USE OF RSM TO OPTIMIZE DRYING TEMPERATURE AND LENGTH OF LEMONGRASS *(Cymbopogan Citratus)* AND ENHANCE SENSORY PROFILE OF GREEN TEA (*Camellia Sinensis)*

by

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A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech in Food Technology

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Approval Letter

The dissertation entitled Use of RSM to Optimize Drying Temperature and Length of Lemongrass *(Cymbopogan Citratus)* and Enhance Sensory Profile of Green Tea *(Camellia Sinensis)* presented by Bipana Gyawali has been accepted as the partial fulfillment of the requirements for the B. Tech degree in Food Technology.

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Abstract

The objective of this study was to optimize the drying temperature and length of lemongrass (*Cymbopogon citratus*) with respect to phytochemical content and antioxidant activity, and to evaluate its suitability for incorporation into green tea (*Camellia sinensis*) as a functional beverage. Fresh lemongrass leaves were cut into different lengths (1, 3, and 5 cm) and dried at varying temperatures (30°C, 45°C, and 60°C). Response Surface Methodology (RSM) using a Central Composite Design was applied to assess the combined effect of drying temperature and length on total phenolic content, total flavonoid content, tannin content, and DPPH radical scavenging activity. The optimized dried lemongrass was blended with green tea at different ratios, and the resulting tea samples were evaluated for sensory attributes using a nine-point hedonic scale.

The results showed that both drying temperature and length significantly influenced the phytochemical composition and DPPH radical scavenging activity of lemongrass. The optimum condition was identified as drying lemongrass at 47.5°C with a length of 3.8cm, yielding the highest DPPH radical scavenging activity (79.42 ± 0.18%) along with elevated flavonoid (22.56 ± 0.92 mg QE/100 g), polyphenol (17.9 ± 0.03 mg GAE/100 g), and tannin contents (0.40± 0.02 mg/100 g). Higher drying temperatures and larger length led to significant losses of bioactive compounds ($p < 0.05$). Sensory evaluation revealed that green tea incorporated with optimally dried lemongrass showed superior acceptability compared to the control (6±0.408). The study concludes that optimized drying conditions effectively preserve the functional properties of lemongrass and enhance the sensory quality of green tea.

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List of Abbreviations

Abbreviation	Full form
AA	Antioxidant Activity
ANOVA	Analysis of Variance
CCD	Central Composite Design
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EC	Epicatechin
ECG	Epicatechin-3-gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
FDA	Food and Drug Administration
GAE	Gallic Acid Equivalent
GRAS	Generally Recognized As Safe
HSD	Honestly Significant Difference
IC50	50% Inhibitory Concentration
LDPE	Low Density Polyethylene
mg	Milligram
ml	Millilitre
nm	Nanometre
QE	Quercetin Equivalent
RSM	Response Surface Methodology
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
SPSS	Statistical Package for the Social Sciences
TAE	Tannic Acid Equivalent
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
TTC	Total Tannin Content
UV	Ultraviolet
WHO	World Health Organization

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Part I

Introduction

1.1 General Introduction

Tea *(Camellia sinensis)* is the most popular beverage globally and can be brewed in many different styles. It belongs to the *Camellia* genus within the *Theaceae* family. There are two main varieties of this plant: the Chinese type, *Camellia sinensis* var. *sinensis*, and the Assamese type, *Camellia sinensis* var. *assamica* (Chang *et al.*, 2017). Green, black, and oolong teas are made in different ways. To make green tea, the leaves are picked fresh and quickly steamed or fried to stop them from fermenting. This keeps the tea fresh and dry. Green tea gets its color and taste mainly from natural compounds called epicatechins (Mukhtar and Ahmad, 2000). As people around the world are living longer, research shows that making changes to diet and lifestyle no matter your age can help improve heart health, metabolism, and brain function, while also reducing the impact of chronic diseases (Calder, 2018). More studies suggest that a healthy diet should focus on plant-based foods like fruits, vegetables, whole grains, nuts, and healthy oils, while cutting back on red and processed meats and sugary drinks (Schulze *et al.*, 2018).

Green tea has caught the attention of both scientists and everyday people because of its wide range of health benefits. It's known for its antioxidant properties and its potential to fight cancer, reduce blood pressure, prevent obesity, and protect against harmful cell changes (Cabrera *et al.*, 2006). Herbal teas have been used for centuries to support health and treat various ailments. Unlike traditional teas made from *Camellia sinensis*, these are brewed from different parts of other plants like their leaves, flowers, seeds, fruits, stems, or roots. People often drink herbal teas for their calming and revitalizing effects. They're known to aid digestion, soothe stomach problems, help detoxify the body, and strengthen the immune system (Chatterjee *et al.*, 2012).

Lemon grass (*Cymbopogon citratus*), is a member of poaceae family. It is a medicinal plant with compounds capable of controlling pathogens and increasing herbal resistance to pathogenic diseases. Lemongrass is widely used in the herbal teas and other non-alcoholic beverages in baked food, and also in the confections. Essential oil from the lemongrass is

commonly used as a fragrance in the perfumes and cosmetics, such as creams and soaps. Due to the presence of various chemical constituents present in lemon grass oil, it uses in different pharmaceutical industries for its anti-depressant, analgesic, antipyretic, bactericidal, anti- septic, carminative and astringent properties (Wifek *et al.*, 2016). Lemongrass grows best in moderately fertile soil with regular watering. Sandy loam that drains well is ideal for its growth. The plant can adapt to different soil types, from loamy to less fertile laterite soils. However, it doesn't do well in chalky or waterlogged conditions, so those should be avoided for successful cultivation (Nambiar and Matela, 2012).

Lemongrass is known for its positive effects on digestive health, aiding in the relief of bloating, gas and gastric ulcers. It also supports cardiovascular health by helping to regulate blood pressure and lower cholesterol levels. Its neuroprotective and stress reducing properties make it a popular ingredient in aromatherapy for managing anxiety and promoting relaxation.

1.2 Statement of problem

Lemongrass *(Cymbopogon citratus)* is widely valued for its potent phytochemicals and antioxidant properties, which make it a promising candidate for functional food applications such as green tea *(Camellia sinensis)*. However, the drying process particularly the temperature and length can significantly influence the retention of these bioactive compounds. Inappropriate drying conditions may lead to the degradation of essential nutrients, reducing the therapeutic potential of lemongrass. Despite its popularity, there is limited research focused on optimizing these parameters to preserve its health-promoting qualities during processing and incorporation into beverages. It demonstrated that higher drying temperatures accelerate moisture removal but can also degrade sensitive compounds (Nguyen et al., 2019). Hence, there is the need for controlled drying to maintain both efficiency and phytochemical integrity. Without proper optimization, the antioxidant capacity and nutritional value of lemongrass may be compromised, limiting its effectiveness in green tea formulations (Rahman et al., 2013).

In functional beverage development, processing techniques such as optimized drying and size reduction have the potential to enhance antioxidant extraction, improve physicochemical stability, and influence sensory characteristics. Nevertheless, limited research has systematically examined how specific combinations of drying temperature and

lemongrass particle size affect antioxidant capacity, phenolic content, and sensory acceptability when incorporated into green tea formulations. This lack of optimized processing parameters restricts the effective utilization of lemongrass as a functional ingredient.

Furthermore, green tea is naturally associated with bitterness and astringency due to its catechin and caffeine content, which can intensify during thermal processing and negatively affect consumer acceptance. Incorporation of lemongrass may improve flavor balance and functional quality. however, inappropriate processing conditions can also lead to aroma loss and undesirable mouthfeel. Currently, there is insufficient evidence identifying the optimal drying and length conditions that simultaneously preserve phytochemical integrity and enhance sensory attributes in lemongrass green tea beverages (McDougall *et al.*, 2005). Such processing induced changes may negatively affect aroma balance and mouthfeel, masking desirable flavor characteristics of green tea (Martin *et al.*, 2013). To improve palatability and enhance functional value, herbal ingredients such as lemongrass are commonly incorporated into green tea formulations.

1.3 Objective

1.3.1 General Objective

The general objectives of this dissertation were to Optimization of Drying Temperature and Length of Lemongrass *(Cymbopogan Citratus)* Using RSM And Enhancing Sensory Profile of Green Tea (*Camellia Sinensis).*

1.3.2 Specific Objectives

1. To optimize drying temperature and length to maximize phytochemical retention and antioxidant activity in lemongrass under different drying conditions.
2. To prepare lemongrass samples under varying drying temperatures and length for analysis.
3. To determine the impact of lemongrass addition on the antioxidant profile of green tea.
4. To study the sensory attributes (aroma, flavor, color) of green tea infused with treated lemongrass.

1.4 Significance of study

There has been growing concern over the safety of synthetic antioxidants, leading to increased interest in natural, plant-based alternatives (Taghvaei and Jafari, 2015). Lemongrass (*Cymbopogon citratus*) is widely recognized for its rich content of bioactive compounds such as citral, geraniol, and flavonoids, which exhibit strong antioxidant, antimicrobial, and anti-inflammatory properties. These phytochemicals act as free radical scavengers and contribute to the prevention of oxidative stress related diseases (Aberoumand and Deokule, 2008).

Drying is a critical post-harvest process that influences the retention of these bioactive compounds. However, improper drying conditions can lead to the degradation of essential oils and antioxidants (Setareh *et al.*, 2023). Therefore, optimizing drying temperature and particle size is essential to preserve the therapeutic potential of lemongrass. In this study, lemongrass was cut into different sizes (1 cm, 3 cm, and 5 cm) and dried at varying temperatures 30°C, 45°C and 60°C (Tran and Nguyen, 2018).

Green tea (*Camellia sinensis*) is already known for its high antioxidant content, primarily due to catechins and polyphenols. By incorporating optimized lemongrass into green tea, a synergistic blend was developed that enhances the health promoting properties of the beverage. This fusion not only improves the antioxidant potential but also introduces a refreshing flavor profile, making it more appealing to health-conscious consumers. Herbal teas are gaining popularity globally due to increasing awareness of the role of diet in overall well-being (Joubert *et al.*, 2017).

Lemongrass *(Cymbopogon citratus)* offers a wide range of health benefits that further support its use as a functional food ingredient. It has traditionally been used to aid digestion, relieve gastrointestinal discomfort, reduce fever, and alleviate anxiety and stress due to its mild sedative properties. The presence of citral and geraniol also contributes to antimicrobial activity, helping inhibit the growth of pathogenic bacteria and fungi, while its anti-inflammatory effects support immune function and may reduce the risk of chronic diseases. Regular consumption of lemongrass-based beverages has additionally been associated with improved lipid metabolism and mild blood pressure regulation, highlighting its potential role in cardiovascular health. However, the development of functional herbal teas offers a promising avenue for public health improvement. This study contributes to the scientific

validation of lemongrass as a functional ingredient and supports the commercialization of blended green tea products. It also provides valuable insights for tea entrepreneurs and small-scale producers seeking sustainable and energy-efficient processing methods. Ultimately, this research aims to promote the value of indigenous medicinal plants, support local economies, and contribute to the socioeconomic development of Nepal.

Part II

Literature review

2.1 Green tea

2.1.1 Introduction of green tea

Green tea (Camellia sinensis) ranks among the most widely consumed beverages globally and is the predominant tea variety produced in China (Fu *et al.*, 2020). As a non-fermented and non-oxidized tea, it is celebrated for its refined taste, distinctive aroma, and notable health benefits, including antioxidant, anti-cancer, anti-inflammatory, and anti-atherosclerotic properties (Tan *et al.*, 2019). It is estimated that over two-thirds of the global population regularly consumes green tea (Hsu, 2005). A key group of bioactive compounds in green tea are catechins, flavanol monomers also known as flavan-3-ols which are prevalent in many plants derived foods and beverages. The major catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG), with EGCG being the most potent in terms of biological activity (Kaur *et al.*, 2015).

Numerous studies have linked green tea consumption to a reduced risk of various cancers, such as those affecting the lungs, colon, esophagus, oral cavity, stomach, small intestine, kidneys, pancreas, and mammary glands. Epidemiological data and clinical trials suggest that green tea and to a lesser extent, black and oolong teas may lower the incidence of chronic diseases due to their high polyphenol content (Tsuneki *et al.*, 2004). The manufacturing process of green tea differs from that of black tea. Freshly harvested leaves are steamed to prevent enzymatic oxidation, preserving the green color and bioactive compounds. This method helps retain polyphenols and their associated health benefits (Hursel *et al.*, 2009). Polyphenols, particularly phenolic compounds, are recognized for their antioxidant capabilities, functioning as reducing agents, hydrogen donors, and metal ion chelators.

2.1.2 History of green tea

Green tea is believed to have originated in China and has since become a staple beverage across Asia (Thasleema, 2013). The majority of global green tea production occurs in Asia, with India being the leading producer, followed by China, Kenya, and Sri Lanka (Lee *et al.*,

2014). Within India, the northeastern regions particularly West Bengal and Assam contribute the largest share, followed by Himachal Pradesh in the north and smaller quantities from Tamil Nadu in the south. Differences in harvest timing, processing methods, tea plant varieties, and geographic conditions result in unique flavor profiles, aromas, and appearances among green teas from different regions (Jung, 2004).

Tea is derived from the young leaves and shoots of the *Camellia sinensis* plant, which exists in two main botanical varieties: *C. sinensis var.* sinensis and *C. sinensis var*. *assamica*. The sinensis variety is native to southeastern China, Darjeeling, and Japan, while the *assamica* variety originates from Assam, Thailand, and Sri Lanka (Macfarlane and Macfarlane, 2009). The three major types of tea green, oolong, and black are distinguished primarily by their degree of fermentation, which in tea processing refers to enzymatic and oxidative transformations within the leaves (Gunathilaka and Tularam, 2016).

Table 2.1 The taxonomy of *Camellia sinensis*

Classification	Taxonomy
Kingdom	Plantae
Sub Kingdom	Magnoliopsida
Division	Theales
Order	Theaceace
Family	*Camellia (L.)*
Genus	*Camellia Sinensis (L.) Kuntze*
Species	*Camellia sinensis var. assamica*
Main Varities	*Camellia sinensis var. sinensis*

Source: McCully (2013)

2.1.3 Green tea processing

Green tea is classified as a non-fermented tea, and its processing methods vary notably between Japanese and Chinese traditions. Japanese green tea is typically produced by harvesting, briefly withering, steaming to halt enzymatic activity, rolling or shaping, and drying. In contrast, Chinese green tea undergoes pan firing after withering, followed by similar subsequent steps. The key distinction lies in the drying technique: Japanese teas use steam heating, while Chinese teas rely on dry heat to deactivate oxidases (Kosińska and Andlauer, 2014). White tea, by comparison, requires a significantly longer withering period than black tea approximately 4 to 5 hours (Yang *et al.*, 2009). Rapid post-harvest treatment of tea leaves is essential to preserve flavor and prevent spoilage. Processing influences the final product by altering moisture content, enzyme activity, and the synthesis of volatile compounds, which collectively shape the tea's appearance, aroma, and taste (Ahmed and Stepp, 2013).

