups per day. A case control study among Asian-American women in Los Angeles County found a substantial negative

		correlation between drinking green tea and the risk of breast cancer. Consumption of black tea was found to have a slight inverse relationship with the incidence of breast cancer. Therefore, the meta-analysis concluded that there was a lower risk for breast cancer with green tea consumption and a possible late-stage, promotional effect of black tea on breast cancer (Sun et al., 2006).
4	Tea and Other Cancer	In a case-control study of patients with esophageal cancer in Shanghai, green tea consumption was linked to a reduced chance of developing the disease. The association between green tea consumption and colorectal cancer risk was evaluated in a population-based prospective cohort stud which included 60,567 Chinese menaged 40–74 years at baseline. Regular green tea consumption of at least three times/week for more than six consecutive months was related with reduced risk of colorectal cancer in non-smokers and the risk decreased with the increased amount of green tea consumption. The association between green tea drinking and the risk of pancreatic cancer was investigated in a population-based case control study in urban Shanghai with recruitment of 908 patients of pancreatic cancer and1067 healthy controls. Regular green tea drinking was associated with 32% reduction of pancreatic cancer risk as compared to those who did not drink tea regularly in women with increased consumption and longer duration of tea drinking associated with reduced pancreatic cancer risk.

5	Tea and Cardiovascular Diseases	Tea consumption is increasingly being linked to improved cardiovascular and metabolic health. Green tea drinking was linked to a lower incidence of coronary artery disease in male patients who had coronary arteriography for the first time in China, with an adjusted odds ratio of 0.62 when compared to those who did not consume green tea. In contrast to female patients, where there was no inverse correlation between green tea consumption and coronary artery disease, male patients had identical dose-response associations for frequency, duration, concentration, and starting age of green tea consumption (Q. M. Wang et al., 2010). In a dose-response study, weight gain from the ages of 20 to 40 was positively related with myocardial infarction and stroke whereas drinking alcohol and drinking tea had the opposite effects on hemorrhagic and ischemic stroke.
6	Tea and Diabetes	The potential effects of tea drinking on diabetes metabolism and insulin signalling have attracted attention in a number of research. In a double-blind controlled trial, the effects of continuous consumption of a beverage high in catechins on individuals with type 2 diabetes who were not receiving insulin therapy were examined. For 12 weeks, the patients were given green tea with either 582.8 mg or 96.3 mg of catechins per day. The potential effects of various daily dosages of black tea consumption on several metabolic, inflammatory, and oxidative stress parameters in people with type 2 diabetes mellitus. Black tea extract (BTE) was administered to the patients at doses of 150, 300, 450, and 600 ml during the first,

	second, third, and fourth weeks, respectively. The control group received 150 ml of BTE throughout the intervention period.
7	Coffee, tea, and caffeine consumption were evaluatedas risk factors for rheumatoid arthritis onset among older women in a prospective cohort study. Compared with those reporting no use, subjects drinking more than or equal to 4cups/day of decaffeinated coffee were at increased risk of rheumatoid arthritis. In contrast, women consuming more than or equal to 3 cups/day of tea displayed a decreased risk of rheumatoid arthritis compared with women who never drank tea, while caffeinated coffee and daily caffeine intake were not associated with the development of rheumatoid arthritis. The associations of rheumatoid arthritis onset with the highest categories of decaffeinated coffee and tea consumption were stronger in women with seropositive disease compared with those with seronegative disease (Mikuls et al., 2002).

Green tea's anti-photo aging properties

Green tea has strong ROS scavenging action due to its polyphenol content, which makes it a possible option for photoaging treatment. In a recent study, mice that had undergone UV-mediated photoaging were fed tea polyphenols. In vitro, it was discovered that the amount of hydroxyproline had significantly risen, and both catalase activity and the amount of protein carbonyl that was present had significantly decreased.

Green tea's aqueous extract was discovered to help mice with photo-aged skin. It was discovered to boost collagen and elastin levels while decreasing the production of MMP-3 enzymes that break down collagen, potentially having anti-wrinkle benefits (Y. Wang et al., 2019).

Stress resistance properties of green tea

ROS is necessary for regular cellular signalling and metabolism. However, a change in the amount of ROS can cause oxidative stress, which harms cells and consequently the entire body. By acting as a radical scavenger or through other indirect antioxidant mechanisms, exposure to antioxidants under oxidative stress helps to protect the host. Under these many physiological circumstances, the high antioxidant content of green tea confers stress resistance. The anti-oxidative, anti-hypertensive, anti-inflammatory, anti-proliferative, anti-thrombogenic, and lipid-lowering action of green tea polyphenols is one of the key roles they play in protecting the arteries from damage. They can change lipid biosynthesis enzymes and lessen intestinal lipid absorption. They can also scavenge free radicals, bind redox active transition metal ions, and block redox active transcription factors. They can stop vascular inflammation, which stops atherosclerotic plaques, stops vascular smooth muscle cells from proliferating, and stops platelet adhesion. These qualities enable green tea to lower the body's level of stress and so protect against cardiovascular diseases (Y. Wang et al., 2019).

Noon chai

Traditionally created in Kashmir, Noon Chai is a pink-colored salted tea beverage. Noon Chai is the Kashmiri word for this beverage (salted tea), whereas Sheer Chai is the Central Asian term used by the ethnic Kashmiri community. It is known as Namkeen (salty) Chai or Pink

Chai or Pink Tea because it has a salty flavour. Noon chai. In Kashmir, noon chai is the most popular traditional salty food, and virtually everyone drinks it every day (Khuroo et al., 1992).

The most often consumed beverage in Kashmir is this one. Almost every family in Kashmir has been known to partake in the extremely old tradition of sipping Noon Chai for many generations. This tea is frequently served in a flask or huge samovar (a metal device that burns coal to keep the contents warm). This strange beverage is made using a special technique that is only used in the Kashmir valley. To make "tueth," green tea leaves are steeped in sodium bicarbonate until a thick reddish-brown extract is produced. The secret to achieving excellent pink tea is accurate baking soda measurement. Less soda will not render tea pink, whereas too much sodium bicarbonate will turn it black. It takes between 45 and 2 hours for tueth to develop to the brown colour stage. After diluting this tea with water, salt and milk are then added. The tea is served hot and may be garnished with cream

on top depending on the personal liking. The cream is taken from the top layer that forms on boiled milk after the milk is cooled. The formed salted tea may be boiled again before drinking. Noon Chai is first beverage taken in the morning and later in the afternoon with the Naan (a type of bread, also called tchot or telvur) brought fresh from the bakers. A typical naan recipe involves mixing white flour with salt, baking soda and enough yogurt to make a smooth, elastic dough which is cooked in a tandoor (a clay oven) in which burning wood is used for cooking. Salt and baking soda added in all of these native naan recipies, contributes to further salt ingestion along with the Noon Chai. The per capita daily consumption of Noon Chai ranges from 200 to 2500 mL and most of the population likes to take it at high temperature particularly during winters. The practice of Noon Chai intake is slightly more prevalent in rural regions where agriculture is main profession. In these areas besides morning and afternoon, it is also often taken during working hours. Noon Chai intake increases in winter particularly in rural regions for giving warmth and indoor enjoyment. Salt and baking soda added in all of these native naan recipies, contributes to further salt ingestion along with the Noon Chai. The average daily consumption of Noon Chai is between 200 to 2500 mL per person, and most people like to drink it hot, especially in the winter. The average daily consumption of Noon Chai is between 200 to 2500 mL per person, and most people like to drink it hot, especially in the winter (Wani et al., 2013). In rural areas where agriculture is the primary industry, Noon Chai use is slightly more common. It is frequently consumed during working hours in these locations in addition to the morning and afternoon. In the winter, people drink more chai in the afternoon, especially in rural areas where it provides warmth and indoor enjoyment. According to Kashmiri mythology, Noon Chai is cooling in the summer and fights the cold in the winter. It is thought to have diaphoretic qualities, perhaps as a result of the soda bicarbonate concentration. Consumption of salt in noon tea Variations in salt consumption levels among Kashmir valley residents are a crucial problem when understanding the relationship between salt intake (in Noon Chai) and risk of stomach cancer. The quantity of salt added to Noon Chai is determined by the subject's judgement of their own personal salt preferences. The amount of salt consumed with Noon Chai varies depending on how much salt is added and how many cups are consumed everyday. An increased risk for stomach cancer is independently linked to high salty tea drinking (>4 cups per day) (Gerolis et al., 2017). Additionally, the naan that is served with Noon Chai has salt added, which raises the intake of salt.

