

# THE COMPARISON OF EPIGALLOCATECHIN GALLATE LEVELS IN MATCHA POWDERS IN CEREMONIAL, PREMIUM AND CULINARY GRADE BY HPLC METHOD

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MASTER OF SCIENCE

IN

ANTI-AGING AND REGENERATIVE SCIENCE

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# THE COMPARISON OF EPIGALLOCATECHIN GALLATE LEVELS IN MATCHA POWDERS IN CEREMONIAL, PREMIUM AND CULINARY GRADE BY HPLC METHOD



# THIS INDEPENDENT STUDY IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

IN

ANTI-AGING AND REGENERATIVE SCIENCE

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# INDEPENDENT STUDY APPROVAL MAE FAH LUANG UNIVERSITY FOR

# MASTER OF SCIENCE IN ANTI-AGING AND REGENERATIVE SCIENCE

Independent Study Title: The Comparison of Epigallocatechin Gallate Levels in

Matcha Powders in Ceremonial, Premium and Culinary

Grade by HPLC Method

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#### **ACKNOWLEDGEMENTS**

I would like to express my sincere gratitude to Mae Fah Luang University for the opportunity to pursue my Master's degree in Anti-Aging and Regenerative Science, and for providing a supportive academic environment throughout my studies.

I am deeply thankful to my advisor, Associate Professor Dr. Phakkharawat Sittiprapaporn, for his continuous guidance, expertise, and encouragement throughout the research process. His insight and mentorship were invaluable to the success of this study.

My sincere appreciation also goes to the members of my examination committee, Dr. Ariya Sarikaphuti and Associate Professor Dr. Wongdyan Pandii. Thank you for your time, critical feedback, and valuable suggestions, which have strengthened the quality of this work. I would also like to extend my heartfelt thanks to Miss. Wanpen Kanthathong for her technical support and expertise in laboratory procedures, particularly during the HPLC analysis phase of the study.

Lastly, I am deeply grateful to my family for their unwavering love, support, and encouragement throughout this journey.

Ravinda Landau

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Culinary Grade by HPLC Method

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**Degree** Master of Science (Anti-Aging and Regenerative Science)

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#### **ABSTRACT**

This study compared EGCG content across ceremonial, premium, and culinary-grade matcha powders from Uji, Shizuoka, and Kagoshima using high-performance liquid chromatography (HPLC). A total of 27 samples were analyzed, and results showed that premium-grade matcha had the highest average EGCG concentration (19.28 mg/g), followed by culinary (13.60 mg/g) and ceremonial (8.19 mg/g). However, statistical analysis revealed no significant differences between the grades. Contrary to popular belief, ceremonial-grade matcha did not consistently contain the highest EGCG levels. These findings suggest that matcha grade and price are not reliable indicators of antioxidant potency. Premium-grade matcha may offer better value for those seeking health benefits associated with EGCG. Further studies are recommended to explore additional bioactive compounds and a larger sample size.

**Keywords:** Matcha, Ceremonial Grade, Premium Grade, Culinary Grade, EGCG, Catechin, Antioxidant, HPLC

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#### **CHAPTER 1**

#### INTRODUCTION

## 1.1 Background

Tea is arguably the most widely consumed beverage globally, second only to water (Jakubczyk et al., 2020). In recent years, there has been growing interest in exploring the connection between diet and health. Consequently, numerous studies have been conducted to examine the relationship between green tea consumption and its impact on health (Weiss & Anderton, 2003). Matcha, a finely ground powder made from specially grown and processed green tea leaves (*Camellia sinensis*), has captured global attention for its distinct flavor, vibrant green hue, and numerous health benefits (Pastoriza et al., 2017). With roots deeply connected to Japanese culture, matcha has been a cornerstone of traditional tea ceremonies since the 12th century (Wolf et al., 2008). In recent years, its popularity has grown due to its recognition as a superfood and its versatility in both traditional and contemporary culinary applications.

The health benefits of matcha are largely attributed to its rich content of catechins, particularly epigallocatechin gallate (EGCG), a potent antioxidant (Farooq & Sehgal, 2018). These catechins are known to reduce inflammation, fight cancer, and support cardiovascular health, making matcha a desirable addition to health-conscious diets worldwide (Phuah et al., 2023). Additionally, matcha contains other bioactive compounds, including L-theanine, which enhances relaxation and cognitive focus, further solidifying its status as a holistic wellness beverage (Devi & Bhasin, 2023).