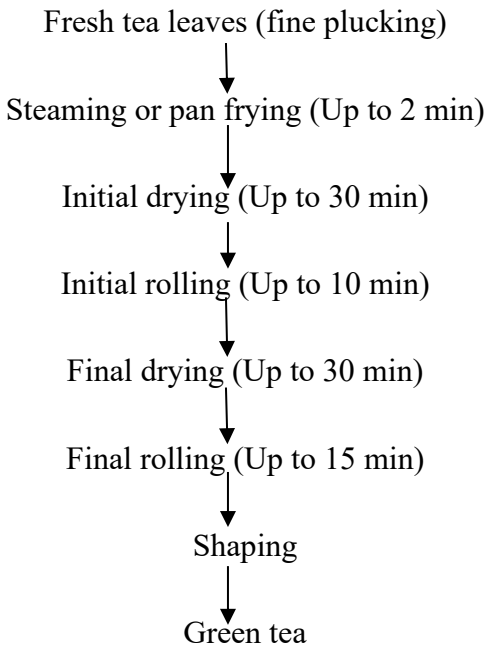


Fig 2.1 Flowchart of processing of green tea

Source: Singh *et al.* (2014)

2.1.3.1 Plucking

Producing premium-quality green tea requires the careful selection of young buds and shoots bearing two to three leaves. Tender, uniform leaves are preferred for high-grade varieties such as *Longjing*, *Biluochun*, and *Gyokuro*, while coarse or immature leaves are avoided due to their negative impact on quality standards (Singh *et al.*, 2014). After harvesting, the shoots are typically withered for 1-3 hours on bamboo trays or the ground to develop a grassy aroma and achieve optimal moisture levels.

Green tea is generally made from delicate, young leaves, with the plucking unit varying by cultivar some include a terminal bud and up to three adjacent leaves, while others use a single leaf. Mature leaves are excluded due to their toughness and astringency. Harvesting can be manual or mechanized, though hand-plucking is preferred for premium teas despite its labor intensity, with skilled workers collecting up to 30 kg per day. Tea bushes are harvested every 4 to 14 days across three seasons. Spring tea (late February to April) is aromatic and slightly bitter, summer or pre-monsoon tea (mid-May to June) is less desirable and autumn tea (September to October) is full-bodied and smoother than other seasonal harvests (Ahmed, 2011).

2.1.3.2 Fixing (Pan frying/steaming)

Fixing is a critical step in green tea processing that involves applying heat to freshly harvested leaves to deactivate enzymes responsible for fermentation and oxidation, thereby preserving the tea's green color and bioactive compounds (Xu and Chen, 2002). This process typically lasts 10-15 minutes and targets enzymes such as polyphenol oxidase, catalase, peroxidase, and ascorbic acid oxidase, which are highly active post-harvest (Obanda *et al.*, 1992). Fixing methods vary by region pan-frying, originating in China, involves heating leaves on a dry pan at approximately 180°C, while steaming, common in Japan, uses moist heat at around 100°C. Steaming is generally more effective at retaining polyphenols and antioxidant activity. However, improper temperature control either too low or excessively high can lead to undesirable changes in leaf color, texture, and flavor. Over-drying may also compromise the rolling process, resulting in fragmented leaves and reduced quality (Adnan *et al.*, 2013).

2.1.3.3 First Drying

Drying is a critical step in green tea processing, typically involving the rotation of leaves in wooden or metal drums for approximately 30 min at 55°C, during which about half of the moisture content is removed. This stage induces both physical and chemical transformations that significantly influence the final tea quality. To prevent undesirable flavors such as burnt notes and to conserve energy, precise temperature control is essential (Xie *et al.*, 2006). Prolonged exposure to high temperatures can trigger non-enzymatic browning reactions, which are common in food processing and may degrade product quality (Liu *et al.*, 2018). Various drying techniques are employed, including hot-air blast ovens, vacuum drying, and microwave-assisted drying, each offering different advantages in moisture removal and quality retention (Lou, 2002).

2.1.3.4 First rolling

During green tea processing, freshly cleaned leaves are rolled for approximately 10 min under varying pressures to initiate structural breakdown and enhance extractability. The rolling process is followed by mechanical treatments including rotor vane crushing, curl-turn-cut refinement, and roll breaking to untangle compacted leaf balls that hinder fermentation. Proper rolling is essential to ensure uniform particle size and preserve chemical integrity; both under- and over-rolling can negatively affect enzyme interactions and final tea quality. Roll breaking also facilitates faster drying, which is typically repeated to reduce moisture, intensify flavor, and improve product stability. Green tea leaves are then heat-fixed in perforated drums, resulting in needle-shaped, dark green dried leaves. Compared to black and oolong teas, green tea retains higher vitamin C content due to minimal oxidation during processing (Naheed *et al.*, 2007).

2.1.3.5 Final drying, rolling, and polishing

In the final stages of green tea processing, the leaves undergo hot air drying for approximately 30 min to reduce residual moisture. This is followed by a 15 min rolling phase between two rotating metal plates, which helps shape and flatten the leaves. The process concludes with polishing, where the leaves are pressed against a heated plate to enhance smoothness and brightness. This polishing step is essential for improving the visual appeal of the finished product, contributing significantly to its aesthetic quality.

2.1.4 Factors affecting green tea quality

Tea quality, especially in partially fermented varieties, is shaped more by environmental and biochemical factors than by yield. Soil type, climate, elevation, and cultivation practices such as fertilization, irrigation, and harvesting play a crucial role in determining quality. Processing techniques and cultivar selection further influence the concentration of key metabolites. Non-volatile compounds like caffeine, catechins, and amino acids contribute to taste, while volatile compounds define aroma (Rawat *et al.*, 2007). Leaf maturity, geographic origin, and manufacturing methods also affect sensory attributes (Han *et al.*, 2016). Nitrogen fertilization enhances amino acid levels, improving liquor color through catechin interaction. Tender leaves, with higher enzymatic activity, require more intensive fixing to deactivate enzymes and promote protein hydrolysis, which improves flavor and nutritional value. High quality teas are typically aromatic, sweet, and delicate, while bitterness and astringency are considered undesirable (Wen *et al.*, 2020).

2.1.5 Harmful effect of green tea

While green tea is widely recognized for its health-promoting properties, excessive intake may lead to adverse effects, particularly due to its bioactive compound EGCG. Individual responses to green tea catechins can vary, and high doses have been linked to liver cytotoxicity (Schmidt *et al.*, 2005) and oxidative DNA damage in the pancreas and liver of animal models (Takabayashi *et al.*, 2004). EGCG may act as a pro-oxidant in pancreatic beta cells, potentially impairing glycemic control in diabetic subjects (Yun *et al.*, 2006). High-dose green tea extract has also been associated with thyroid enlargement and altered hormone levels in rats (Sakamoto *et al.*, 2001). Although such effects are unlikely in humans under normal consumption, caution is advised. Overconsumption of green or black tea may pose risks due to caffeine content, aluminum accumulation, and reduced iron bioavailability (Hamdaoui *et al.*, 2003). Vulnerable populations such as individuals with cardiovascular conditions, renal impairment, or those pregnant or lactating should limit intake to avoid complications like heart rhythm disturbances, neurological issues, and nutrient deficiencies (Costa *et al.*, 2002).

2.1.6 Bioactive Constituents and Therapeutic Potential of Green Tea

Green tea is rich in bioactive compounds, particularly catechins like EGCG, which account for up to 80% of its polyphenol content and are known for their potent antioxidant and antitumor properties. Animal studies have demonstrated green tea's protective effects against degenerative diseases, hepatotoxicity, and various cancers, including those of the kidney, pancreas, and mammary glands (Roomi *et al.*, 2005) (Koo and Cho, 2004). Its polyphenols also exhibit hypolipidemic, antiproliferative, and neuroprotective activities, with evidence suggesting benefits in conditions like Parkinson's and Alzheimer's (Unno *et al.*, 2007). Green tea enhances immune function and may inhibit hepatic stellate cell proliferation, potentially slowing liver fibrosis progression (Sakata *et al.*, 2004). Additionally, green tea polyphenols support cardiovascular health by lowering blood pressure, glucose levels, and body weight (Tsuneki *et al.*, 2004). Its chemical composition includes proteins, amino acids (e.g., theanine, glutamic acid), carbohydrates, lipids, vitamins (B, C, E), minerals, pigments, and volatile compounds, all of which vary with season, cultivar, and growing conditions (Mukhtar and Ahmad, 2000). Epidemiological studies confirm green tea's superior health benefits compared to black and oolong teas, largely due to its high polyphenol content (Zaveri, 2006).

2.1.7 Hazard Effects of Bioactive Compounds

Bioactive compounds such as polyphenols, flavonoids, tannins, and catechins are widely recognized for their antioxidant, anti-inflammatory, and disease-preventive properties. However, excessive intake or high-dose exposure may produce adverse health effects. Certain polyphenols, particularly epigallocatechin gallate (EGCG) from green tea, have been reported to exhibit pro-oxidant behavior at elevated concentrations, leading to oxidative stress and potential liver toxicity (Schmidt *et al.*, 2005)

Animal studies have demonstrated that high doses of green tea catechins may induce hepatic cytotoxicity and oxidative DNA damage in pancreatic and liver tissues (Takabayashi *et al.*, 2004). Furthermore, EGCG has been shown to interfere with pancreatic β-cell function and glucose metabolism, posing risks for individuals with diabetes when consumed in excessive amounts (Yun *et al.*, 2006). Prolonged consumption of concentrated green tea extracts has also been associated with thyroid enlargement and hormonal imbalance in experimental models (Sakamoto *et al.*, 2001).

Tannins, although beneficial for their antioxidant and antimicrobial activity, may reduce mineral bioavailability by forming insoluble complexes with iron and other trace elements, potentially contributing to nutritional deficiencies when consumed in large quantities (Hamdaoui *et al.*, 2003). Similarly, excessive intake of herbal bioactives may cause gastrointestinal discomfort, nausea, or allergic reactions in sensitive individuals.

Despite these potential hazards, such adverse effects are generally associated with concentrated extracts or pharmacological doses rather than normal dietary consumption. When consumed as part of functional beverages such as lemongrass-incorporated green tea, bioactive compounds remain within safe physiological limits. Therefore, controlled processing and moderate consumption are essential to maximize health benefits while minimizing possible risks.

2.2 Lemongrass

Lemongrass *(Cymbopogon citratus)* is a tropical, perennial grass widely cultivated for its aromatic essential oil, rich in citral and other bioactive compounds such as terpenoids, benzenoids, and sulfur and nitrogen containing organics. Lemongrass is grown on marginal lands and contributes to soil and water conservation through its extensive root system (Joy *et al.*, 2006). Its dried leaves are commonly used in herbal teas and culinary preparations, offering a lemon like flavor without altering biochemical parameters in the body. Lemongrass is also popular in global cuisines, especially Thai, and is used in syrups, sauces, and spice blends. The essential oil and oleoresin are extensively applied in food flavoring ranging from beverages and desserts to meat products and in perfumery, cosmetics, and pharmaceuticals.

Medicinally, lemongrass exhibits antispasmodic, anti-inflammatory, antimicrobial, antioxidant, and digestive properties. It is traditionally used in various cultures to treat stomach ailments, promote uterine health, and relieve pain. Nutritionally, it is a source of essential vitamins (A, B-complex, C, folate) and minerals (Ca, K, Mg, Fe, Zn) (Majewska *et al.*, 2019). Due to its recognized safety status (GRAS) by the FDA, lemongrass essential oil is increasingly explored as a natural food preservative, aligning with consumer demand for healthier and safer alternatives to synthetic additives (Avoseh *et al.*, 2015).

Table 2.2 Taxonomical classification of lemongrass

Taxonomical classification	
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae
Genus	*Cymbopogon Spreng*

Source: ceriferus Hack (2007)

2.2.1 Multifunctional Applications and Ethnomedicinal Significance of Lemongrass

Lemongrass *(Cymbopogon citratus)* is a versatile plant widely utilized across tropical and subtropical regions for its culinary, cosmetic, and medicinal properties. In the culinary domain, it serves as a key ingredient in traditional beverages and dishes. Known as "*Takrai*" in Thailand, it is extensively used in soups, curries, and marinades, while in Vietnam, fresh leaves are added to salads. In Western cuisine, lemongrass is incorporated into seafood broths and spice blends. Pharmacological studies have validated some of these claims. For instance, Puatanachokchai *et al.* (2002) reported chemo preventive potential against colon carcinogenesis, while Stehmann and Brandão (1995) highlighted its efficacy in alleviating urinary retention. In South America and Southeast Asia, lemongrass is also used in traditional drinks such as sherbets and soft beverages (Burkill, 1935).

In cosmetics, the essential oil is valued for its refreshing aroma and is incorporated into soaps, perfumes, candles, and insect repellents. Ethnobotanical records reveal its widespread use in folk medicine for treating digestive disorders, menstrual irregularities, rheumatism, and nervous conditions (Agbafor and Akubugwo, 2007; Nambiar and Matela, 2012) . In

Brazil, it was the most cited plant for psychoneurological ailments among women attending health centers.

Recent reviews emphasize lemongrass's therapeutic potential, attributing its bioactivity to essential oils and phenolic compounds. It has shown promise in managing gastrointestinal disorders, enhancing circulation, regulating menstrual cycles, and promoting restful sleep (Majewska *et al.*, 2019). Its vitamin C content and antimicrobial properties make it effective against respiratory infections, skin conditions, and urinary tract infections (Coelho *et al.*, 2016). Furthermore, lemongrass is used in Ayurvedic medicine as a febrifuge and nervine tonic, aiding in the treatment of headaches, muscle cramps, and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Wifek *et al.*, 2016).

Table 2.3 Country wise use and application of Lemon Grass

Country	Purpose	Reference
India, Nepal	Used for gastrointestinal problems. A decoction made from the leaves in recommended as diaphoretic in fever	(Alves and Souza, 1960)
China	As ansiolitic	(Xaio, 1983)
Mauricio islands, Malay Peninsula	Common to use the lemongrass tea against flu, fever, pneumonia, and to solve gastric and sudorific problems	(Nambiar and Matela, 2012)
Nigeria	As antipyretic and for its stimulating and antispasmodic effects.	(Olaniyi *et al.*, 1975)
Indonesia	Indicated to help the digestion, to promote diuresis, sweating and as emmenagogue	(HH, 1983)
Cuba and other Caribbean region	For analgesic and anti-inflammatory actions.	(Soto Ortiz *et al.*, 2002)
Africa and Asia	Considered as antitussive, antiseptic, sudorific, stomachis, anti-rheumatic and to treat backache, sprain and haemoptysis	(Alves and Souza, 1960)
London	The grass has been reported to revitalize the body and promote good health. It aids digestion and inhibits chemical induced carcinogenesis by modulating xenobiotic metabolizing enzymes in the liver and intestine.	

Source: Nambiar and Matela (2012)

2.2.2 Chemical composition and characteristic of lemongrass

Lemongrass is structurally composed of cellulose, hemicellulose, and lignin, which contribute to its fibrous integrity and functional versatility. Cellulose features a linear molecular structure with both crystalline and amorphous regions, while hemicellulose is more branched and less polymerized. Lignin, a complex aromatic polymer, binds these components together, enhancing rigidity and resilience (Kaur and Dutt, 2012). The plant's elemental composition is predominantly carbon and oxygen, and its essential oil yield typically 1-2% of dry weight is influenced by genotype, cultivation conditions, and drying methods. Notably, oven drying increases oil yield, whereas shade drying preserves citral concentration, a key marker of oil quality (Carlson *et al.*, 2001; Hanaa *et al.*, 2012)

Chemically, lemongrass essential oil is rich in citral, comprising two isomers: geranial and neral, which together account for over 45% of the oil content. Additional constituents include terpenes, alcohols, ketones, esters, and a wide spectrum of phytochemicals such as flavonoids, alkaloids, tannins, and phenolic acids. These compounds contribute to its broad therapeutic potential, including antifungal, antibacterial, anti-inflammatory, and anti-nociceptive properties (Ekka *et al.*, 2024). The presence of trace bioactives like myrcene, citronellol, and quercetin further enhances its pharmacological value, making lemongrass a promising candidate for functional food and medicinal applications.