Salt and gastric cancer

The high risk of stomach cancer in societies that commonly consume processed salty foods appears to be caused by high sodium intake. In numerous association studies and case-control studies, excessive salt consumption has been mentioned as a potential risk factor for gastric cancer (Tsugane, 2005). Consuming salt is positively correlated with a higher risk of developing stomach cancer. When comparing moderate to high salt intake to low salt intake, a substantial upward trend in risk of gastric cancer is seen (D'Elia et al., 2012). According to an epidemiological study of Japanese immigrants from other countries who stopped eating salty food, the incidence of stomach cancer dramatically decreased over time. In Hawaii, where eating of salted fish was discovered to be connected with stomach cancer, the involvement of food in cancer incidence was observed. In Lithuania, it had been discovered that eating salted mushrooms increased the risk of stomach cancer. In Serbia, eating a lot of salty food was linked to an increased risk of stomach cancer. Most Costa Ricans consume salted black beans regularly, and the country has one of the highest rates of stomach cancer ever documented in international literature (Rojas-Campos et al., 1990).

Thermal effects of Noon Chai

High-temperature tea consumption has been linked to thermal damage to the stomach mucosa (Pütz et al., 2002). If consumed at a high temperature, noon chai may damage the stomach epithelium thermally (Lee et al., 1995). Inflammatory reaction then causes inflammation and the production of oxygen and nitrogen free radicals, which support carcinogenesis. An extensive case-control study on healthy controls in Mongolia found that nearly three drinking hot tea increases your risk of developing gastric cancer by times. Strong and hot tea drinkers had a greater chance of developing stomach cancer than those who did not (Pourfarzi et al., 2009).

Noon Chai carcinogens:

It has been demonstrated that drinking hot, salty tea frequently exposes one to extremely high levels of the animal carcinogens methylamine, ethylamine, diethylamine, pyrrolidine, and methylbenzylamine. In addition to the preformed N-nitrosodimethylamine (NDMA), the preparation of salted tea using traditional methods in Kashmir results in significant amounts of N-nitrosoproline (NPRO) (360 g/kg), N-nitroso pipecolic acid (NPIC) (5870 g/kg), and three additional yet unidentified non-volatile N-nitroso compounds. There is no reason to believe that humans are immune to nitrosamines, which have been demonstrated

to serve as strong carcinogens in a wide range of animal species (Pourfarzi et al., 2009). N-nitroso compounds, their possible endogenous formation due to high consumption of salted tea may be a critical risk factor for the high occurrence of gastric cancer in Kashmir. Tannins isolated from salted tea have been found to give a positive result in ribosomal degranulation tests and extract showed genotoxicity to rat hepatocytes in alkaline elucidative assays. Tannins may also be a risk factor causing gastric cancer in Kashmir. (Wani et al., 2013) The extract so obtained is concentrated and needs dilution and addition of milk to prepare the final beverage (Higdon et al., 2003). A hot cup of tea is provided. Pink tea is another name for the prepared beverage because of its pink tint. However, the hue is creamy to yellowish if sodium bicarbonate is not added during the extraction process. Traditional Kashmiri drinks like Kashmiri chai or green tea, water, salt, baking soda, and milk are used to make pink tea, also known as Noon chai. To enhance the extraction of polyphenols when making Noon chai, tea leaves are cooked for a lengthy period of time (approximately an hour). It is not advised to use tea bags since they may reduce the effectiveness of polyphenol extraction. The tea extract turns red after adding baking soda, then turns pink with the last addition of milk. In the case of Kashmiri chai or green tea, the extract changes colour from a light brown to a dark brown when the pH rises. For this reason, adding baking soda is necessary to provide the distinctive pink hue. Green tea or a Kashmiri chai mix work well for making pink tea since they have greater polyphenol contents than other types of tea. White tea, on the other hand, contains a relatively low level of polyphenols (Cabrera et al., 2003). In fact, adding baking soda makes a white tea infusion, which first appears pale-yellow, light brown. Although there is a noticeable shift in colouring, it is not quite enough to produce the fancy pink tea. Anthocyanins are a group of polyphenolic substances found in many herbal teas, including hibiscus blossoms, which also function as pH indicators. The acid and base hues in the case of hibiscus flowers are red and green, respectively. Other herbal teas like Red Zinger or Mandarin Orange exhibit a similar behaviour. In this instance, adding baking soda would result in a "green" tea extract rather than a pink one.

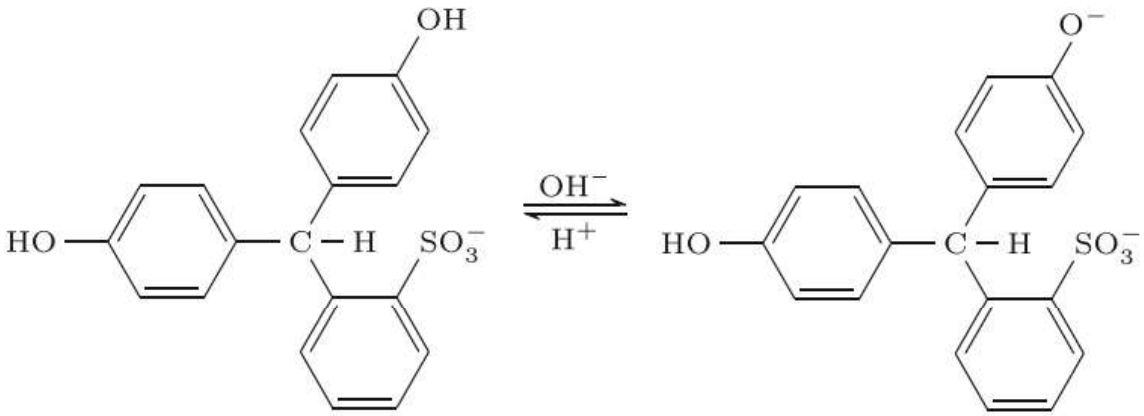


Figure 3**,** Acid-base equilibrium of phenolsulfonphthalein (Phenol red). Phenolsulfonphthalein is yellow in acidic conditions and turns red in alkali (D'Ulivo, 2018)

Development in tea industry

The discovery of the native tea plant in Assam by Robert Bush in 1823 heralded the beginning of the Indian tea industry.

The East India Company lost its monopoly on the tea trade in China in 1833, which gave the production of tea a boost. When a scientific committee was dispatched to Assam in 1835 to report on the possibilities for the tea business, the team discovered several tea plants in the hills between Assam and Burma.

C.A. Bruce received the position of Superintendent of Tea Forests in 1836. The first shipment of Indian tea (eight chests) was sent to London in 1839, when it was auctioned off at a price per pound that ranged from six to thirty-four shillings. Two thirds of the experimental teas were given to the new firm in 1840. The first tea firm in India distributed its final profits in 1852. Jorhat Company, the second limited company to be established, was established in Assam in 1859.

Tea growing began in Chittagong and Chotta Nagpur between 1862 and 1867. Ultimately, wherever there was a chance of success, tea planting was started in numerous Indian areas. India and Sri Lanka quickly took control of the global tea trade and market (Karmakar and Banerjee, 2005).