However, not all matcha is created equal. The quality and grade of matcha vary significantly, influencing its taste, color, and nutritional value (Meyer et al., 2023). The market categorizes matcha into grades, with ceremonial-grade matcha often commanding significantly higher prices than culinary-grade versions. Ceremonial-grade matcha is characterized by its vibrant color, smooth texture, and umami flavor, attributed to meticulous cultivation and processing methods. Key

factors affecting matcha grades include the cultivation process, shading duration, leaf selection, and grinding method. Higher-grade ceremonial matcha is cultivated with meticulous care, involving extended shading to enhance chlorophyll and amino acid content, which contributes to its vibrant green hue and umami-rich flavor (Koláčková et al., 2019).

In contrast, culinary grades, while still nutritious, often have a more robust taste and are better suited for cooking and blending with other ingredients. The differences in quality not only impact its sensory attributes but also its cost and application, making it essential to understand what constitutes premium matcha. This raises a critical question: does the highest-grade ceremonial matcha offer a higher catechin content and, by extension, more health benefits than its lower-grade culinary counterpart?

This independent study seeks to investigate whether the premium price of ceremonial-grade matcha is justified by a greater concentration of catechins, particularly epigallocatechin gallate (EGCG), compared to culinary-grade matcha. By examining the EGCG content across different matcha grades, this research aims to provide clarity on whether consumers gain superior health benefits from investing in higher-grade matcha or if lower-grade options deliver comparable nutritional value.

# 1.2 The Importance of Research

The importance of this research is to provide a clearer understanding of whether high-priced matcha contains significant EGCG content, leading to more health benefits compared to low grades, and whether price is a reliable indicator of matcha's nutritional value.

# 1.3 Research Objective

To investigate and compare the EGCG content across different grades of matcha powder: ceremonial, premium, and culinary.

## 1.4 Research Hypothesis

Hypothesis: Ceremonial-grade matcha has significantly higher EGCG content, leading to more pronounced health benefits compared to premium and culinary-grade matcha.

## 1.5 Scope of Research

The scope of this independent study focuses on evaluating the EGCG content in different grades of matcha powder to determine if higher-grade, more expensive matcha contains more catechins than lower-grade, cheaper matcha. The study will analyze the relationship between matcha's price and its potential health benefits, primarily by the concentration of the catechin epigallocatechin gallate, or EGCG.

Key Elements Within the Scope Include

#### 1.5.1 Matcha Grades

- 1.5.1.1 Ceremonial-grade matcha (high-quality, expensive).
- 1.5.1.2 Premium-grade matcha (mid-range, cafe quality).
- 1.5.1.3 Culinary-grade matcha (lower-quality, more affordable).

#### 1.5.2 Primary Focus

- 1.5.2.1 Catechin Content: The main catechin, EGCG, will be analyzed across the different matcha grades.
- 1.5.2.2 Comparison of EGCG Levels: Differences in EGCG concentrations between expensive ceremonial, premium, and culinary-grade matcha.

#### 1.5.3 Methodology

- 1.5.3.1 Laboratory analysis of catechin content using high-performance liquid chromatography (HPLC).
- 1.5.3.2 Comparisons between different brands and grades of matcha to ensure a wide range of samples.

#### 1.5.4 Factors Affecting Catechin Content

- 1.5.4.1 Shading practices.
- 1.5.4.2 Harvesting periods.

#### 1.5.4.3 Processing methods.

#### 1.5.5 Outcome

- 1.5.5.1 Determine if price correlates with catechin content: The study aims to answer whether more expensive matcha contains more catechin concentrations, which increases health benefits compared to cheaper alternatives.
- 1.5.5.2 Practical Recommendations: Offering insights for consumers on whether investing in higher-grade matcha is justified based on its catechin content and health benefits.

#### 1.5.6 Limitations

- 1.5.6.1 The study will focus exclusively on the catechin EGCG as the primary marker of health benefits and will not cover other compounds like caffeine, L-theanine, or vitamins.
- 1.5.6.2 The study will not account for other potential subjective factors, such as taste or texture differences between matcha grades.

# 1.6 Conceptual Framework

The conceptual framework provides the underlying structure for understanding the relationship between different grades of matcha powder and their EGCG content, specifically addressing the research question: Does the highest-grade ceremonial matcha have more catechin content than the lower-grade culinary matcha?

#### 1.6.1 Independent Variable (IV)

The grade of matcha refers to the quality of the tea powder, which is categorized based on factors such as cultivation techniques, leaf selection, and processing methods. The grades can be broadly divided into ceremonial-grade matcha, premium, and culinary-grade matcha.

#### 1.6.2 Dependent Variable (DV)

Catechin content refers to the concentration of the primary catechins found in matcha, such as EGCG, ECG, EGC, and EC. This study will focus on EGCG.

#### 1.6.3 Moderating Variables

Moderating variables include the cultivation method, harvesting period, and processing techniques, which impact the grade and amount of catechins in the matcha powder.