Table 2.4 Nutritional content of lemon grass

S. No.	Nutritional component	Quantity
1.	Carbohydrates	55.00%
2.	Crude fat	5.10%
3.	Crude protein	4.56%
4.	Crude fiber	9.28%
5.	Energy	360.55 kcal/100g

Source: (Ekka *et al.*, 2024)

2.2.3 Health benefits of lemongrass

Lemongrass is a widely utilized medicinal and culinary herb known for its diverse therapeutic properties, largely attributed to its rich phytochemical profile, particularly citral. It exhibits potent anti-hyperlipidemic and anti-hypercholesterolemic effects, contributing to reduced LDL cholesterol and triglyceride levels, which may help prevent atherosclerosis and other cardiovascular disorders. Its diuretic action supports detoxification by enhancing urinary output and regulating liver and kidney function, while also lowering uric acid levels. Lemongrass has demonstrated anticancer potential, with citral showing selective cytotoxicity against hepatic, breast, and skin cancer cells through mechanisms involving apoptosis and inhibition of cell proliferation. Its antimicrobial and antifungal activities are effective against pathogens such as Staphylococcus aureus, Helicobacter pylori, and Escherichia coli, making it beneficial for treating infections and gastrointestinal disorders. Additionally, lemongrass tea is known for its sedative and hypnotic effects, promoting sleep and calming the nervous system, while also alleviating symptoms of anxiety, convulsions, and fatigue. In respiratory health, it provides relief from cough, cold, and bronchial asthma, supported by its vitamin C content and anti-inflammatory properties (Ekka *et al.*, 2024).

Lemongrass also plays a role in managing type-2 diabetes, with animal studies indicating improved insulin regulation and glucose tolerance. Its analgesic and anti-inflammatory effects help relieve headaches, muscle cramps, and rheumatic pain, while its antioxidant activity supports cellular health, DNA synthesis, and skin regeneration. Beyond medicinal uses, lemongrass is employed in aromatherapy, cosmetics, insect repellents, and pet care products due to its essential oils and antimicrobial effects. It is also used in culinary applications across Southeast Asia and in the preservation of ancient manuscripts, owing to its hydrophobic and protective qualities. These multifaceted benefits underscore lemongrass's significance in functional food development and its potential for broader nutraceutical applications (Toungos, 2019)**.**

2.3 Phytochemicals in plants

Phytochemicals are bioactive substances, present in small quantities in food, exert beneficial effects depending on their dosage (Kris-Etherton *et al.*, 2002). Plant metabolites are broadly categorized into primary (e.g., carbohydrates, proteins, lipids) and secondary metabolites (e.g., polyphenols, alkaloids, steroids), with the latter playing no direct role in growth or

development but serving as adaptive responses to environmental stressors. Secondary metabolites are typically low molecular weight compounds with distinct biological activity. Phytochemicals such as flavonoids, terpenoids, alkaloids, and plant sterols vary structurally and functionally, contributing significantly to the therapeutic potential of plants (Kumar *et al.*, 2018). Research suggests that over 10,000 phytochemicals may influence chronic conditions including cancer, stroke, and metabolic syndrome. The major phytochemical constituents include fatty acids, polysaccharides, glycosides, steroids, sesquiterpenoids, aliphatic compounds, and sesquiterpenes underscoring their relevance in functional food development and nutraceutical applications.

2.3.1 Classification of Phytochemicals

2.3.1.1 Phenolic/Polyphenols

Polyphenol phytochemicals represent the most abundant and widely distributed class of bioactive compounds in the plant kingdom. Structurally, they are characterized by one or more hydroxyl groups (-OH) attached directly to aromatic hydrocarbon rings (Altıok, 2010). These compounds are classified into various subgroups depending on the number of carbon atoms in their molecular framework, which influences their chemical behavior and biological activity as shown in table

Table 2.5 Major classes of phenolic compounds in plant

S. N	No. of Carbon atom	Basic Skeleton	Class
1	6	C_6	Simple Phenols Benzoquinones
2	7	C_6-C_1	Phenolic acids
3	8	C_6-C_2	Acetophenones Tyrosine derivatives
4	9	C_6-C_3	Hydroxycinnamic acid, Coumarin
5	10	C_6-C_4	Naphthoquinones
6	13	C_6-C_1-C_6	Xanthones
7	14	C_6-C_2-C_6	Stilbenes
8	15	C_6-C_3-C_6	Flavonoids
9	18	$(C_6$-$C_3)^2$	Lignans
10	30	$(C_6$-C_3-$C_6)^2$	Bioflavonoids
11	N	$(C_6$-C_3-$C_6)^n$	Condensed tannins

Source: Saxena *et al.* (2013)

2.3.1.2 Flavonoids

Flavonoids are a prominent class of polyphenolic compounds widely distributed in fruits, vegetables, seeds, olive oil, tea, and red wine. As secondary metabolites, they do not directly contribute to plant growth but serve ecological roles such as pigmentation particularly in autumn foliage and defense against microbial and insect threats (Watson, 2000). Structurally, flavonoids are low molecular weight compounds composed of a three-ring backbone with variable substitutions, and they exist both in free form and as glycosides (Kesarkar *et al.*, 2009). Over 4,000 flavonoids have been identified, with approximately 500 occurring in their free state. These compounds exhibit a range of biological activities, including anti-inflammatory, antioxidant, anti-allergic, antithrombotic, and Vaso protective effects. Their antioxidant capacity is closely linked to their molecular structure, particularly the number and position of hydroxyl groups, which influence their ability to neutralize free radical (Heim *et al.*, 2002).

2.3.1.3 Tannins

Tannins are a widely distributed class of polyphenolic compounds in the plant kingdom, distinguished by their capacity to bind with proteins, pigments, large biomolecules, and metal ions, while also exhibiting notable antioxidant properties (Bernhoft *et al.*, 2010; Okuda and Ito, 2011); . Although present in numerous plant species including tea leaves, coffee beans, and herbs their concentration is minimal in lower organisms such as algae, fungi, and mosses. Tannins are broadly categorized into hydrolysable and condensed types. Hydrolysable tannins yield gallic and ellagic acids upon hydrolysis and include compounds like theaflavins, daidzein, genistein, and glycerin (Bressani *et al.*, 1983). These tannins also produce pyrogallic acid when heated. In contrast, condensed tannins show variable correlations with phenolic content depending on structural characteristics (Baldwin *et al.*, 1987).

Tannin-rich plant extracts possess diverse pharmacological properties, including anti-inflammatory, antibacterial, antioxidant, and hemostatic effects (Anesini *et al.*, 2008). Industrially, tannins are utilized in dye production (e.g., iron gallate ink), food clarification processes, and as additives in textiles, rubber, and beverages for their stabilizing and antioxidant roles. Their therapeutic potential has gained renewed scientific attention due to

rising global health concerns such as cancer and AIDS, prompting research into tannin-based bioactive compounds for drug development (Saxena *et al.*, 2013).

2.3.2 Factors Affecting Phytochemical Content

2.3.2.1 Cultivar Effect

The genetic makeup of a plant is a primary determinant of its phytochemical profile. Studies have shown that variations in phytochemical composition among different cultivars are more pronounced than those observed within the same cultivar grown under varying environmental conditions (Hu, 2012; Tiwari and Cummins, 2013); . This genetic influence is particularly evident in horticultural crops such as potato, faba bean, tomato, garlic, globe artichoke, and cardoon, where phenolic content and antioxidant capacity are significantly shaped by cultivar-specific traits (Rouphael *et al.*, 2016).

2.3.2.2 Extraction Method and Solvent Used

The method of extraction and the type of solvent employed play a crucial role in determining the yield and concentration of phytochemicals. Methanol based extractions have consistently produced higher levels of bioactive compounds. Additionally, agro-climatic conditions such as altitude and aridity affect antioxidant activity, with samples from Highland and Semi-arid zones showing greater potency than those from Tropical regions (Kumar *et al.*, 2017). Comparative studies have demonstrated that soxhlet extraction yields significantly higher phytochemical concentrations than ultrasound-assisted methods (Vidic *et al.*, 2014). Variations in results may also stem from differences in solvent polarity, plant maturity stages, and environmental conditions during cultivation and harvest (Aboshora *et al.*, 2014; Rababah *et al.*, 2010);

2.3.2.3 Environmental Conditions

Phytochemical accumulation in plants is significantly influenced by environmental factors such as sunlight exposure, temperature variation, and regional climatic conditions (Oh *et al.*, 2009). Soil type, mineral composition, water availability, and light intensity also play crucial roles in determining overall phytochemical levels (Rajbhar *et al.*, 2015). Fertilization practices are particularly impactful while nitrogen, phosphorus, and potassium fertilizers enhance vegetative growth, excessive application may reduce phytochemical concentrations

(Santana *et al.*, 2015; Tiwari and Cummins, 2013) . Seasonal changes further contribute to fluctuations in phytochemical profiles due to temperature shifts (Usano-Alemany *et al.*, 2014).

2.3.2.4 Growth Conditions

The developmental stage of a plant directly affects its phytochemical composition. Early growth stages typically exhibit lower levels of phenolics and flavonoids, while alkaloid content tends to increase progressively during maturation (Hu, 2012). Variations in species and maturity stages also contribute to differences in phytochemical concentrations (Sivakumar and Rajan, 2010).

2.3.2.5 Post-Harvest Factors

Phytochemicals are highly sensitive to post-harvest conditions. Factors such as fungal decay, pest infestation, mechanical damage, over ripeness, and improper temperature or humidity during storage and transport can lead to significant degradation of bioactive compounds (Yahia *et al.*, 2017).

2.3.2.6 Post-Harvest Storage Conditions

Storage temperature, atmospheric gas composition, and chemical treatments substantially influence the stability and concentration of phytochemicals. Lower temperatures help preserve phenolic, flavonoid, and antioxidant activity, whereas higher temperatures accelerate degradation. Drying methods and exposure duration to heat also affect compound retention (Li *et al.*, 2012).

2.3.2.7 Other Factors

The efficiency of phenolic compound extraction is determined by several variables, including the chemical nature of the compounds, the type and condition of raw materials, storage duration, and extraction protocols. The choice of analytical standards and potential interference during quantification also play a critical role (Alara *et al.*, 2021).

2.3.3 Importance of Phytochemicals

Phytochemicals are bioactive compounds in plants that contribute to health-promoting functions through diverse mechanisms. They may inhibit microbial growth, interfere with metabolic pathways, or modulate gene expression signaling networks (Manson, 2003). These compounds are recognized for their dual role in chemotherapy and chemoprevention, particularly in cancer management, as they often share molecular targets with conventional anticancer agents.

Essential oils and plant extracts exhibit antimicrobial activity by disrupting the phospholipid bilayer of microbial membranes, increasing permeability, and impairing cellular integrity. Their action may also involve enzyme inhibition, genetic material degradation, and interference with energy metabolism (Kotzekidou *et al.*, 2008). Additionally, phytochemicals serve ecological roles in plants such as defense, reproduction, and signaling through compounds like phytoalexins, allelochemicals, and antifeedants. Dietary intake of phytochemicals has been associated with reduced risk of chronic diseases, including type 2 diabetes, cardiovascular disorders, and cancer, supported by extensive in vitro and in vivo studies over the past two decades (Doughari *et al.*, 2009; Liu *et al.*, 2018).

2.4 Antioxidant Activity

Antioxidants are bioactive compounds that inhibit or delay oxidative reactions, thereby enhancing food stability and protecting biological systems from oxidative stress. In functional beverages such as lemongrass infused green tea, antioxidants play a dual role they prevent quality deterioration during processing and storage, and they contribute to health benefits by neutralizing reactive oxygen species (ROS) like superoxide anions, hydroxyl radicals, and singlet oxygen (Pisoschi and Negulescu, 2011; Upadhyay *et al.*, 2011)

Lemongrass contains key phytochemicals such as citral, geraniol, and flavonoids, which exhibit potent antioxidant properties. When incorporated into green tea a rich source of catechins and polyphenols the synergistic effect may enhance the beverage's ability to scavenge free radicals and reduce oxidative stress. This is particularly relevant in preventing degenerative conditions such as cardiovascular diseases, neurodegeneration, and diabetes (Doughari *et al.*, 2009). Endogenous antioxidant systems like glutathione, catalase, and ubiquinol help regulate ROS levels in the body. However, dietary supplementation through

antioxidant rich beverages is essential to compensate for physiological deficiencies (Jayaprakash *et al.*, 2015). Optimizing the drying parameters of lemongrass is critical to preserving these bioactive compounds, as thermal degradation can significantly reduce antioxidant capacity. Plant derived antioxidants, including vitamin C, β-carotene, flavonoids, and polyphenols, exhibit broad biological activities such as anti-inflammatory, antiviral, anticarcinogenic, and immune-modulating effects (Graf *et al.*, 2005; Omojate Godstime *et al.*, 2014) .

2.5 Characteristics of Drying

Drying is a critical post-harvest operation that significantly influences moisture removal, shelf life, sensory quality, and retention of bioactive compounds in plant materials. The characteristics of drying depend on temperature, air velocity, particle size, and drying duration, all of which affect heat and mass transfer mechanisms.

At lower drying temperatures, phytochemicals such as phenolics and flavonoids are better preserved; however, drying time becomes prolonged, increasing microbial risk and energy consumption. Conversely, higher temperatures accelerate moisture removal but may cause thermal degradation of heat-sensitive compounds, essential oils, and pigments (Sellami *et al.*, 2012). In lemongrass, excessive drying temperatures have been shown to reduce citral content and antioxidant activity due to volatilization and oxidative degradation.

Moderate drying temperatures (40–50°C) are reported to provide optimal balance between drying efficiency and phytochemical retention, maintaining aroma, color, and antioxidant properties (Mabai *et al.*, 2018). Length also plays a vital role, as smaller length enhance surface area for moisture diffusion but may increase exposure to oxidative damage, whereas larger segments retain moisture longer, leading to uneven drying.

Different drying methods such as sun drying, oven drying, microwave drying, and solar drying produce varying effects on product quality. Oven drying offers better control over temperature and airflow, resulting in more uniform drying and improved preservation of bioactive compounds compared to traditional sun drying (Salimi *et al.*, 2024). Proper drying therefore ensures structural stability, minimizes enzymatic activity, and preserves functional constituents.In functional beverage development, optimized drying characteristics are essential to retain antioxidant capacity, enhance extraction efficiency, and improve sensory

attributes. Controlled drying of lemongrass contributes directly to the stability, quality, and health-promoting potential of lemongrass-incorporated green tea.

2.6 Impact of Drying Techniques on Lemongrass Quality

Drying is a vital postharvest process that significantly affects the moisture content, sensory properties, and phytochemical retention of lemongrass. It is widely used to extend shelf life, reduce microbial activity, and minimize packaging and transportation costs (Sellami *et al.*, 2012). Among various drying methods sun, solar, oven, and microwave each has a distinct impact on the preservation of color, aroma, essential oils, and antioxidants. Sun drying, though cost effective, often leads to uneven moisture loss and degradation of heat sensitive compounds due to prolonged exposure to ambient conditions. Solar drying offers better control but still poses challenges in volatile oil preservation. Microwave drying is rapid and efficient but may cause structural damage and loss of aroma if not carefully optimized. In contrast, oven drying at 40°C has consistently shown superior results in retaining sensory attributes and bioactive compounds (Mabai *et al.*, 2018).