Additionally, the states of Karnataka, Tripura, Himachal Pradesh, Uttarakhand, Arunachal Pradesh, Manipur, Sikkim, Nagaland, Meghalaya, Mizoram, Bihar, and Orissa are linked

with small businesses in this sector. Despite being in modest quantities, a particular type of tea originates from small growers of Kangra Valley in the picturesque Himachal Pradesh.

The specialty of this valley is green tea, which is well-known worldwide. The tea business contributes a significant amount of foreign currency to the agricultural economy. As a result, since 1950, there has been a rise in the number of nations producing tea globally. It is significant to note that, on the global scale, India placed first in terms of the area planted with tea (Soni et al., 2015).

In the contemporary food business, tea is typically ground or ultra-micro crushed into powders with granularities as small as a few microns, which may then be consumed or processed into a variety of snacks, drinks, bakery goods, and other items (Kayama et al., 2022). In many products where their solubility is crucial, such as reconstituted milk, newborn formula, cocoa, and high protein beverages, dry powder is a crucial component. Dissolution of food powder will directly affect how customers judge the overall quality of the product. The qualities of food powders' reconstitution have been measured in a variety of ways. When working with bulk commodities, the industry's adopted worldwide standards are reasonably simple and helpful. The generated results, however, are at best semi-quantitative and frequently not repeatable or comparable to measurements made by various operators or using different methodologies. These techniques in the dairy sector were frequently created based on traditional goods like instant whole milk or quick skim milk powders. As a result, they might not be appropriate to evaluate the quality of other speciality goods, which have been increasingly popular in recent years (such as high fat content powders or whey protein concentrates) (Fang et al., 2008).

In India, the tea business is significant and contributes significantly to the country's economy. India is currently the world's largest producer of tea and one of the main exporters of several types of tea (Kidwai, 1999)

Over the past century, there have been various iterations of tea processing, from loose tea to blended tea to packet tea to tea bags to instant teas, ready-to-drink teas, and flavoured teas. Tea has the potential to become the beverage of the twenty-first century as science and technology's perceptive eyes explore deeper the chemical and metabolic processes triggered by intake. Tea may be brewed in a variety of methods, although it is often made into black, green, and oolong tea. The first instant tea, a product made from dried infusions of tea, was created in 1940 in England using black tea. In a method that has been developed

and patented by Hindustan Lever Limited, fresh green tea leaves are heated to a temperature that inactivates the enzymes before being ground up, extracted with hot water, and dried using customary techniques like freeze- or spray-drying. Another technique created by the Tea Research Institute of Ceylon for the creation of cold water soluble tea concentrates and powders involves the extraction of tea leaves using hot water, followed by gel filtration to separate out non-phenolic substances like proteins, polypeptides, and chlorophylls while keeping the phenolic substances. The filtrate that results is then concentrated to produce a powder that is soluble in cold water.

CHAPTER 3
MATERIAL & METHODS

Chemicals

Sodium carbonate, methanol was of analytical grade. Folin–Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH). Mineral water obtained from a Reverse osmosis (Ro) Aquagrad system with the TDS value of 2000 ppm was used throughout the study.

Sample Procurement

The Green Tea or Noon Chai was procured from the Kamal Food Spices Pvt. Ltd. Kashmir.

Method

Tea Extraction

Kashmiri noon Chai was brewed in water in the ratio of (1:10 w/v) at the temperature of 100 °C for 15- 120 min. The mixture was added with sodium bicarbonate with 250 mg-350 mg. The extract was sieved to remove the leaves. Further the extraction was dried in two ways Freeze dried and oven dried.

Experiment Design

Response Surface Methodology (RSM) and a three level, three component Box-Behnken design were selected to analyse the effects of time, sodium bicarbonate, and aeration in order to optimise Noon Tea using the Design Expert 11.1.0.1 software's central composite design (CCD), the experiment was constructed (State-Ease, Minneapolis, MN, USA) with Twenty different configurations, including eight duplicates of the centre point (Table 5). The water-to-tea ratio was expressed as 50 millilitre of water per 5 g of tea (50 mL/5 g). Each sample was prepared according to method used in Kashmir, by boiling green tea leaves (5 g) in 50 ml of water in the presence of sodium bicarbonate in different concentrations across a period of time. The water was pre-heated to the designated temperature prior to brewing.

Box-Behnken Design

Optimization of Noon Tea extraction was performed with the aid of Design Expert 11.1.0.1 software's central composite design (CCD), the experiment was constructed (State-Ease, Minneapolis, MN, USA) software. Box Behnken was employed for assessing the time, sodium bicarbonate, and aeration in order to optimise Noon Tea. Extract tea was estimated

as response variables. Table 4 represents the value of independent variables, and table 5 represents their levels. The whole experimental design consisted Twenty runs, including eight duplicates of the

centre point conducted in a randomized manner to decrease the chance of unpredicted variations. The data of extraction yield depend on A, B and C (independent Variables) was evaluated by using quadratic model.

Table 6, Box Behnken the time, sodium bicarbonate and aeration

Std	Run	Factor 1	Factor 2	Factor 3
		A:Time	B:Sodium Bicarbonate	C:Aeration
		min	mg	sec
18	1	35	250	165
16	2	35	250	165
19	3	35	250	165
20	4	35	250	165
8	5	50	300	240
9	6	9.77311	250	165
17	7	35	250	165
10	8	60.2269	250	165
15	9	35	250	165
11	10	35	165.91	165
3	11	20	300	90
4	12	50	300	90
14	13	35	250	291.134
1	14	20	200	90
6	15	50	200	240
12	16	35	334.09	165
2	17	50	200	90
7	18	20	300	240
13	19	35	250	38.8655
5	20	20	200	240

Tea Powder

After a design expert optimised the combination of variable components for the highest extraction yield, the Kashmiri Noon Tea solution was then made and dried using two alternative methods, freeze drying and oven drying. The dried powders were crushed in a form of fine Kashmiri Noon Tea Powder.

Freeze drying of Kashmiri tea extract

In freeze-drying experiments, the freeze dryer (Gold Sim Model: FD-5 3T) was used. The temperature of heating plates of the freeze dryer used in the drying experiment can be adjusted from −5 to 57 °C while the condenser temperature can be set as low as −57 °C. The four trays of the freeze dryer are capable to hold 17 L of solution. The condenser where sublimated and desorbed water vapour removed from the drying chamber. The vacuum system of the freeze dryer equipped with one compressor with a power 2×10^4.

Hundred grams of Kashmiri Noon tea was added into 1000 ml of boiling RO water in a steel container to allow soluble solids of the tea to infuse into boiling water. Solution was sieved and poured in the tray (diameter 180 mm, No of plates 3) of the freeze dryer. The solution was stored in -20°C for overnight. The heating plates temperature of the freeze dryer was set at -57 °C. On completion of freezing, the drying chamber pressure was set to 3 Pa and kept constant during the drying.

Oven Drying

For oven-drying, hundred grams of Kashmiri Noon tea was added into 1000 ml of boiling RO water in a steel container to allow soluble solids of the tea to infuse into boiling water. Solution sieved and concentrated to 10% (w/w). The solution was poured in glass Petri plate (diameter 180 mm, No. of plates 2) and kept in hot air oven at 100 °C.

Antioxidant

The DPPH method, which is quick, easy, and independent of sample polarity, has been found to be the most practical method for quickly screening a variety of samples for radical scavenging activity (Koleva et al., 2002). In order to conduct the analysis, Burits & Bucar's 2000 standard DPPH assay was used. The capacity of the tea extracts to donate hydrogen atoms or electrons was quantified.

To 1ml of the diluted tea liquor extract (dilution of 1:4), 1ml of 0.004% methanol solution of DPPH was added and incubated for 30 min. at room temperature. After thirty minutes, the violet colour of DPPH was completely changed to yellow. The test tubes were shaken properly to make sure that the colour change was complete. After the stipulated 30 min., the absorbance of the samples was determined (OD) in a spectrophotometer, against a

control, at 765nm. The control was 1ml DPPH solution + 1ml. methanol. The tests were carried out in quintuplet for each variable and the mean OD was calculated.

Inhibition of free radical, DPPH,

Inhibition Percentage (IP)% = [(A control – A sample)/A control] × 100 where

A control is the absorbance of the control and A sample is the absorbance of the sample.

Total Polyphenols

Determination of Total Phenols Total phenol content was determined according to the modified method of (Y. Wang et al., 2019). Briefly 1.0 mL of the diluted (1:2) sample extracts were added to tubes containing 5.0 mL of a (1:10) dilution of Folin–Ciocalteu reagent in water. After 4 min, 4.0 mL of a 7.5% Na2CO3 was added and mixed thoroughly, and samples were incubated for 1 hour at ambient temperature. The absorbance was read at 765 nm using a UV–Visible Nanodrop (Onec, Thermo Scientific). Total phenolic content was determined with the use of an external standard curve of Gallic acid in the concentration range of 20–100 μg/mL (Pearson's correlation coefficient: R2=0.99) and expressed as μg Gallic acid equivalents per mg. Mc Donald et al. (2001)

Fourier transform infrared spectroscopy (FTIR) analysis

To access the influence of brewing conditions on tea leaves, infrared spectra of dried green tea leaves and tea infusion prepared at the optimum experimental conditions were recorded using a FTIR spectrometer (FTIR-Bruker, Alpha II). The powdered tea leaves and tea extract% were placed on the ATR crystal and were scanned in the range of 4000–400 cm−1 to analyze the group frequencies.

Colour analysis

Hot milk and water were combined with the instant tea powder to dissolve it. By clicking on several sample sites, the L, a, and b values were measured using the Color Analysis tool. The dried product's colour is a crucial quality factor that affects consumer acceptance. In the L*a*b* color space, the average of colour parameters including L*(lightness), a*(red/green coordinates) and b*(yellow/blue coordinates) were calculated for whole pixels in the images that had been processed. One of the validation methods for color values extracted by CVSs is calibration using Hunter-Lab colorimeter (Imaniar et al., 2019). In order to calibrate the color values obtained by means of the CVS system with Hunter Lab

(A60-1010-615 model colorimeter, Reston, VA), the color values of 12 color chart were used (Figure 3).

Sensory Analysis

Sensory analysis of Kashmiri Noon Tea Instant Powder was conducted using the hedonic testing method. The instant powder sensory test was conducted on 10 trained panelists before start of the evaluation a training session of 15 minutes was conducted with the panelist. Afterwards, one sample at a time was offered to each member. The sensory testing was made in the panel room with controlled temperature and relative humidity. The panel room was completely free of food/chemical odours, unnecessary sounds. Judges were provided with prescribed questionnaire to record their sensory observations on the basis of colour, Taste and aroma. The information contained on the sensory performa was indicated as 9 = Like extremely; 8 = Like very much; 7 = Like; 6 = Like slightly; 5 = Neither like nor dislike;4 = Dislike slightly;3 = Dislike moderately;2 = Dislike;1 = Dislike extremely (Larmond, 1977) (Susanti et al., 2021).

Statistical Analysis

All experiments were run in triplicate. Statistical analysis of the response surface model was conducted using analysis of variance (ANOVA) to know the influence of extract parameters on yield using Design Expert Software. Statistical significance was tested at $P<0.05$.

CHAPTER 4
RESULTS AND DISCUSSION

Extraction varying three factors (Time, Sodium Bicarbonate and aeration) was evaluated in terms of tea extraction. The result clearly shows that the extraction yield obtained after 20 runs are presented in Table 6 and it was observed that the extraction yield increased from 2.26 nm to 2.64 nm. The maximum yield value was obtained via combination of Time 50 min, 300 mg Sodium Bicarbonate, 165 sec aeration.

Table 7, Box-Behnken Design

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	A:Time	B:Sodium Bicarbonate	C:Aeration	Yield
		min	mg	sec	765 nm
18	1	35	250	165	2.48
16	2	35	250	165	2.48
19	3	35	250	165	2.48
20	4	35	250	165	2.47
8	5	50	300	240	2.64
9	6	9.77311	250	165	2.24
17	7	35	250	165	2.48
10	8	60.2269	250	165	2.7
15	9	35	250	165	2.48
11	10	35	165.91	165	2.39
3	11	20	300	90	2.35
4	12	50	300	90	2.54
14	13	35	250	291.134	2.45
1	14	20	200	90	2.26
6	15	50	200	240	2.58
12	16	35	334.09	165	2.51
2	17	50	200	90	2.53
7	18	20	300	240	2.4
13	19	35	250	38.8655	2.38
5	20	20	200	240	2.26

Models Fitting

RSM was used to optimize the extraction yield from Kashmiri Noon Tea. The influence of Time, Sodium Bicarbonate and Aeration on extraction yield was investigated by a Box-Behnken Design. The predicted yields of extraction (Table 7) were estimated using the mathematical models and compared with the observed results. Regression analysis was conducted to determine the effect of dependent variables and the likely interactions between them and to evaluate the statistical significance of the models. The regression equations for yield were given as:

Yield = 2.47827 + 0.131335 * A + 0.0367446 * B + 0.0232649 * C + -0.02 * AB + 0.0125 * AC + 0.0125 * BC + -0.00251959 * A^2 + -0.00959065 * B^2 + -0.021965 * C^2

Table 8, ANOVA quadratic of extraction yield of Kashmiri Noon Tea optimization

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.2749	9	0.0305	679.20	< 0.0001	significant
A-Time	0.2356	1	0.2356	5239.13	< 0.0001	
B-Sodium Bicarbonate	0.0184	1	0.0184	410.09	< 0.0001	
C-Aeration	0.0074	1	0.0074	164.40	< 0.0001	
AB	0.0032	1	0.0032	71.17	< 0.0001	
AC	0.0013	1	0.0013	27.80	0.0004	
BC	0.0012	1	0.0012	27.80	0.0004	
A^2	0.0001	1	0.0001	2.03	0.1842	
B^2	0.0013	1	0.0013	29.48	0.0003	
C^2	0.0070	1	0.0070	154.64	< 0.0001	
Residual	0.0004	10	0.0000			
Lack of Fit	0.0004	5	0.0001	4.40	0.0650	not significant
Pure Error	0.0001	5	0.0000			
Cor Total	0.2753	19				
R^2					0.9895	
Adjusted R^2					0.9969	

The Model F-value of 679.20 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 4.40 implies there is a 6.50% chance that a Lack of Fit F-value this large could occur due to noise. The Predicted R^2 of 0.9895 is in reasonable agreement with the Adjusted R^2 of 0.9969; i.e. the difference is less than 0.2.

Influence of Independent Variable on Extraction Yield:

To understand the interaction between several factors, we have generated individual Response Surface Graph. Each response surface shows a function and effect of three factors through RSM in the perturbation graph shown in figure 4.

From the graph as shown in fig 4, the maximum yield (2.64 nm) is achieved with increase in the factor A (Time- 50 min) and increase in the concentration of factor B (Sodium Bicarbonate- 300 mg), while the maximum yield was achieved with slightly increase in the factor C (Aeration Time -165 sec).