Lemongrass tea prepared from samples dried at this temperature was rated highest in color, aroma, taste, and overall acceptability, making it ideal for tea production (Mabai *et al.*, 2018) Additionally, oven drying at 45°C was found to yield the highest essential oil content, preserving key volatile compounds such as geranial and neral, which contribute to lemongrass's antioxidant and antimicrobial properties (Salimi *et al.*, 2024). Dried lemongrass leaves are also rich in carbohydrates, vitamin C, and volatile oils like piperitone, elemol, α-eudesmol, and limonene, which enhance both flavor and medicinal value (Priyanka *et al.*, 2022). The drying process not only stabilizes these compounds but also ensures year-round availability and supports traditional medicinal uses, including treatment of cold, cough, fever, gastrointestinal disorders, and rheumatism.

2.7 Design and Optimization of Functional Tea Blends Incorporating Lemongrass

The concept of synergy in functional foods emphasizes the advantage of using whole plant materials, where multiple bioactive compounds interact to produce enhanced health benefits compared to isolated components (Malongane *et al.*, 2017). Blending green tea with lemongrass exemplifies this principle, as the combination improves both nutritional value and sensory appeal. Compared to green tea alone, the infusion of lemongrass contributes to

a more balanced distribution of nutrients and a distinct citrusy flavor profile, which enhances consumer acceptability (Nambiar and Matela, 2012).

This herbal blend is rich in antioxidants, including catechins, flavonoids, and vitamin C, which collectively contribute to its therapeutic properties. The synergistic interaction between green tea polyphenols and lemongrass phytochemicals enhances antimicrobial activity, particularly against foodborne pathogens, and helps protect cells from oxidative damage (Jain *et al.*, 2011). These compounds work together to inhibit microbial growth and support immune defense mechanisms. Moreover, the green tea lemongrass blend is associated with improved metabolic health, digestive function, and stress relief, making it a promising natural alternative for health-conscious consumers seeking functional beverages (Nambiar and Matela, 2012). The synergistic effects of this combination not only elevate its bioactivity but also enhance its market potential in the herbal tea segment.

2.8 Sensory Evaluation

Sensory science within the field of food research explores how individuals perceive and respond to food products through their senses particularly taste, aroma, and texture. It provides a systematic and empirical framework for understanding human reactions to various food components (Barthomeuf *et al.*, 2009). The discipline has expanded notably since the mid-20th century, paralleling the growth of the processed food and consumer goods industries (Lawless and Heymann, 2010).

Sensory evaluation involves consumers assessing products based on their preferences, which informs product development and menu decisions. Items that receive favorable responses are retained, while those with low acceptability are reconsidered or removed (Wichchukit and O'Mahony, 2015). This approach minimizes external biases such as brand influence and ensures that consumer feedback is based solely on sensory attributes. It equips food scientists, developers, and industry professionals with reliable data on how products are perceived (Tuorila and Monteleone, 2009). A key metric in sensory analysis is consumer acceptability, typically measured using hedonic scales with untrained panelists. However, sensory research extends beyond simple preference ratings. It also encompasses consumer perceptions, emotional responses, and the relationship between descriptive sensory data, instrumental measurements, and consumer attitudes toward a product (Venturi *et al.*, 2016).

2.8.1 9-Point hedonic scale rating

The 9-point hedonic scale is one of the most widely adopted sensory evaluation tools in food science, used for over six decades to assess consumer preferences and product acceptability (Lim, 2011). Originally developed by the U.S. Army to improve menu planning in military canteens, the scale consists of nine verbal categories ranging from “dislike extremely” (1) to “like extremely” (9), which are later converted into numerical values for statistical analysis (Peryam and Pilgrim, 1957).

This scale is considered a balanced bipolar tool, with four positive and four negative categories symmetrically arranged around a neutral midpoint (Lim, 2011). It allows researchers to quantify consumer responses and identify preference trends across different product variations. A typical hedonic test involves 75–150 untrained consumers, often conducted in centralized sensory labs to ensure consistency and minimize external bias (Nicolas *et al.*, 2010).While the traditional scale uses verbal descriptors reassigned as numbers, some versions employ purely numerical formats with labeled endpoints. These two formats are not interchangeable, as they engage different cognitive strategies during evaluation (Wichchukit and O'Mahony, 2015). Larger sample sizes are often recommended for affective tests to account for individual variability and enhance statistical reliability. Beyond measuring liking, the scale also helps uncover consumer segments, flavor preferences, and reasons for product rejection, making it a valuable tool in food product development and quality optimization.

Part III

Materials and Methods

3.1 Materials

The materials that are included in this work are as follow:

3.1.1 Lemongrass

Fresh leaves of lemongrass were selected as the primary plant material for drying optimization. The samples were collected from a local farm in Rupandehi district with the assistance of local growers. The leaves were harvested during their optimal maturity stage and immediately transported for pretreatment and drying.

3.1.2 Green Tea

Green tea *(Camellia sinensis)* was selected as the base material for tea blending. Green tea was collected from two locations the village of Ilam, known for its high-altitude tea cultivation, and the surrounding area of the Central Campus of Technology, Dharan. The samples were stored in airtight containers to preserve freshness and prevent oxidation prior to analysis.

3.1.3 Packaging Materials

Dried lemongrass samples were stored in polyethylene bags to prevent moisture absorption. For sensory evaluation, tea blends were packed in food-grade nonwoven fabric tea bags to ensure hygiene and consistency.

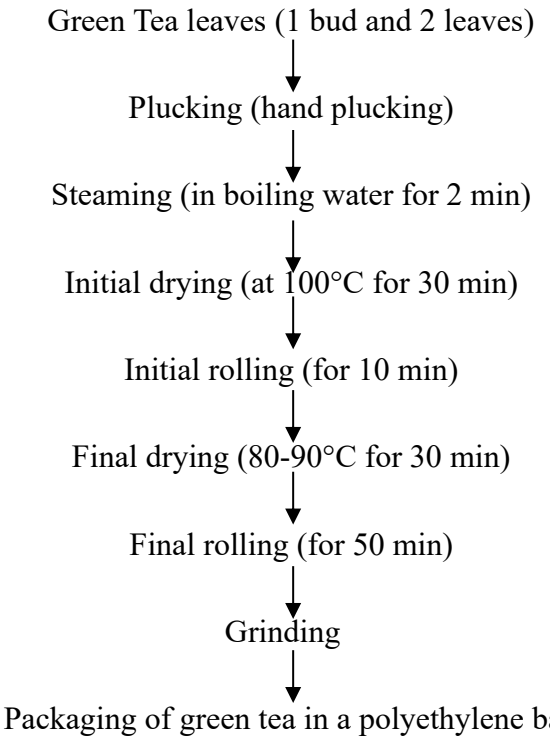
3.1.4 Equipment and Chemicals

All equipment, materials, and chemicals required for drying, extraction, and analysis were obtained from the laboratory of the Central Campus of Technology, Dharan. This includes drying ovens, analytical balances, glassware, and reagents used for phytochemical and antioxidant assays. A complete list of equipment and chemicals is provided in Appendix A.

3.2 Methods

3.2.1 Preparation of dried green tea

The flowchart for the preparation of dried green tea is as follows



Source: Singh *et al.* (2014)

Fig 3.1 Flowchart for the preparation of dried green tea

Green tea was prepared by harvesting fresh green leaves with two leaves and a bud.The leaves were briefly steam treated for two minutes to inactivate enzymes and preserve their green color. Initial drying was carried out at 100°C for 30 min to reduce moisture, followed by rolling for at least 10 min to break leaf structure and enhance flavor. A second drying phase at 80-90°C for 30 min ensured optimal moisture content. Final rolling for up to 50 min improved texture and product quality. The resulting dried green tea was crushed and packed in polyethylene bags to maintain freshness during storage and transport.

3.2.2 Preparation of Dried Lemongrass leaves

The flowchart for the preparation of dried lemongrass leaves as follows:

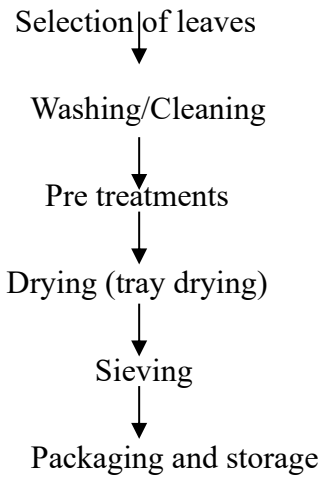


Fig 3.2 Flowchart for the preparation of dried lemon grass leaves

The dried samples were crushed and were sealed in LDPE bags and stored at ambient temperature.

3.3 Experimental Design

A statistical software Design Expert® version 13.0 (Stat-Ease Inc., USA) was used for experimental design, model building, and data analysis. The lemongrass incorporated green tea was prepared with variation in: (a) lemongrass segment size and (b) drying temperature. The range for drying temperature was 30-60°C, while for lemongrass segment size it was 1-5 cm. These ranges were selected based on relevant literature and several preliminary laboratory trials.

Total of 13 runs were generated by Design Expert® version 13.0 using Response Surface Methodology (RSM) with a Central Composite Design (CCD) and a quadratic design model. The experiment was randomized to minimize the effects of uncontrolled factors. The responses selected for the study were total phenolic content (TPC), total flavonoid content (TFC), tannin content and antioxidant activity (AA).

Table 3.1 Experimental design for length of leaves and drying temperature

Run	Length of leaves (cm)	Drying temperature (ºC)
1	1	45
2	3	45
3	3	45
4	3	30
5	1	30
6	5	30
7	3	45
8	3	45
9	3	45
10	5	60
11	5	45
12	3	60
13	1	60

3.4 Selection of Optimum Drying Temperature and Particle Size

To determine the optimum drying temperature and particle size of lemongrass for incorporation into green tea, freshly harvested lemongrass leaves were thoroughly washed, cleaned, and cut into uniform sizes (1, 3, 5cm). The leaves were subjected to drying at different temperatures of 40°C, 50°C and 60°C in a hot-air oven until constant weight was achieved. After drying, the samples were ground. The dried powders were stored in airtight containers under dry conditions.

3.5 Phytochemical Analysis

3.5.1 Total Phenolic Content (TPC)

The total phenolic content of fresh lemongrass, powdered lemongrass, fresh green tea, powdered green tea and samples of green tea incorporated with lemongrass was determined using a modified spectrophotometric method adapted by Sangeetha *et al.* (2014). 1 mg/ml aqueous solution of the extract was prepared and mixed with 0.5 ml of the sample, 2.5 ml of 10% Folin Ciocalteu reagent, and 2.5 ml of 7.5% sodium carbonate solution. The reaction mixture was incubated at 45°C for 45 min. Absorbance was measured at 765 nm using a UV-visible spectrophotometer. All samples were analyzed in triplicate, and the average absorbance was used to calculate phenolic content. A standard curve was constructed using gallic acid, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

3.5.2 Total Flavonoid Content (TFC)

Total flavonoid content of fresh lemongrass, powdered lemongrass, fresh green tea, powdered green tea and samples of green tea incorporated with lemongrass was assessed using a modified aluminum chloride colorimetric assay based on the method described (Shraim *et al.*, 2021). In this procedure, 2 ml of sample solution was mixed with 0.2 ml of 5% sodium nitrite and allowed to stand for 5 min. Then, 0.2 ml of aluminum chloride solution was added, followed by another 5 min incubation. Subsequently, 2 ml of 1N sodium hydroxide was added, and the final volume was adjusted to 5 ml. After 15 min, absorbance was recorded at 510 nm. A quercetin standard curve (20-100 μg/ml) was used for calibration, and results were expressed as milligrams of quercetin equivalents (mg QE/g).

3.5.3 Total Tannin Content (TTC)

The total tannin content of fresh lemongrass, powdered lemongrass, fresh green tea, powdered green tea and samples of green tea incorporated with lemongrass was measured using the Folin–Ciocalteu method as outlined by Blainski *et al*. (2013). A 10 ml volumetric flask was used to prepare the reaction mixture containing 0.1 ml of sample extract, 7.5 ml of distilled water, 0.5 ml of Folin Ciocalteu reagent, and 1 ml of 35% sodium carbonate solution. The volume was made up to 10 ml with distilled water. After thorough mixing, the solution was allowed to stand at room temperature for 30 min. Gallic acid standard solutions

(20–100 µg/ml) were prepared similarly. Absorbance was measured at 725 nm, and tannin content was expressed as mg GAE/g of extract.

3.5.4 DPPH Radical Scavenging Activity

The antioxidant potential of fresh lemongrass, powdered lemongrass, fresh green tea, powdered green tea and samples of green tea incorporated with lemongrass was evaluated using the DPPH radical scavenging assay, following the protocol of Reena Patel *et al*. (2015). A mixture of 1 ml of sample extract and 4 ml of 0.01% methanolic DPPH solution was prepared and kept in the dark at ambient temperature (28°C) for 30 min. Absorbance was measured at 517 nm using a UV-visible spectrophotometer. Methanol was used as the control. The percentage of DPPH radical scavenging activity was calculated using the formula:

DPPH scavenging activity (%) = $\frac{Ac - As}{Ac}$

Where, A_c represents for absorbance of control;

A_s represents for absorbance of test sample.

The optimization process was based on the evaluation of phytochemical and antioxidant properties of the lemongrass powders. The moisture content of each sample was determined by the oven-drying method at 105°C until a constant weight was reached. Samples showing the highest TPC and TFC values, strongest antioxidant activity (lowest IC_{50}), and acceptable moisture content were considered to represent the optimum drying temperature and particle size combination. The optimum condition obtained was used for further formulation of lemongrass incorporated green tea blends and subsequent sensory evaluation.

3.6 Preparation of Lemongrass incorporated Green Tea in Teabag Packaging

To prepare the lemongrass incorporated green tea, optimized lemongrass powder was blended with green tea powder at specific ratios as presented in Table 3.2. The components were mixed thoroughly to obtain a uniform and homogeneous blend. Subsequently, 3 grams of the blended tea were packed into individual teabags. The open ends of the teabags were securely tied with strings, properly sealed, and labeled to maintain sample identity. Care was

taken to ensure the teabags were tightly sealed to prevent any leakage or contamination during storage and handling.

Table 3.2 Different formulations of lemongrass powder and green tea powder

Product	Formulation
Control (C)	100% Green tea
A	5% Lemongrass + 95% Green tea
D	10% Lemongrass + 90% Green tea
B	15% Lemongrass + 85% Green tea
E	20% Lemongrass + 80% Green tea

3.7 Sensory Evaluation of Lemongrass incorporated Green Tea

The sensory quality of the lemongrass incorporated green tea samples was assessed using a nine-point hedonic scale to evaluate parameters such as color, aroma, flavor, taste, mouthfeel, and overall acceptability. The hedonic rating method is a widely accepted approach for determining consumer preference and product acceptability (Lawless and Heymann, 2010). Untrained panelists participated in the sensory evaluation to ensure unbiased and representative assessment.