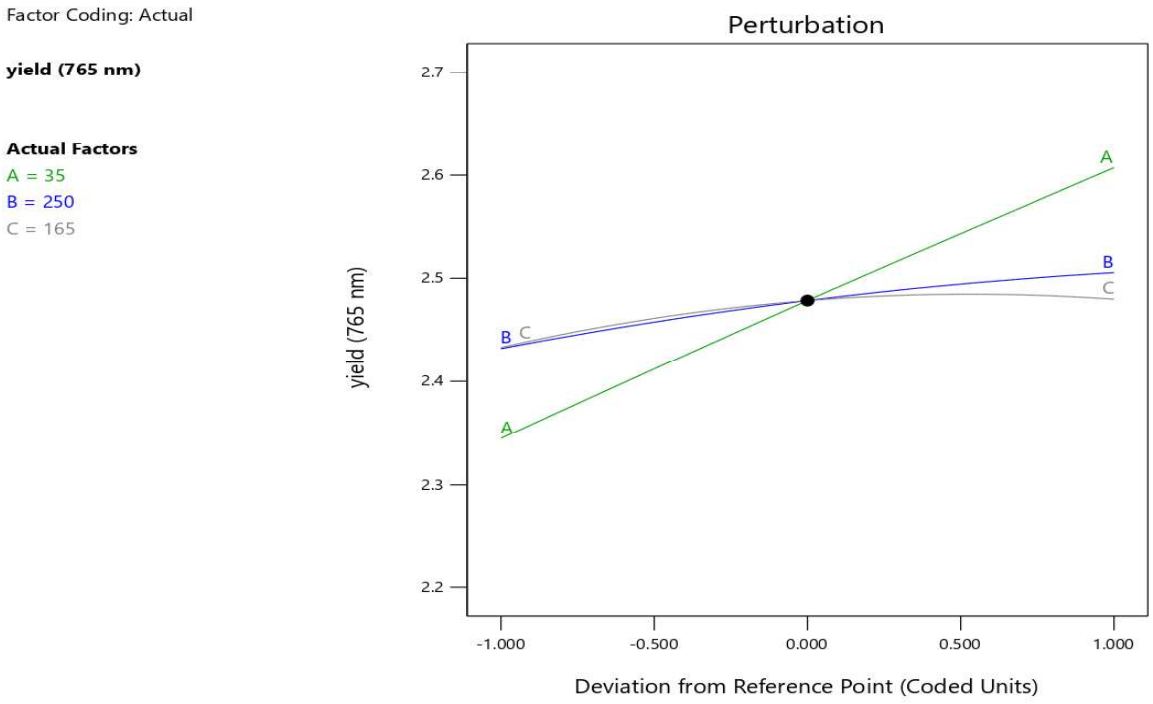


Figure 4, Perturbation graph Response surface analysis

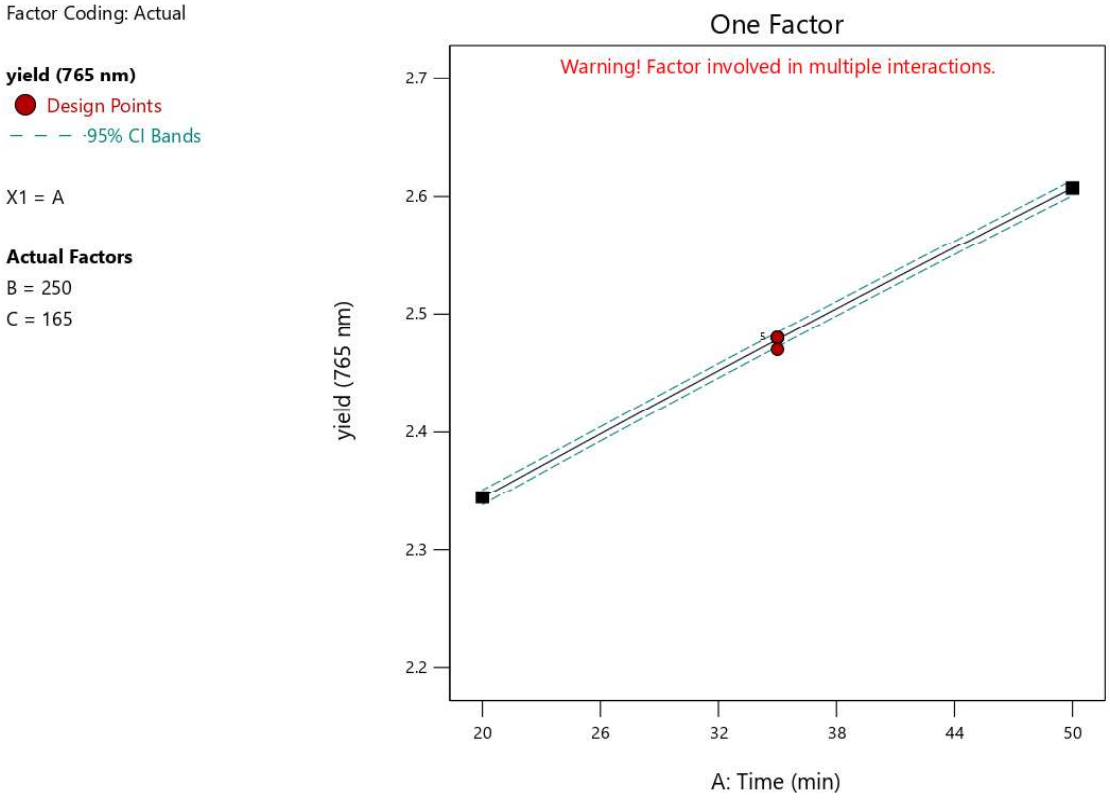


Figure 5, One factor graph of time (min)

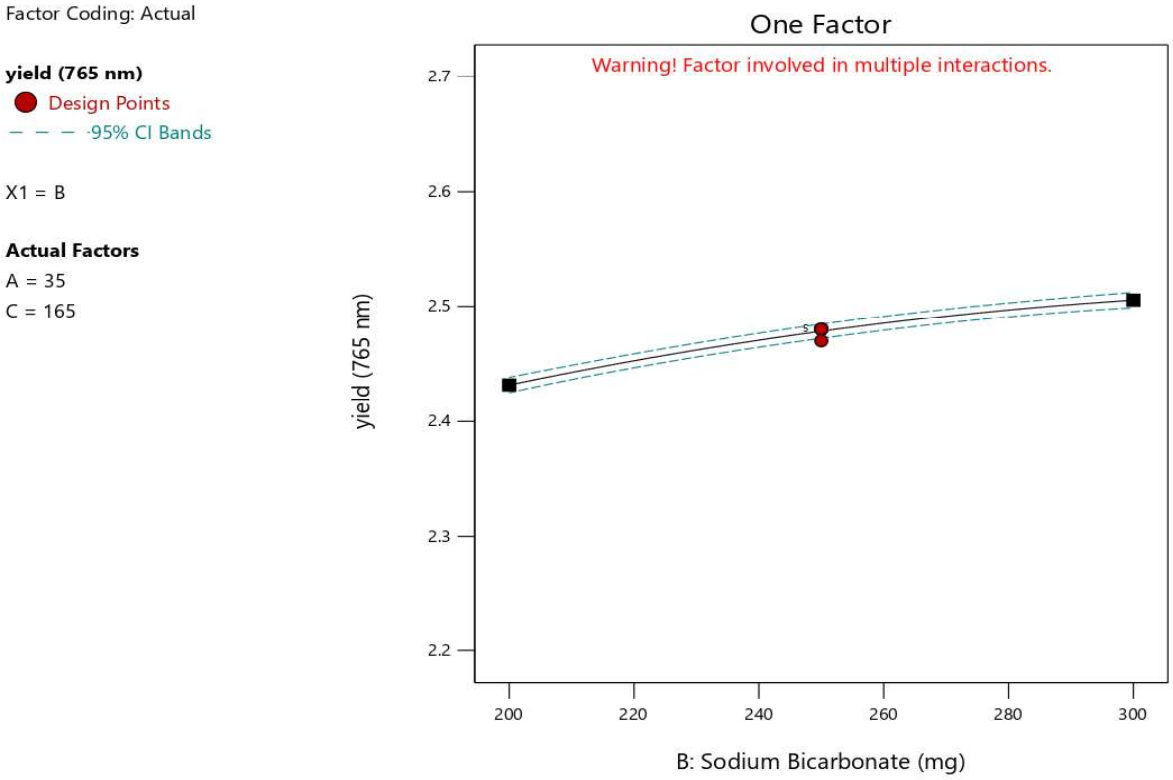


Figure 6, One factor graph of sodium bicarbonate (mg)

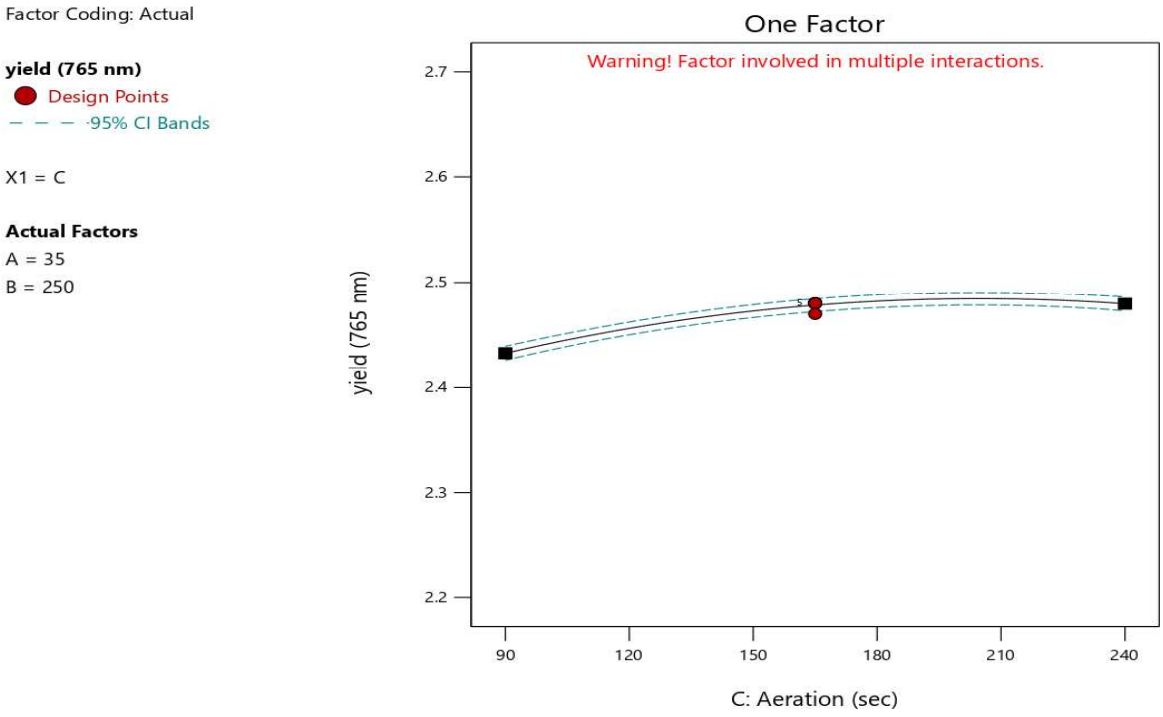


Figure 7, One factor graph of aeration (sec)

Optimization Graph:

The optimization value was obtained by differentiating the quadratic model using Design-Expert software. The fundamental goal of optimization is to determine the amounts of independent variables that will result in the maximum protein yield and functional properties. The combination of Time 50 min, 300 mg Sodium Bicarbonate, 165 sec aeration at 765nm were predicted to provide the maximum yield of 2.64 nm.

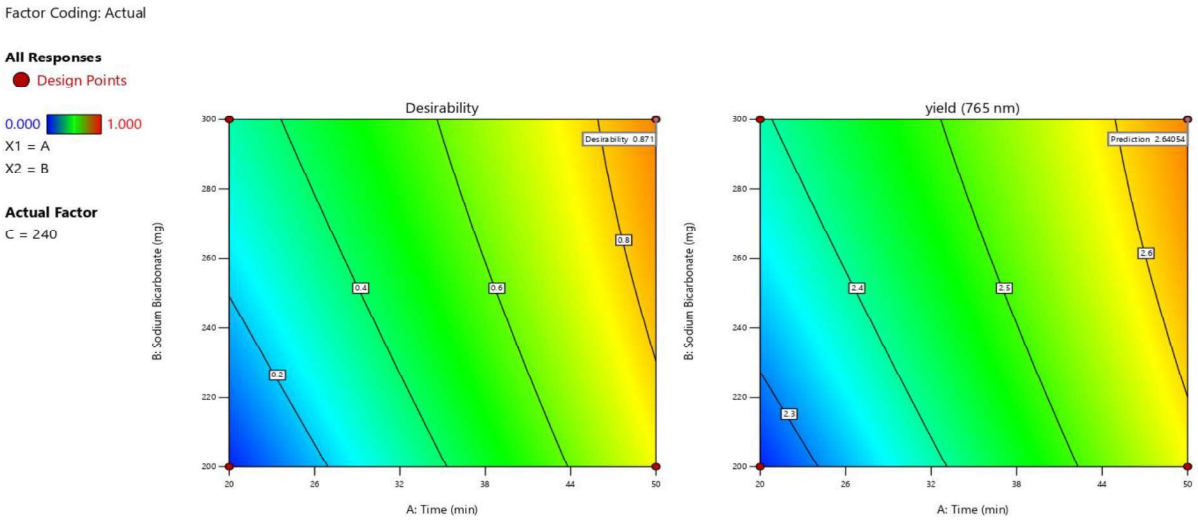


Figure 8, Optimization graph

Instant Tea Powder

Total amount of Kashmiri tea powder was obtained from freeze drying (FD) was 3.25 g and oven dried (OD) was 3 g. The tea powder of FD and OD were used in further analysis of the Kashmiri Noon Tea.

Antioxidant:

Radical scavenging activity of Extract 1 (OD) and Extract 2 (FD) extract was evaluated through DPPH scavenging assay. The radical scavenging activity of OD inhibited 39.54% . Intriguingly, the scavenging potential of FD extract indicate an elevated 45.24% inhibition at a wavelength of 517 nm (fig. 9).

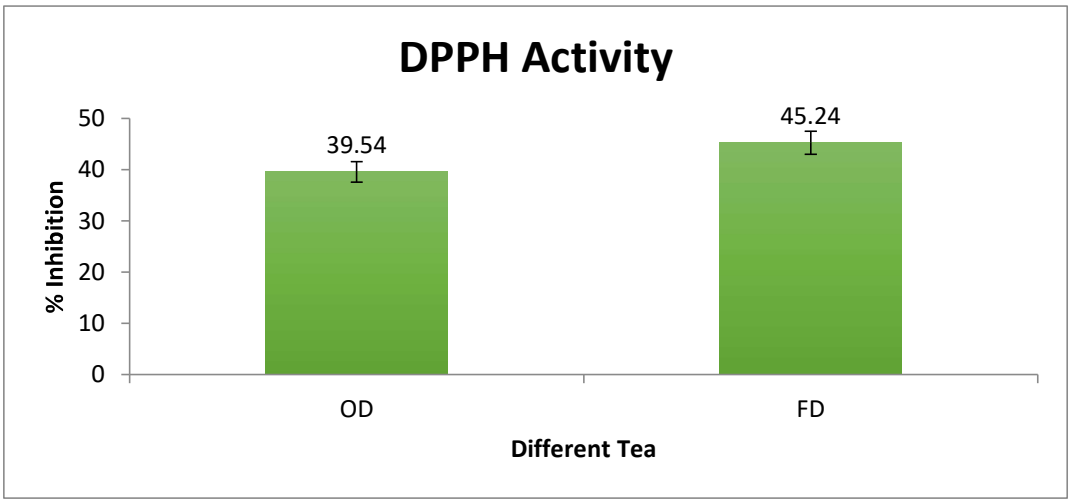


Figure 9, Antioxidant graph showing the scavenging activity of Freeze dried (FD) and oven dried (OD) extract.