For each evaluation, 3 g of the blended tea sample were brewed with 150 ml of freshly boiled water for 3 min. The brewed tea samples were served in cups under warm conditions at approximately 80–90 °C to maintain consistency. The evaluation room was maintained free from distracting odors, noise, and external light to provide a neutral testing environment. Each panelist was provided with an evaluation sheet (Appendix B) to record sensory observations individually. Potable water was supplied for rinsing between the samples, and conversation among panelists was restricted during evaluation to minimize bias. The sensory ratings were based on a nine-point hedonic scale, where 9 = “like extremely,” 8 = “like very much,” 7 = “like moderately,” 6 = “like slightly,” 5 = “neither like nor dislike,” 4 = “dislike slightly,” 3 = “dislike moderately,” 2 = “dislike,” and 1 = “dislike extremely”. The sensory results were used to determine the most acceptable blend ratio of lemongrass and green tea.

3.8 Statistical Analysis

All experimental data were recorded in triplicate and statistically analyzed using IBM SPSS Statistics version 27. A one-way analysis of variance (ANOVA) was conducted to determine the significant differences among treatments. Mean comparisons were performed using Tukey's HSD test at a 5% significance level ($p < 0.05$). Microsoft Excel was used for graphical representation and data visualization of the results.

Part IV

Result and discussion

Lemongrass *(Cymbopogon citratus)* was processed under controlled drying conditions with varying length to examine the effects of drying temperature and length on phytochemical retention and antioxidant activity. Response Surface Methodology (RSM) was applied to optimize these processing parameters using total phenolic content, total flavonoid content, tannin content, and antioxidant activity as response variables.

The optimized lemongrass powder was subsequently incorporated into green tea *(Camellia sinensis)* at different proportions. The developed formulations were evaluated for phytochemical properties and sensory attributes using a nine-point hedonic scale. The findings are discussed in relation to the influence of processing conditions and formulation levels on functional and sensory quality.

4.1 Analysis of raw materials

The raw materials used in this study, comprising lemongrass and green tea, were analyzed in both raw and dried forms to evaluate their phytochemical characteristics. The analysis focused on key bioactive components, including total phenolic content (TPC), total flavonoid content (TFC), DPPH Radical Scavenging Activity, and Tannin content. This assessment provides baseline information on the effect of drying on the phytochemical composition of the raw materials and supports their selection for subsequent product formulation. The analysis of lemongrass and green tea is given in Table 4.1.

Table 4.1 Analysis of lemongrass and green tea

Parameters	Lemongrass (raw)	Green tea (raw)	Green tea (dried)
TPC (mg GAE/g)	20.29±0.73	18.24±1.20	10.47±0.57
TFC (mg QE/g)	15.02±0.30	10.92±0.36	7.94±0.35
TTC (mg TAE/g)	1.52±0.04	1.29±0.05	1.23±0.04

DPPH Radical Scavenging Activity (% RSA)	55.92±3.01	63.34±0.83	47.95±0.82

The phytochemical composition of lemongrass and green tea in raw and dried forms showed noticeable variations in total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and tannin content. Phenolic compounds are key contributors to antioxidant properties in plant materials, and their levels are known to be influenced by processing conditions such as drying (Singleton *et al.*, 1999).

Higher TPC and TFC values observed in lemongrass and raw green tea compared to dried green tea can be attributed to the degradation and oxidation of heat-sensitive polyphenolic compounds during drying. Similar reductions in phenolic and flavonoid contents due to thermal processing have been reported for green tea and other herbal materials (Chan *et al.*, 2010).

The DPPH radical scavenging activity followed a trend comparable to TPC, indicating a strong relationship between phenolic concentration and antioxidant capacity. The higher antioxidant activity of raw green tea aligns with previous findings highlighting catechins and related polyphenols as major antioxidants in minimally processed green tea (Brand-Williams *et al.*, 1995).

Tannin content showed relatively minor variation among samples, suggesting greater thermal stability compared to other phenolic fractions. This observation is consistent with reports that tannins are less susceptible to moderate heat treatments (Hagerman *et al.*, 1998)

Overall, these results demonstrate that drying significantly affects the phytochemical profile of green tea, while lemongrass retains appreciable bioactive content, supporting their suitability as functional ingredients for product formulation.

4.2 Optimization of length of leaves and drying temperature for TPC, TFC, DPPH scavenging activity and TTC

The response surface methodology (RSM) was employed to investigate how the length of leaves and drying temperature affected TPC, TFC, DPPH scavenging activity and TTC. Table 4.2 contains results from 13 experimental runs that used central composite design

(CCD) and two independent factors: length (cm, A) and drying temperature (ºC, B) and four response TPC, TFC, DPPH scavenging activity and TTC.

Table 4.2 Experimental design for TPC, TFC, DPPH scavenging activity and TTC with the results obtained

	Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 5
Run	A: Length of leaves	B: Drying temperature	Total polyphenol	Total Flavonoids	Dpph Radical Scavenging Activity	Tannin
	cm	C	mg GAE/g	mg QE/g	%	mg TAE/g
1	1	45	6.41	14.75	79.69	0.81
2	3	45	13.62	18.84	80.22	0.83
3	3	45	15.5	20.8	79.56	0.84
4	3	30	14.6	15.9	64.26	0.66
5	1	30	8.56	12.96	65.63	0.69
6	5	30	18.64	14.75	66.98	0.68
7	3	45	15.1	21.84	80.68	0.82
8	3	45	13.41	18.76	79.89	0.79
9	3	45	16.12	20.5	78.26	0.801
10	5	60	14.07	21.75	62.39	0.74
11	5	45	16.79	17.58	79.28	0.77
12	3	60	14.2	23.24	64.25	0.76
13	1	60	7.48	21.48	66.38	0.79

4.3 Effect of length of leaves and drying temperature on total polyphenol of lemongrass

The parameter range was determined using Design-Expert software, and the effect of length of leaves and drying temperature on the total polyphenol content of lemongrass was evaluated. The coefficients of the model and other statistical attributes for total polyphenol content are presented in Appendix. The total polyphenol content of lemongrass ranged from 6.41 to 18.64 mg GAE/g.

A quadratic model was suggested to represent the experimental data. The Model F-value of 22.46 implies that the model is significant. P-values less than 0.0500 indicate significant model terms. In this case, A (length of leaves) and A^2 (quadratic effect of length of leaves) were found to be significant model terms. Factors B (drying temperature), AB (interaction), and B^2 showed no significant effect on total polyphenol content, as their P-values were greater than 0.05. The lack-of-fit F-value of 0.94 indicates that the lack of fit was not significant (P = 0.50) relative to the pure error.

The coefficient of determination (R^2 = 0.9413) indicates that 94.13% of the variability in total polyphenol content was explained by the model. The predicted R^2 value of 0.7156 was in reasonable agreement with the adjusted R^2 value of 0.8994, as the difference between them was less than 0.2, indicating acceptable predictive ability of the model. The coefficient of variation (C.V. = 8.72%) suggests good precision and reliability of the experimental results.

ANOVA for Quadratic model

Response 1: Total polyphenol

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	153.96	5	30.79	22.46	0.0004	significant
A-Length of leaves	121.95	1	121.95	88.97	< 0.0001	
B-Drying temperature	6.10	1	6.10	4.45	0.0728	
AB	3.05	1	3.05	2.22	0.1797	
A^2	20.10	1	20.10	14.66	0.0065	
B^2	0.0291	1	0.0291	0.0212	0.8883	
Residual	9.59	7	1.37			
Lack of Fit	3.96	3	1.32	0.9372	0.5012	not significant
Pure Error	5.63	4	1.41			
Cor Total	163.55	12				

Factor coding is Coded.
Sum of squares is Type III - Partial

The Model F-value of 22.46 implies the model is significant. There is only a 0.04% 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, A^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 0.94 implies the Lack of Fit is not significant relative to the pure error. There is a 50.12% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Fit Statistics

Std. Dev.	1.17	**R^2**	0.9413
Mean	13.42	**Adjusted R^2**	0.8994
C.V. %	8.72	**Predicted R^2**	0.7156
		Adeq Precision	13.8719

The Predicted R^2 of 0.7156 is in reasonable agreement with the Adjusted R^2 of 0.8994; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 13.872 indicates an adequate signal. This model can be used to navigate the design space.

The change in total polyphenol content of lemongrass was represented by the following equation:

Total polyphenol (mg GAE/g) = $1.81+ 7.61A - 0.021B - 0.029AB - 0.674A^2 + 0.00046B^2$

Where A and B represent the actual values of length of leaves (cm) and drying temperature (°C) respectively. From the developed quadratic model, total polyphenol content showed a significant positive linear effect of length of leaves (A) and a significant negative quadratic effect of leaf length (A^2), indicating that polyphenol content increased with leaf length up to an optimum level and decreased thereafter. Drying temperature and its interaction with leaf length showed no significant effect on total polyphenol content at the 95% confidence level.

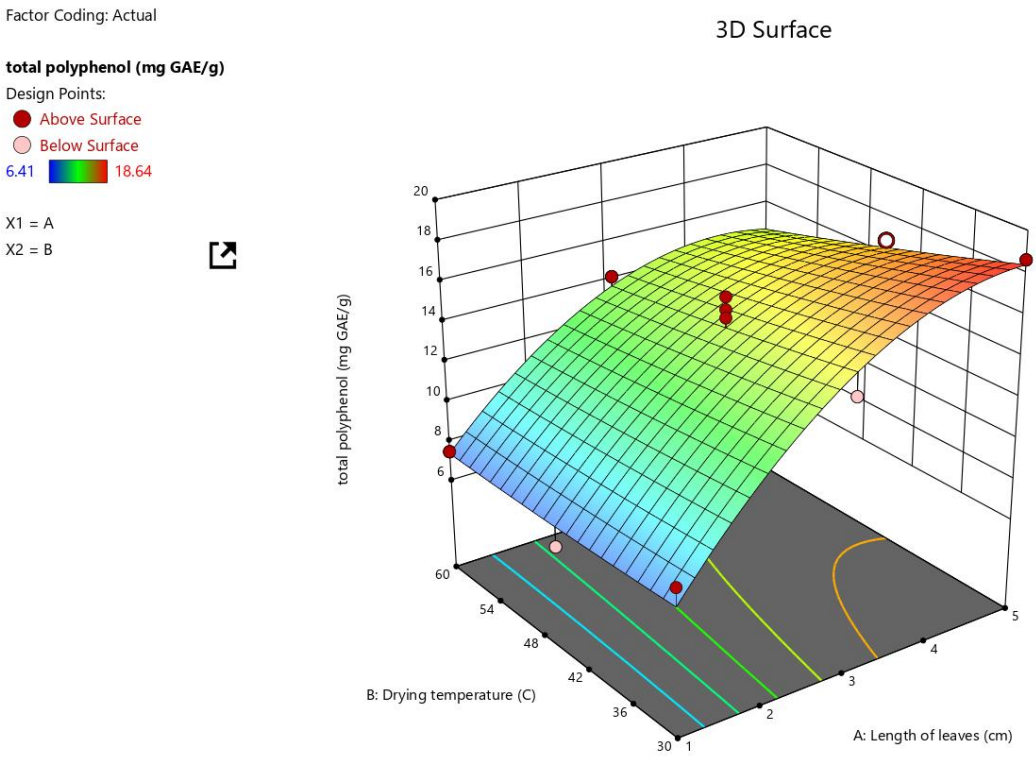


Fig 4.1 Response curve 3D plot for the effect of length of leaves and drying temperature on TPC of lemongrass

4.4 Effect of length of leaves and drying temperature on total flavonoids of lemongrass

The parameter range was determined using Design-Expert software, and the effect of length of leaves and drying temperature on the total flavonoids of lemongrass was evaluated. The coefficients of the model and other statistical attributes for total flavonoids are presented in Appendix. The total flavonoids of lemongrass ranged from 12.96 to 23.24 mg QE/g.

A quadratic model was suggested to describe the experimental data. The Model F-value of 14.41 implies that the model is significant. P-values less than 0.0500 indicate significant model terms. In this case, B (drying temperature) and A^2 (quadratic effect of leaf length) were identified as significant model terms. The linear effect of A (length of leaves), the interaction term AB, and the quadratic effect of B^2 were found to be non-significant ($P > 0.05$), indicating that these factors had no significant influence on total flavonoid content within the studied range. The lack-of-fit F-value of 0.82 indicates that the lack of fit was not significant ($P = 0.55$) relative to the pure error.

The coefficient of determination ($R^2 = 0.9115$) indicates that 91.15% of the variability in total flavonoids was explained by the model. The predicted R^2 value of 0.6607 was in reasonable agreement with the adjusted R^2 value of 0.9115, as the difference between them was less than 0.25, indicating acceptable predictive ability of the model. The coefficient of variation (C.V. = 6.81%) suggests good precision and reliability of the experimental results.

ANOVA for Quadratic model

Response 2: Total Flavonoids

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	117.05	5	23.41	14.41	0.0014 significant
A-Length of leaves	3.99	1	3.99	2.45	0.1612
B-Drying temperature	87.10	1	87.10	53.62	0.0002
AB	0.5776	1	0.5776	0.3556	0.5697
A^2	23.97	1	23.97	14.76	0.0064
B^2	0.5818	1	0.5818	0.3582	0.5684
Residual	11.37	7	1.62		
Lack of Fit	4.32	3	1.44	0.8173	0.5479 not significant
Pure Error	7.05	4	1.76		
Cor Total	128.42	12			

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**

The Model F-value of 14.41 implies the model is significant. There is only a 0.14% 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 B, A^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 0.82 implies the Lack of Fit is not significant relative to the pure error. There is a 54.79% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good we want the model to fit.

Fit Statistics

Std. Dev.	1.27	**R^2**	0.9115
Mean	18.70	**Adjusted R^2**	0.8482
C.V. %	6.81	**Predicted R^2**	0.6607
		Adeq Precision	13.5834

The Predicted R^2 of 0.6607 is in reasonable agreement with the Adjusted R^2 of 0.8482; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 13.583 indicates an adequate signal. This model can be used to navigate the design space.

The change in total flavonoids of lemongrass was represented by the following equation:

Total flavonoids (mg QE/g) = $2.991 + 5.3965A + 0.1084B - 0.0126AB - 0.7365A^2 + 0.00204B^2$

Where A and B represent the actual values of length of leaves (cm) and drying temperature (°C) respectively. From the developed quadratic model, total flavonoid content was found to be significantly influenced by the linear effect of drying temperature (B) and the quadratic effect of leaf length (A^2) at the 95% confidence level. The positive linear coefficient of drying temperature indicates that an increase in drying temperature enhanced flavonoid content, whereas the negative quadratic coefficient of leaf length suggests that flavonoid content increased up to an optimum leaf length and decreased beyond that point. The interaction

between leaf length and drying temperature did not show a significant effect on total flavonoid content.

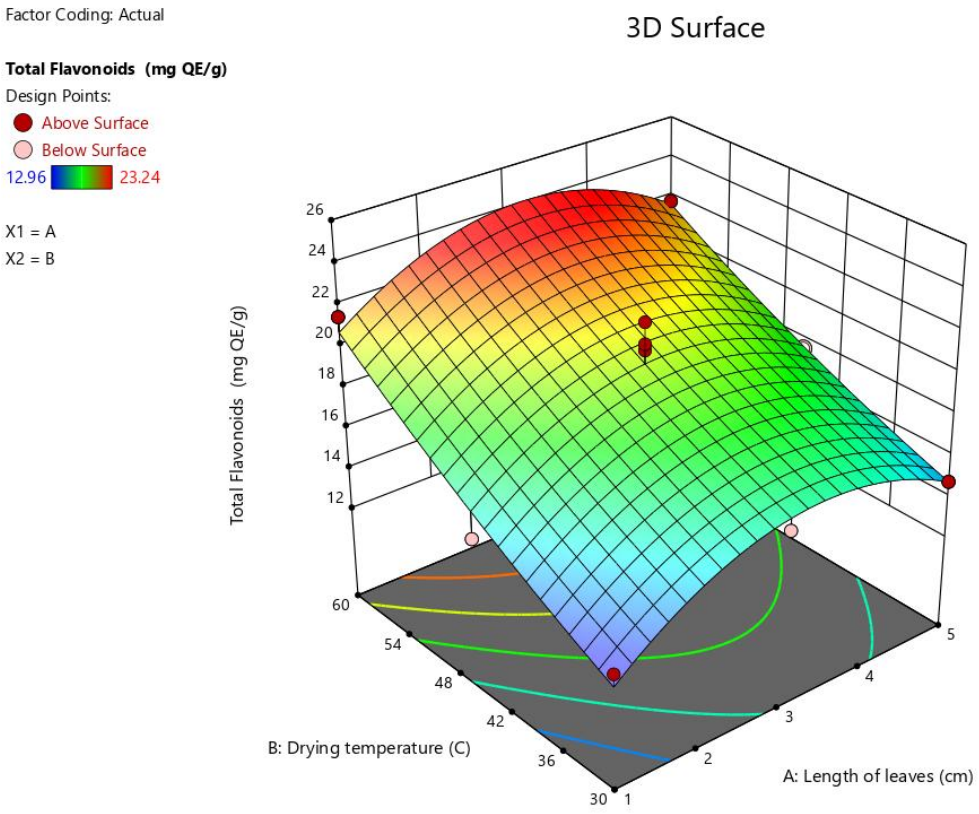


Fig 4.2 Response curve 3D plot for the effect of length of leaves and drying temperature on TFC of lemongrass

4.5 Effect of length of leaves and drying temperature on DPPH radical scavenging activity of lemongrass

The parameter range was determined using Design-Expert software, and the effect of length of leaves and drying temperature on the DPPH radical scavenging activity of lemongrass was evaluated. The coefficients of the model and other statistical attributes for total flavonoids are presented in Appendix. The DPPH radical scavenging activity of lemongrass ranged from 62.39 to 80.28%.

A quadratic model was suggested to describe the experimental data. The Model F-value of 163.25 implies that the model is significant. P-values less than 0.0500 indicate significant model terms. The linear effects of A (length of leaves) and B (drying temperature) were

negative, indicating a slight decrease in DPPH activity with increasing factor levels; however, their 95% confidence intervals included zero, suggesting these linear effects were not significant. The lack-of-fit F-value of 0.9022 indicates that the lack of fit was not significant (P = 0.45) relative to the pure error.

The coefficient of determination ($R^2 = 0.9915$) indicates that 99.15% of the variability in DPPH radical scavenging activity was explained by the model. The predicted R^2 value of 0.9602 was in reasonable agreement with the adjusted R^2 value of 0.9854, as the difference between them was less than 0.024, indicating acceptable predictive ability of the model. The coefficient of variation (C.V. = 1.28%) suggests good precision and reliability of the experimental results.

ANOVA for Quadratic model

Response 3: DPPH Radical Scavenging Activity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	707.14	5	141.43	163.25	< 0.0001	significant
A-Length of leaves	1.55	1	1.55	1.79	0.2228	
B-Drying temperature	2.47	1	2.47	2.85	0.1351	
AB	7.13	1	7.13	8.23	0.0240	
A^2	0.4499	1	0.4499	0.5194	0.4945	
B^2	607.13	1	607.13	700.82	< 0.0001	
Residual	6.06	7	0.8663			

Lack of Fit	2.71	3	0.9022	1.07	0.4542 not significant
Pure Error	3.36	4	0.8394		
Cor Total	713.20	12			

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**

The Model F-value of 163.25 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 AB, B^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 1.07 implies the Lack of Fit is not significant relative to the pure error. There is a 45.42% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Fit Statistics

Std. Dev.	0.9308	**R^2**	0.9915
Mean	72.88	**Adjusted R^2**	0.9854
C.V. %	1.28	**Predicted R^2**	0.9602
		Adeq Precision	28.1812

The Predicted R^2 of 0.9602 is in reasonable agreement with the Adjusted R^2 of 0.9854; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 28.181 indicates an adequate signal. This model can be used to navigate the design space.

The change in DPPH radical scavenging activity of lemongrass can be represented by the following equation:

DPPH radical scavenging activity (%) = -56.310 + 1.1429A + 6.0212B - 0.0445AB + $0.1009A^2$ -$0.06589B^2$

Where A and B represent the actual values of length of leaves (cm) and drying temperature (°C), respectively. From the developed quadratic model, DPPH radical scavenging activity was found to be significantly influenced by the linear effect of drying temperature (B) and the quadratic effects of leaf length (A^2) and drying temperature (B^2) at the 95% confidence level. The positive linear coefficient of drying temperature indicates that an increase in drying temperature enhanced DPPH radical scavenging activity, whereas the positive quadratic coefficient of leaf length suggests that antioxidant activity increased up to an optimum leaf length and then slightly decreased beyond that point. In contrast, the negative quadratic coefficient of drying temperature indicates that excessive temperature reduces DPPH activity after a certain threshold. The interaction between leaf length and drying temperature did not show a significant effect on DPPH radical scavenging activity.

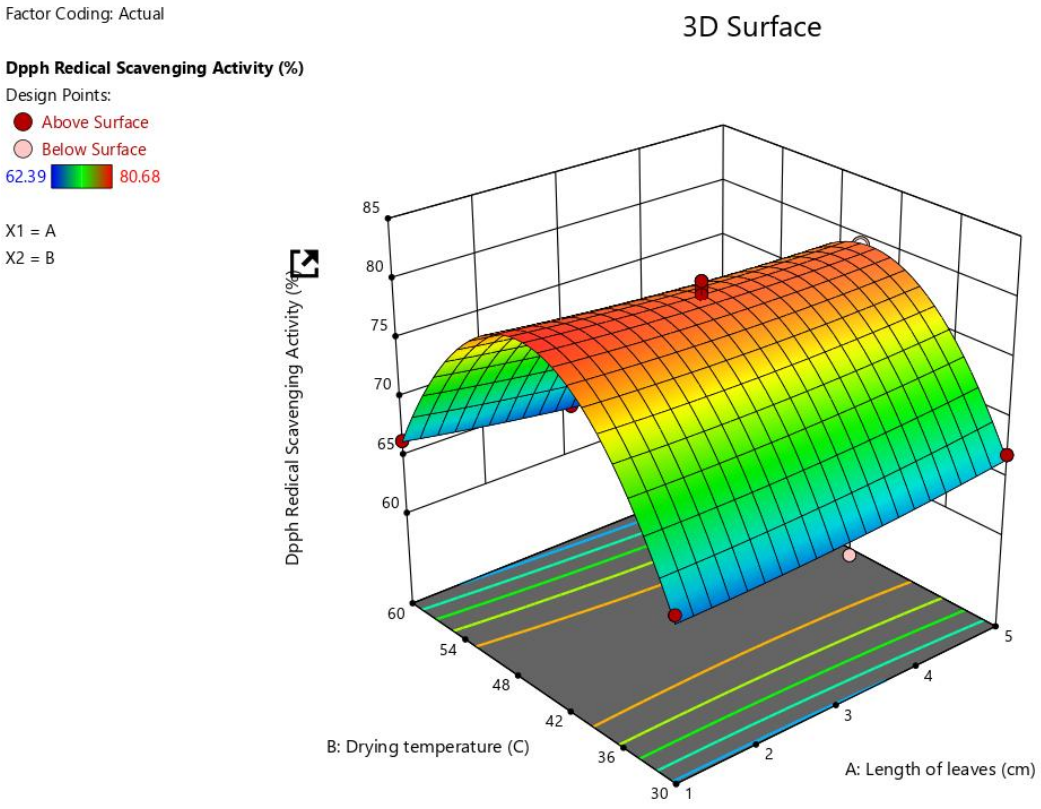


Fig 4.3 Response curve 3D plot for the effect of length of leaves and drying temperature on DPPH radical scavenging activity of lemongrass

4.6 Effect of length of leaves and drying temperature on tannin of lemongrass

The parameter range was determined using Design-Expert software, and the effect of length of leaves and drying temperature on tannin of lemongrass was evaluated. The coefficients of the model and other statistical attributes for tannin are presented in Appendix. The tannin of lemongrass ranged from 0.66 to 0.84 mg TAE/g.

A quadratic model was suggested to describe the experimental data. The Model F-value of 17.98 implies that the model is significant. P-values less than 0.0500 indicate significant model terms. In this case, B (drying temperature) and B^2 (quadratic effect of drying temperature) were identified as significant model terms. The linear effect of A (length of leaves), the interaction term AB, and the quadratic effect of A^2 were found to be non-significant ($P > 0.05$), indicating that these factors had no significant influence on tannin content within the studied range. The lack-of-fit F-value of 1.06 indicates that the lack of fit was not significant ($P = 0.46$) relative to the pure error, confirming that the developed model adequately fits the experimental data.

The coefficient of determination ($R^2 = 0.9278$) indicates that 92.78% of the variability in tannin was explained by the model. The predicted R^2 value of 0.7102 was in reasonable agreement with the adjusted R^2 value of 0.8762, as the difference between them was less than 0.166, indicating acceptable predictive ability of the model. The coefficient of variation (C.V. = 2.71%) suggests good precision and reliability of the experimental results.

ANOVA for Quadratic model

Response 5: Tannin

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.0389	5	0.0078	17.98	0.0007 significant
A-Length of leaves	0.0017	1	0.0017	3.86	0.0904
B-Drying temperature	0.0113	1	0.0113	26.06	0.0014
AB	0.0004	1	0.0004	0.9253	0.3681
A^2	0.0001	1	0.0001	0.2544	0.6295
B^2	0.0206	1	0.0206	47.59	0.0002
Residual	0.0030	7	0.0004		
Lack of Fit	0.0013	3	0.0004	1.06	0.4603 not significant
Pure Error	0.0017	4	0.0004		
Cor Total	0.0419	12			

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**

The Model F-value of 17.98 implies the model is significant. There is only a 0.07% 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 B, B^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 1.06 implies the Lack of Fit is not significant relative to the pure error. There is a 46.03% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good we want the model to fit.

Fit Statistics

Std. Dev.	0.0208	**R^2**	0.9278
Mean	0.7678	**Adjusted R^2**	0.8762
C.V. %	2.71	**Predicted R^2**	0.7102
		Adeq Precision	10.8300

The Predicted R^2 of 0.7102 is in reasonable agreement with the Adjusted R^2 of 0.8762; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 10.830 indicates an adequate signal. This model can be used to navigate the design space.

The change in tannin content of lemongrass can be represented by the following equation:

$$\text{Tannin (mg TAE/g)} = -0.13047 + 0.01613A + 0.0384B - 0.0033AB - 0.00157A^2 - 0.000384B^2$$

Where A and B represent the actual values of length of leaves (cm) and drying temperature (°C), respectively. From the developed quadratic model, tannin content was found to be significantly influenced by the linear and quadratic effects of drying temperature (B and B^2) at the 95% confidence level. The positive linear coefficient of drying temperature indicates that an increase in temperature enhanced tannin content, whereas the negative quadratic coefficient suggests that tannin content decreased beyond an optimum temperature. The effects of leaf length, as well as the interaction between leaf length and drying temperature, were not significant within the studied range.

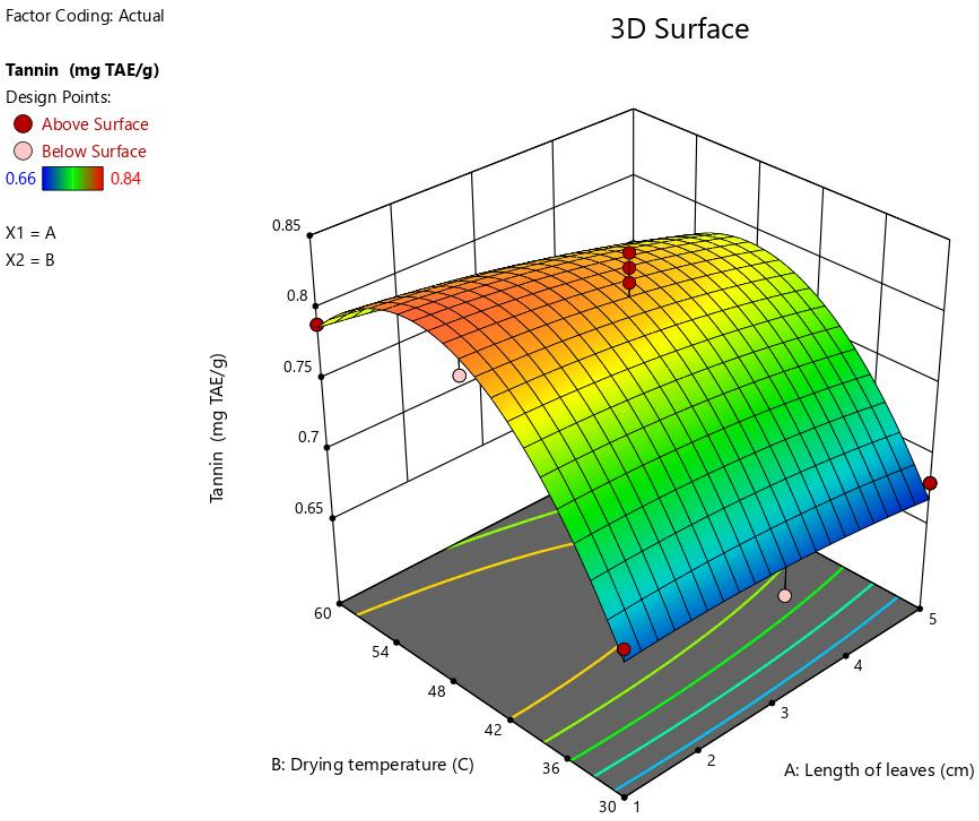


Fig 4.4 Response curve 3D plot for the effect of length of leaves and drying temperature on tannin of lemongrass

4.7 Optimum response condition

Table 4.3 presents the optimized conditions of leaf length and drying temperature for maximizing the phytochemical and antioxidant responses of the formulated tea, as determined through numerical optimization. The recommended processing conditions were a leaf length of 3.79 cm and a drying temperature of 47.52 °C. Under these optimized conditions, the predicted total polyphenol content, total flavonoid content, DPPH radical scavenging activity, and tannin content were 15.76 mg GAE/g, 20.34 mg QE/g, 78.79%, and 0.81%, respectively. The overall desirability value of 0.79 indicates that the selected conditions provide a balanced optimization of all measured responses.

Table 4.3 Parameters for optimization

Parameter	Goal	Lower Limit	Upper Limit
Length of leaves	is in range	1	5
Drying temperature	is in range	30	60
Total polyphenol Content	maximize	6.41	18.64
Total Flavonoid Content	maximize	12.96	23.24
DPPH Radical Scavenging Activity	maximize	62.39	80.68
Tannin Content	is in range	0.66	0.84

4.8 Model Verification

To validate the adequacy of the developed regression models, confirmatory experiments were conducted under the optimized conditions determined by response surface methodology (RSM). The optimized parameters used for validation were a leaf length of 3.8 cm and a drying temperature of 47.5 °C. The predicted values obtained from the Design-Expert software and the experimentally observed responses are presented in Table 4.4.