Total Polyphenol

The solution of Kashmiri Noon tea was then dried in two ways Freeze dried and Ove dried. The freeze-dried yielded the greatest total phenolic content (TPC) value (208± 0.31 g GAE/g), while oven-drying yielded the lowest (153±0.32 g GAE/g). Between freeze drying and oven drying, a significant difference ($p<0.05$) was seen. TPC values published by Atoui et al. (2005) and Chan et al. were comparable to the TPC value achieved in this investigation (2010).

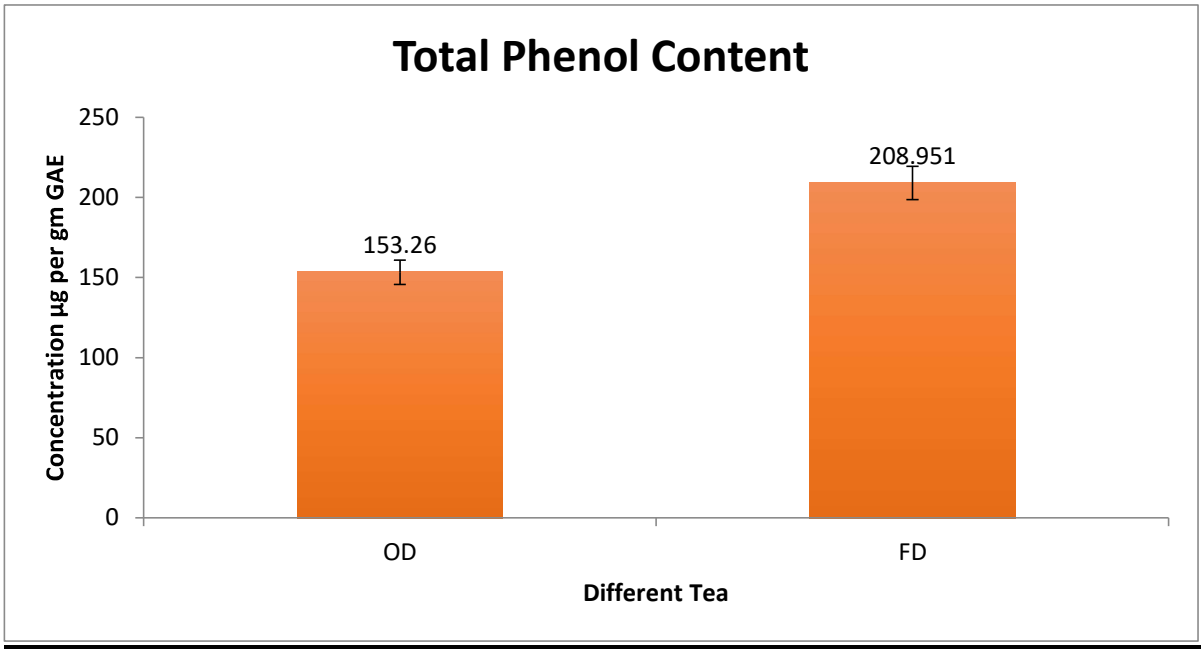


Figure 10, Shows the total polyphenolic content of freeze dried (FD) and OD extract

Fourier Transform Infrared Radiation (FTIR)

In our study, the FTIR spectra of FD powder and oven dried tea powder (OD) are shown in Fig.11 & 12 respectively. The peaks of both the samples did not show much difference. Oven dried (OD) tea leaves shows sharp peak intensities when compared to freeze dried tea extract. The peaks observed in FD were at 3392.69 cm-1, 1384.78 cm-1, 1116.30 cm-1 which were attributed to the vibration of O-H stretching, C-H bonds and STRONG C-O bonds respectively whereas the peaks of IR spectra of OD sample were at 3347, 1606, 1384 and 1073 which were attributed to the vibration of O-H stretching, N-H bends, C-H bend and strong C-O bonds respectively. Same peaks were also reported in [1]

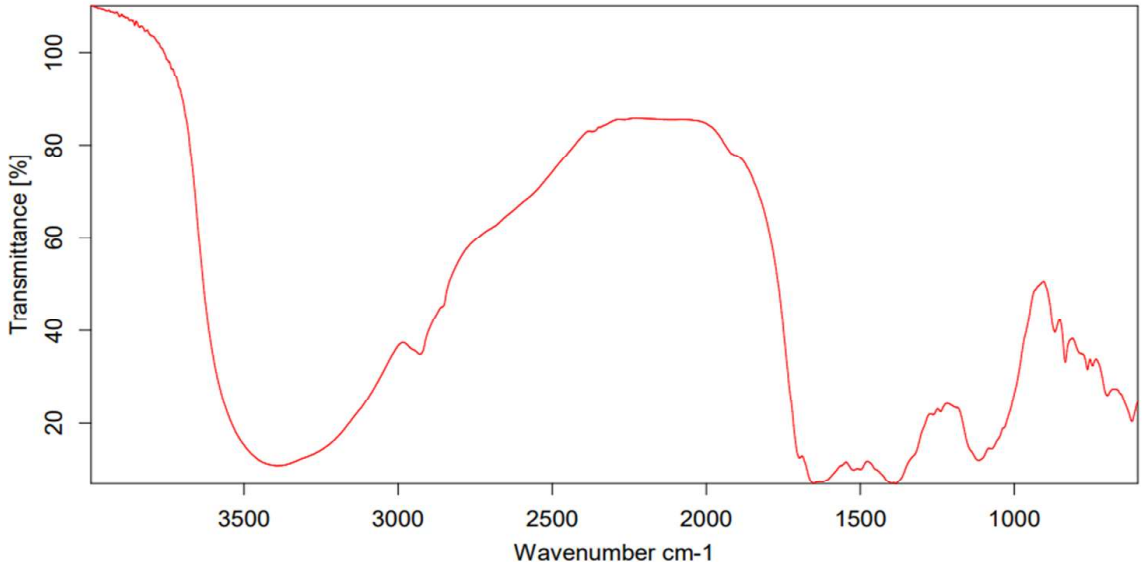


Figure 11, FTIR Graph of freeze dried extract

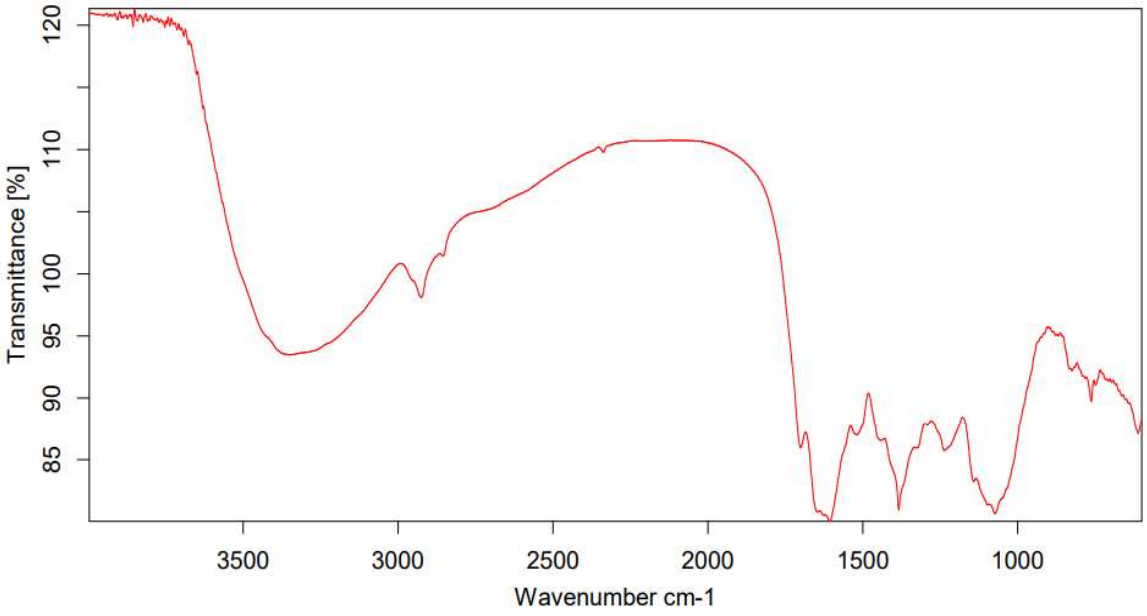
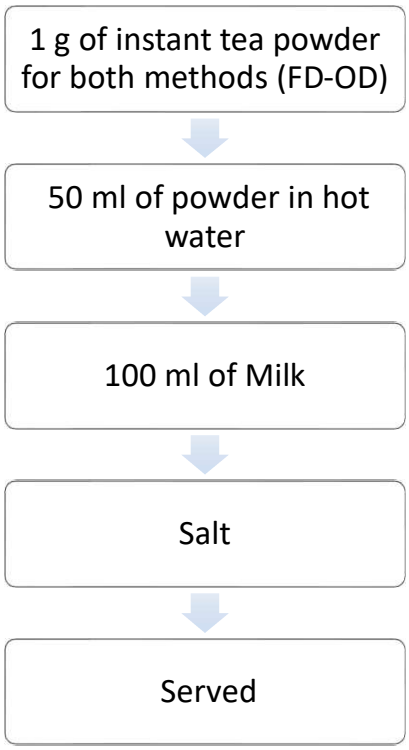