Table 4.4 Predicted and observed values of the responses

Response	Length of leaves	Drying temperature	Predicted value	Observed value
TPC	3.8	47.5	15.79	16.30
TFC	3.8	47.5	20.36	21.00
TTC	3.8	47.5	0.271	0.28
DPPH radical scavenging activity	3.8	47.5	78.98	80.68

The experimentally observed values were slightly higher than the predicted values; however, the results followed the same trend as expected from the models, indicating a reasonable agreement between predicted and actual responses. This confirms the validity and reliability of the developed regression models. The verification results demonstrate that the RSM approach effectively predicted the effects of leaf length and drying temperature on the phytochemical properties and antioxidant activity of lemongrass and green tea, supporting its use for optimization of processing conditions.

4.9 Sensory evaluation of lemongrass incorporated green tea

Five samples of lemongrass incorporated green tea were prepared with the lemongrass concentration of 0, 5, 10, 15 and 20% using the optimized lemongrass sample. The table for the mean scores for the sensory attributes is given in Table B.1. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

4.9.1 Appearance

The mean sensory scores for appearance of samples C, A, D, B, and E ranged from 6.00 to 7.00 (Fig. 4.5). Sample D recorded the highest mean appearance score (7.00), followed closely by samples B and E (6.75) and sample A (6.50), while sample C showed the lowest mean score (6.00). However, statistical analysis indicated that there were no significant differences among the samples ($p > 0.05$), as all treatments shared the same subscript letter "a". This suggests that variations in concentration did not significantly influence the visual appearance of the product within the tested range. The relatively low standard deviation values further indicate consistent panelist agreement across all samples, confirming that the appearance of the product remained stable despite changes in formulation parameters. These findings demonstrate that the RSM-based optimization conditions maintained acceptable and uniform appearance characteristics.

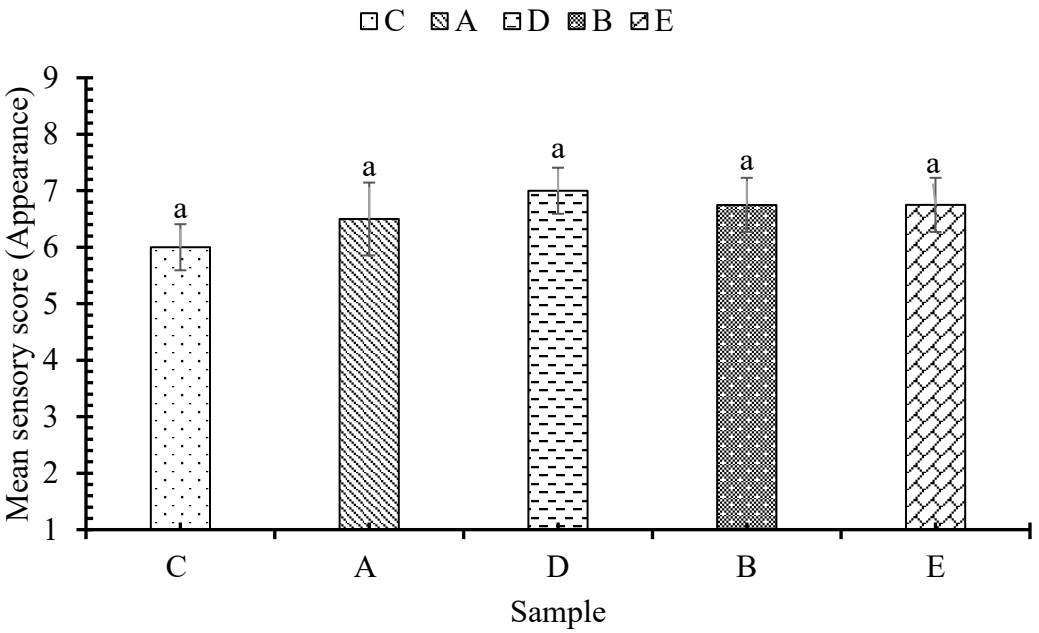


Fig. 4.5 The mean sensory scores for appearance of lemongrass incorporated green tea samples

4.9.2 Taste

The mean sensory scores for taste among the samples ranged from approximately 3.70 to 8.00 (Fig. 4.9). Sample D achieved the highest mean taste score (8.00), followed by sample A (7.00), while sample E recorded a moderate score (4.50). Samples B and C exhibited comparatively lower taste scores of approximately 3.70 and 3.75, respectively. Statistical analysis revealed significant differences among the samples ($p < 0.05$), as indicated by different superscript letters. Sample D (group "c") showed significantly higher taste acceptability compared to samples B and C (group "a"), whereas samples A ("bc") and E ("ab") demonstrated intermediate acceptability. The relatively low standard deviation values suggest reasonable agreement among panelists. Overall, these results indicate that formulation variations significantly influenced taste perception, with sample D showing superior sensory performance compared to the other treatments.

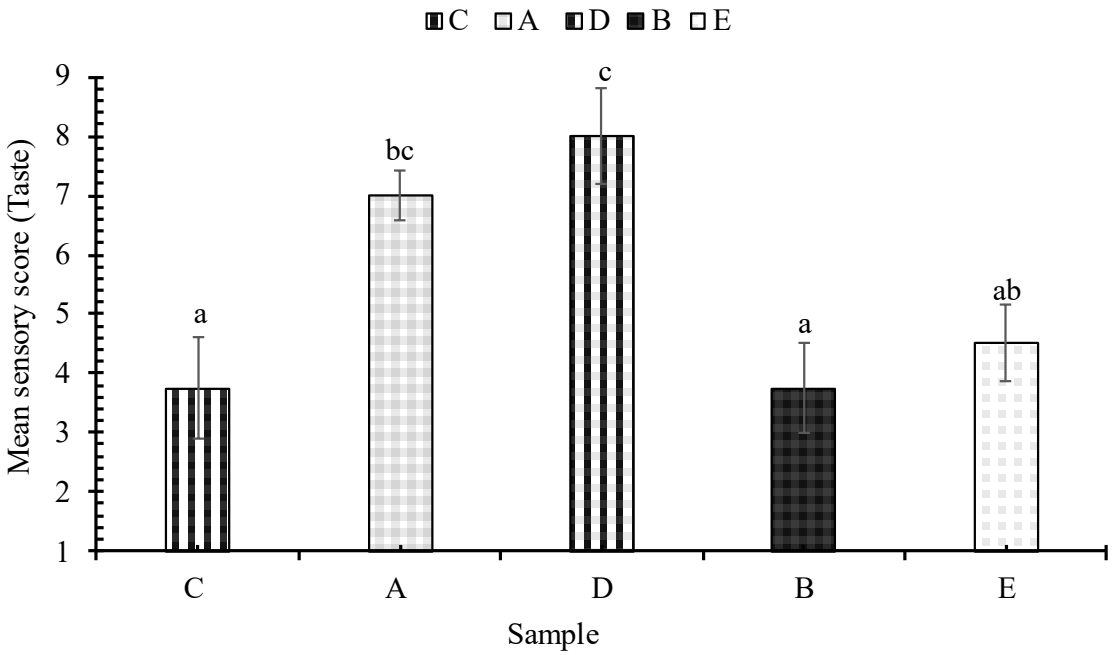


Fig. 4.6 The mean sensory scores for taste of lemongrass incorporated green tea samples

4.9.3 Aroma

The mean sensory scores for aroma of the different samples ranged from 5.25 to 6.75 (Fig. 4.7). Sample D obtained the highest mean aroma score (6.75), followed by sample E (6.25) and sample A (6.00), while samples B and C recorded comparatively lower scores of 5.75 and 5.25, respectively. Despite these numerical differences, statistical analysis showed no significant variation among the samples ($p > 0.05$), as all treatments shared the same group "a". This indicates that changes in formulation conditions did not significantly affect the aroma perception of the product within the experimental range. These results imply that aroma remained relatively stable across different RSM treatments, demonstrating that the optimized processing conditions preserved the aromatic characteristics of the product.

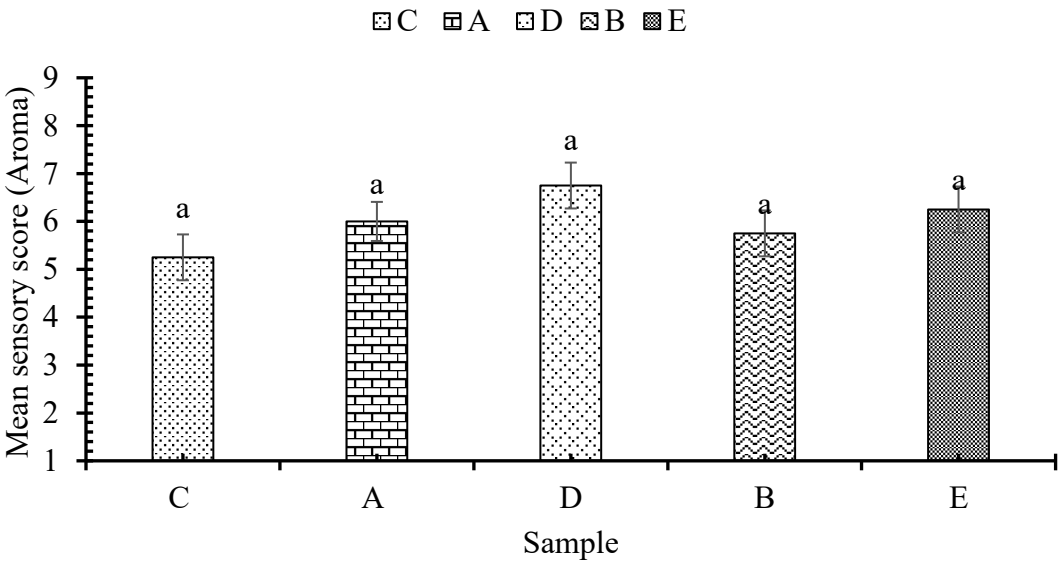


Fig. 4.7 The mean sensory scores for aroma of lemongrass incorporated green tea samples

4.9.4 Mouthfeel

The mean sensory scores for mouthfeel among the samples ranged from 6.00 to 7.00 (Fig. 4.8). Sample B achieved the highest mean mouthfeel score (7.00), followed closely by samples D and E (6.75), while sample A recorded a mean score of 6.25 and sample C showed the lowest value (6.00). Despite these numerical differences, statistical analysis revealed no significant variation among the samples ($p > 0.05$), as all treatments shared the same group "a". This indicates that the changes in formulation conditions did not significantly influence mouthfeel perception within the tested range. The relatively low standard deviation values further demonstrate good consistency among panelist responses. Overall, the results suggest that mouthfeel remained stable across the different RSM treatments, indicating that the optimization variables did not adversely affect the textural properties of the product.

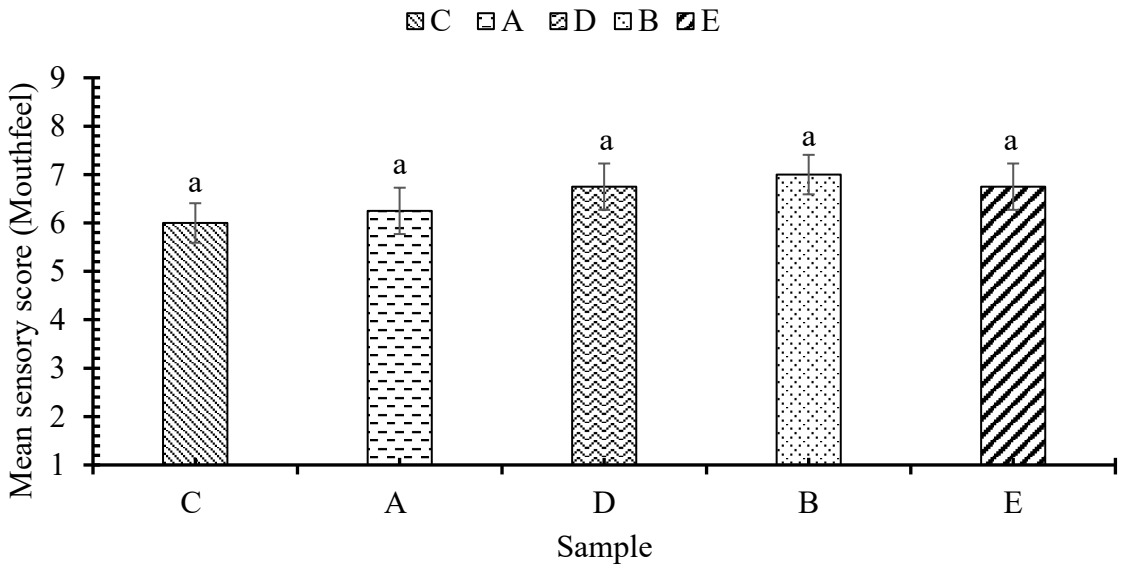


Fig. 4.8 The mean sensory scores for mouthfeel of lemongrass incorporated green tea samples

4.9.5 Overall acceptance

The mean sensory scores for overall acceptability of the samples ranged from 6.00 to 7.00 (Fig. 4.9). Among all formulations, Sample D achieved the highest mean overall acceptability score (7.00), indicating that this formulation was most preferred by the panelists. Samples A and B followed with equal scores of 6.25, while Samples C and E recorded comparatively lower but still acceptable scores of 6.00. Despite these numerical variations, statistical analysis showed no significant differences among the samples ($p > 0.05$), as all treatments shared the group "a". This indicates that changes in formulation conditions did not significantly affect the overall acceptability of the product within the experimental range. The low standard deviation values further suggest good agreement among panelists. Overall, these findings confirm that Sample D represents the most favorable formulation, while all treatments maintained acceptable sensory quality, demonstrating that the RSM-optimized conditions successfully preserved consistent consumer acceptability across samples.

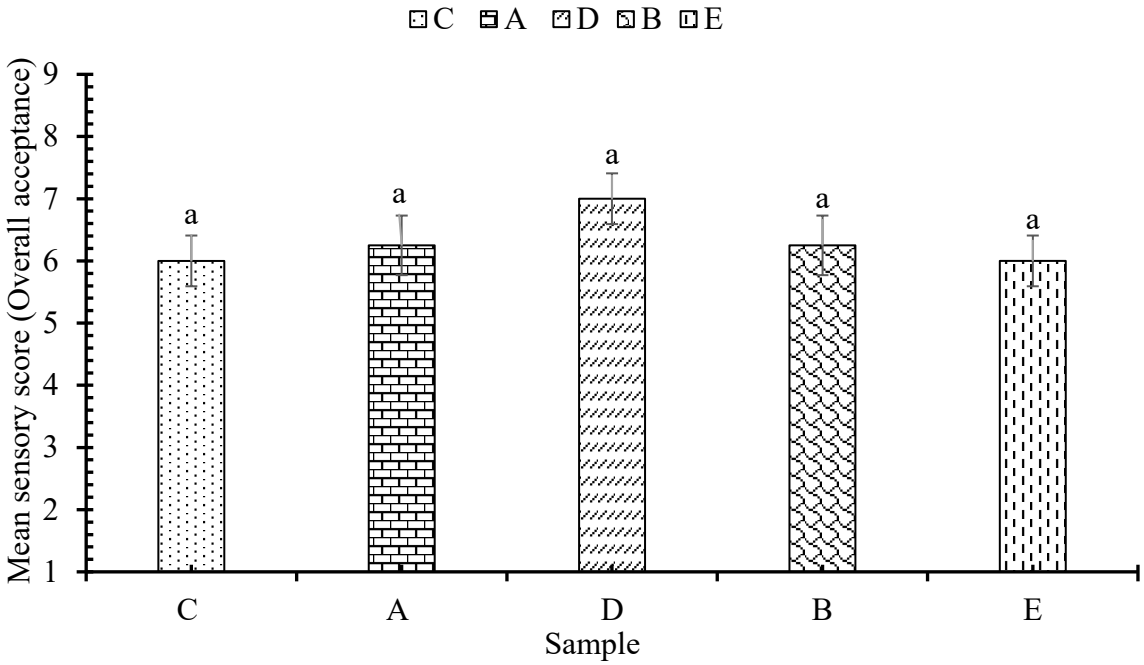


Fig. 4.9 The mean sensory scores for overall acceptance of lemongrass incorporated green tea samples

Part V

Conclusions and recommendations

5.1 Conclusions

This research led to the following conclusions:

1. Drying temperature and length significantly influenced the phytochemical content and antioxidant activity of lemongrass (*Cymbopogon citratus*).
2. Moderate drying conditions were more effective in retaining bioactive compounds compared to higher temperatures and larger length.
3. The optimum condition identified was drying at 47.5°C with a length size of 3.8 cm, which yielded comparatively higher total phenolic content, total flavonoid content, tannin content, and antioxidant activity.
4. Incorporation of optimally dried lemongrass into green tea enhanced the overall phytochemical composition and antioxidant activity of the beverage.
5. Sensory evaluation showed that lemongrass incorporated green tea was generally acceptable, with no significant differences observed in most sensory attributes.
6. Taste exhibited greater variability compared to other sensory parameters, suggesting it to be a more sensitive indicator of formulation changes.

5.2 Recommendations

1. The optimized drying condition (47.5°C and 3.8 cm length size) may be applied in the small-scale production of lemongrass-incorporated green tea to enhance antioxidant properties while maintaining acceptable sensory quality.
2. Moderate drying temperatures are recommended for lemongrass processing to minimize degradation of heat-sensitive phytochemicals while ensuring operational feasibility and consistency in commercial tea formulations.
3. Further investigation on the stability of phytochemicals and sensory attributes during storage is recommended to support product shelf-life evaluation and provide practical information for commercialization.

Part VI

Summary

This study focused on optimizing the drying temperature and length of lemongrass (*Cymbopogon citratus*) to enhance its phytochemical content and antioxidant activity and to evaluate its incorporation into green tea (*Camellia sinensis*) as a functional beverage. The study was conducted in response to growing interest in plant-based antioxidants and the need to preserve bioactive compounds during processing.

Fresh lemongrass leaves were cleaned, cut into different lengths (1, 3, and 5 cm), and dried at varying temperatures (30°C, 45°C, and 60°C). Response Surface Methodology (RSM) using a Central Composite Design was applied to examine the combined effects of drying temperature and particle size on total phenolic content, total flavonoid content, tannin content, and DPPH radical scavenging activity. The optimized lemongrass powder was then incorporated into green tea at different proportions, and the resulting tea blends were evaluated for sensory attributes using a nine-point hedonic scale.

The results indicated that drying temperature and particle size significantly influenced the retention of phytochemicals and antioxidant activity in lemongrass. Moderate drying conditions were found to be more favorable than higher temperatures. The optimum processing condition was identified as drying at 45°C with a particle size of 3 cm, which yielded comparatively higher antioxidant activity and phytochemical content. Incorporation of optimally dried lemongrass into green tea enhanced the functional properties of the beverage while maintaining acceptable sensory quality. Sensory evaluation revealed no significant differences among most attributes, although taste showed greater variability.

The study provides a scientific basis for further research and potential application of lemongrass as a functional ingredient in herbal tea formulations.

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Appendices

Appendix A

Materials, Equipment and Chemicals

1. Materials required

Beakers, Conical flasks, Volumetric Flask, Measuring cylinder, Glass Slides, Coverslips, Petri plates, Inoculating loop, Spatula, Wire gauge, Test tubes, Screwcap tubes, Pipettes, Glass rods, Filter paper, Funnel, etc.

2. Equipments required

Micropipette, Pipette, Microscope, Grinder, Incubator, Refrigerator, Digital Balance, Hot Plate, Cabinet Dryer, Water Bath Shaker, Soxhlet Apparatus, Rotatory Evaporator, Autoclave, Spectrophotometer, Hot air oven.

3. Chemicals required

Methanol, Ethanol, Gallic acid, DPPH, Quercetin, Aluminum chloride, Ascorbic acid, Ferric chloride, Sodium nitrate, Sodium hydroxide, Gram's iodine, Crystal violet, Safranin, DMSO, Conc.H2SO4, Conc. HCl, etc.

Appendix B

Table B.1: ANOVA for quadratic model for total polyphenol

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	153.96	5	30.79	22.46	0.0004 significant
A-Length of leaves	121.95	1	121.95	88.97	< 0.0001
B-Drying temperature	6.10	1	6.10	4.45	0.0728
AB	3.05	1	3.05	2.22	0.1797
A^2	20.10	1	20.10	14.66	0.0065
B^2	0.0291	1	0.0291	0.0212	0.8883
Residual	9.59	7	1.37		
Lack of Fit	3.96	3	1.32	0.9372	0.5012 not significant
Pure Error	5.63	4	1.41		
Cor Total	163.55	12			

Table B.2: ANOVA for quadratic model for total flavonoids

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	117.05	5	23.41	14.41	0.0014	significant
A-Length of leaves	3.99	1	3.99	2.45	0.1612	
B-Drying temperature	87.10	1	87.10	53.62	0.0002	
AB	0.5776	1	0.5776	0.3556	0.5697	
A^2	23.97	1	23.97	14.76	0.0064	
B^2	0.5818	1	0.5818	0.3582	0.5684	
Residual	11.37	7	1.62			
Lack of Fit	4.32	3	1.44	0.8173	0.5479	not significant
Pure Error	7.05	4	1.76			
Cor Total	128.42	12				

Table B.3: ANOVA for quadratic model for DPPH radical scavenging

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	707.14	5	141.43	163.25	< 0.0001	significant
A-Length of leaves	1.55	1	1.55	1.79	0.2228	
B-Drying temperature	2.47	1	2.47	2.85	0.1351	
AB	7.13	1	7.13	8.23	0.0240	
A^2	0.4499	1	0.4499	0.5194	0.4945	
B^2	607.13	1	607.13	700.82	< 0.0001	
Residual	6.06	7	0.8663			
Lack of Fit	2.71	3	0.9022	1.07	0.4542	not significant
Pure Error	3.36	4	0.8394			
Cor Total	713.20	12				

Table B.4: ANOVA for quadratic model for tannin

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0389	5	0.0078	17.98	0.0007	significant
A-Length of leaves	0.0017	1	0.0017	3.86	0.0904	
B-Drying temperature	0.0113	1	0.0113	26.06	0.0014	
AB	0.0004	1	0.0004	0.9253	0.3681	
A^2	0.0001	1	0.0001	0.2544	0.6295	
B^2	0.0206	1	0.0206	47.59	0.0002	
Residual	0.0030	7	0.0004			
Lack of Fit	0.0013	3	0.0004	1.06	0.4603	not significant
Pure Error	0.0017	4	0.0004			
Cor Total	0.0419	12				

Appendix C

Sensory Analysis Score Card

Hedonic rating test

Name of the panelist: ……. Date:

Product name: Lemongrass incorporated green tea

Dear panelist, you are provided with 6 samples of lemongrass incorporated green tea. Please, test the following samples of lemongrass incorporated green tea and check how much you prefer for each of the samples. Give the point for your degree of preference for each sample as shown below:

Sample	Appearance	Taste	Aroma	Mouthfeel	Overall Acceptance
A					
B					
C					
D					
E					

Judge the characteristics on the 1-9 scale as below:

9. Like extremely	6. Like slightly	3. Dislike moderately
8. Like very much	5. Neither like nor dislike	2. Dislike very much
7. Like moderately	4. Dislike slightly	1. Dislike extremely

Any comments:

Signature

APPENDIX D

Table D.1 Table for homogeneity of variances of sensory

Test of Homogeneity of Variances

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Appearance	Based on Mean	.656	4	15	.632
	Based on Median	.583	4	15	.679
	Based on Median and with adjusted df	.583	4	14.727	.680
	Based on trimmed mean	.655	4	15	.632
Taste	Based on Mean	1.438	4	15	.270
	Based on Median	1.327	4	15	.305
	Based on Median and with adjusted df	1.327	4	11.393	.318
	Based on trimmed mean	1.436	4	15	.270
Aroma	Based on Mean	.250	4	15	.905
	Based on Median	.187	4	15	.941
	Based on Median and with adjusted df	.187	4	14.769	.941
	Based on trimmed mean	.249	4	15	.906
Mouthfeel	Based on Mean	.321	4	15	.859

	Based on Median	.265	4	15	.896
	Based on Median and with adjusted df	.265	4	14.695	.896
	Based on trimmed mean	.321	4	15	.860
Overall acceptance	Based on Mean	.281	4	15	.886
	Based on Median	.250	4	15	.905
	Based on Median and with adjusted df	.250	4	14.727	.905
	Based on trimmed mean	.281	4	15	.886

Table D.2 ANOVA table for sensory analysis

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	2.300	4	.575	.595	.672
	Within Groups	14.500	15	.967		
	Total	16.800	19			
Taste	Between Groups	30.300	4	7.575	3.496	.033
	Within Groups	32.500	15	2.167		
	Total	62.800	19			
Aroma	Between Groups	5.000	4	1.250	1.442	.269

	Within Groups	13.000	15	.867		
	Total	18.000	19			
Mouthfeel	Between Groups	2.700	4	.675	.827	.529
	Within Groups	12.250	15	.817		
	Total	14.950	19			
Overall acceptance	Between Groups	2.700	4	.675	.880	.499
	Within Groups	11.500	15	.767		
	Total	14.200	19			

APPENDIX E

E.1 Gallic acid standard curve

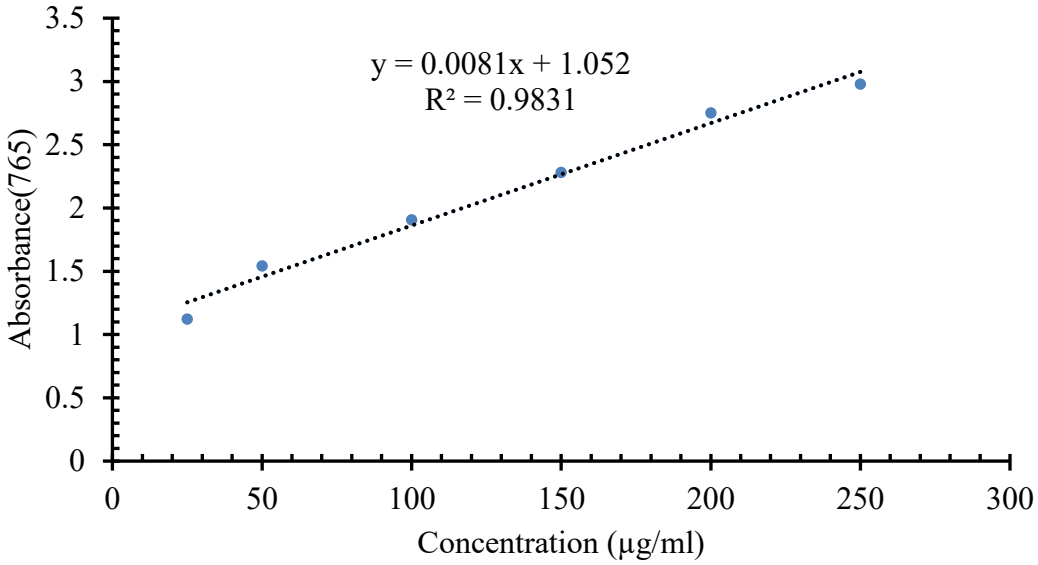

Fig E.1 Standard gallic acid curve

E.2 Standard Quercetic curve

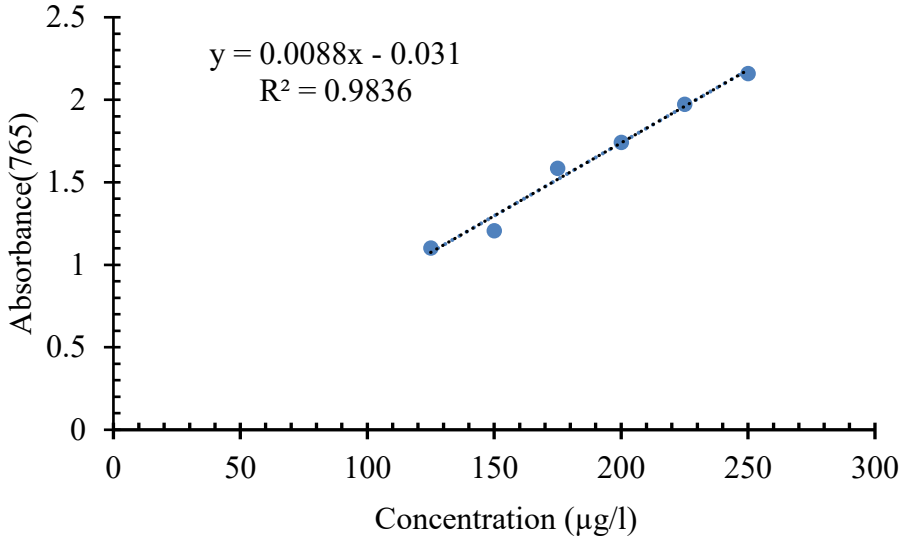

Fig E.2 Standard Quercetic curve

E.3 Ascorbic acid standard curve

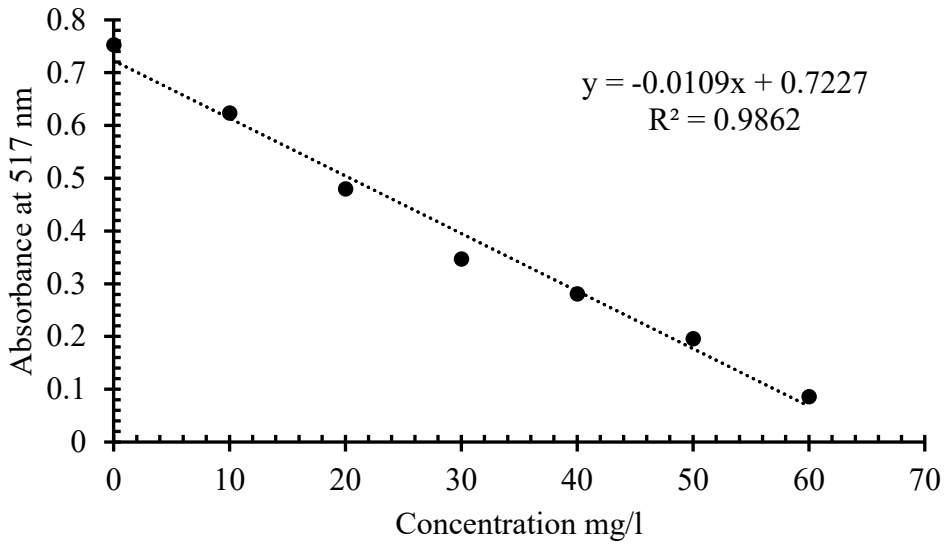

Fig E.3 Standard ascorbic standard curve

Color Plates

Plate 1 Dried lemongrass and green tea sample

Plate 2 Phytochemicals analysis

Plate 3 Sensory analysis of sample

Plate 4 Infusion of samples