Figure 12, FTIR Graph of oven dried extract

Sensory Analysis

Sensory evaluation of over-all acceptance was evaluated. The results were analysed on the basis of scores assigned to colour, odour and taste of freeze dried sample and oven dried on the scale of 10. The sample ranged from 5-8.5 with the highest taste score of 7.5 and a least odour score of 5.



Preparation of Instant Tea Powder

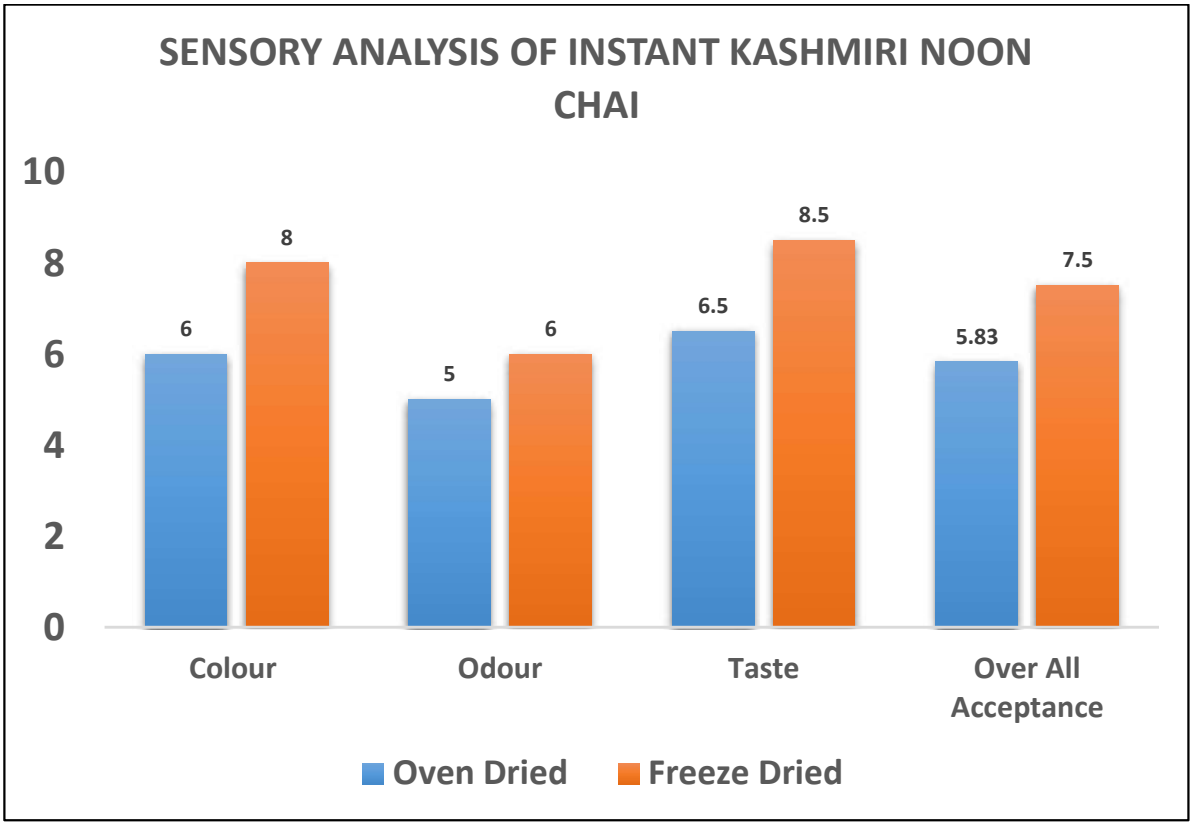


Figure 13, Sensory analysis of instant Kashmiri Noon Tea

Color

The instant tea product's colour is a crucial quality factor that affects consumer acceptance. The L, a, and b values of instant Kashmiri Noon Tea were measured using the Color Analysis tool. In the L*a*b* color, the average of colour parameters including L*(lightness), a*(red/green coordinates) and b*(yellow/blue coordinates) were calculated. The final color of a freeze-dried Kashmiri Noon Tea methods were 70.4 (L*); 15.3 (a*); and 9.3 (b*) and Oven Dried samples were 67.5 (L*);15.5 (a*); and 9.4 (b*) respectively.

Samples	L	a	b
Oven Dried	67±05	15±05	9±4
Freeze Dried	70±03	30±03	9±03

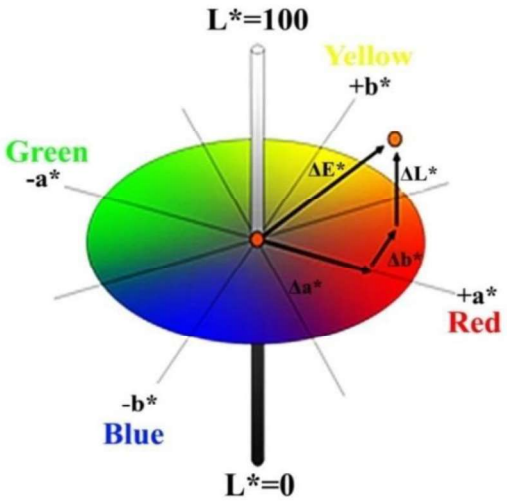


Figure 14, CIELAB Colour Chart

Freeze Dried Instant Kashmiri Chai Powder

Oven Dried Instant Kashmiri Chai Powder

CHAPTER 5

CONCLUSION

In conclusion, the instant powder of Kashmiri Noon Tea was determined by two different methods, Freeze Drying and Oven Drying. The tea extraction was obtained by boiling Kashmiri Noon Tea (1:10 w/v) in the presence of sodium bicarbonate followed by aeration which is a traditional method of making a Kashmiri Noon Tea. RSM was applied to study the process for development to produce Kashmiri Noon tea extract with high antioxidant profile and less epimerization of tea polyphenols rendered the best characteristics of the developed method. In this work, we verified the effect of time, sodium bicarbonate and aeration on total yield, total antioxidant activity, total phenolic content of the tea extract and sensory evaluation and colour of the reconstituted instant tea powder.

The effect of these variables on the response variables was observed in varying degrees. The maximum values of the three output parameters: 2.64 total yield, 45.24% antioxidant activity and 208.30 µg GAE/mg total phenolic content were obtained. The model analysis showed a good fit that was statistically acceptable at $p<0.05$ and adequate with satisfactory R2 value of 0.9895. The FTIR analysis confirmed the functional groups of Kashmiri Noon Tea in the brewed extract. Furthermore, Sensory and colour analysis was performed to confirm the presence of various bioactive compounds with therapeutic efficacies of tea and tea polyphenols.

CHAPTER 6

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