#### 1.6.4 Mediating Variable

The catechin concentration, particularly of EGCG, is expected to mediate the relationship between the grade of matcha and its health benefits. Higher concentrations of catechins lead to stronger antioxidant, anti-inflammatory, and anti-cancer properties.

# 1.7 Framework Diagram

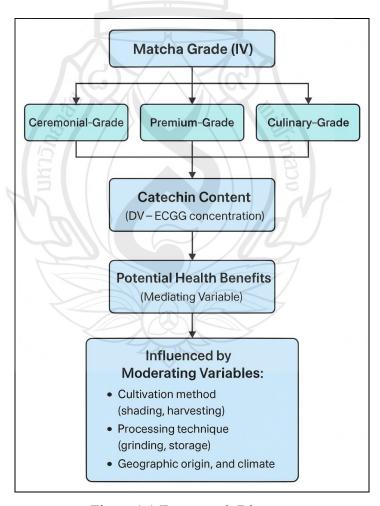


Figure 1.1 Framework Diagram

#### 1.8 Definitions

#### 1.8.1 Matcha

A finely powdered green tea made from specially grown and processed leaves of the *Camellia sinensis* plant. Unlike other teas, matcha is unfermented and consumed whole, maximizing its nutritional benefits.

#### 1.8.2 Ceremonial-Grade Matcha

The highest quality matcha is made from the youngest and most tender leaves, harvested in the first round. It is known for its vibrant green color, smooth texture, and high concentrations of beneficial compounds.

#### 1.8.3 Premium-Grade Matcha

A middle-grade matcha powder made from first to second harvest leaves. It is typically used in cafes for drinks such as lattes and daily consumption.

#### 1.8.4 Culinary-Grade Matcha

A lower-grade matcha made from more mature leaves. It has a coarser texture and a more bitter flavor than higher-grade matcha powder. Typically used as an additive in cooking and baking matcha desserts.

#### 1.8.5 Catechins

A group of polyphenolic compounds found predominantly in tea, known for their potent antioxidant properties. The primary catechins in matcha are:

EGCG: Epigallocatechin gallate

EGC: Epigallocatechin

ECG: Epicatechin gallate

EC: Epicatechin

#### 1.8.6 Epigallocatechin Gallate (EGCG)

The most potent and abundant catechin in matcha is known for its powerful antioxidant, anti-inflammatory, and anti-cancer properties. EGCG is a primary indicator of matcha's health benefits.

#### 1.8.7 Polyphenols

A category of naturally occurring compounds found in plants, including catechins, that act as antioxidants and provide various health benefits such as anti-inflammatory and anti-cancer properties.

#### 1.8.8 Antioxidants

Molecules that inhibit the oxidation of other molecules, thereby preventing cellular damage caused by free radicals.

#### 1.8.9 Oxidative Stress

An imbalance between free radicals and antioxidants in the body, which can lead to cell and tissue damage. Catechins in matcha help reduce oxidative stress by neutralizing free radicals.

#### **1.8.10 Shading**

A cultivation technique where matcha plants are covered with shade nets or bamboo mats for about 3–4 weeks before harvest. This process increases the chlorophyll and L-theanine content while reducing catechin bitterness.

#### 1.8.11 High-Performance Liquid Chromatography (HPLC)

A laboratory technique used to separate, identify, and quantify individual components, such as catechins, in a mixture. HPLC will be used in this study to measure EGCG levels in different grades of matcha.

#### **CHAPTER 2**

#### LITERATURE REVIEW

Matcha, made from shade-grown leaves and processed into a fine powder, has gained global fame for both ceremonial and culinary uses. Its complex flavor profile is attributed to its unique amino acid and catechin composition (Horie et al., 2017). Research by Meyer et al. (2023) reveals significant differences in chemical composition and antioxidant properties among these green tea types. A study by Koláčková et al. (2019) also offers an in-depth analysis of matcha's nutritional composition, phenolic content, antioxidant properties, and digestibility. This independent study will focus on the EGCG content of various grades of matcha. To ensure a comprehensive understanding and to gather all relevant and essential aspects for this study, the literature review has been organized into five key topics: cultivation and processing of matcha powder, chemical composition of matcha, antioxidant activity of matcha, health benefits of EGCG in matcha, and analysis methods of chemical compounds.

# 2.1 Cultivation and Processing of Matcha Powder

The grade of matcha is typically categorized into ceremonial, premium, and culinary grades and is determined by several factors that affect its quality, flavor, nutritional content, and price (Devi & Bhasin, 2023). These factors include the cultivation process, harvest timing, leaf selection, and processing techniques (Fujioka et al., 2016). Matcha plants are grown under shade for the final few weeks before harvest. This shading process plays a significant role in determining the matcha's grade. Ceremonial-grade matcha is usually shaded for a longer period (up to 3-4 weeks), which increases the levels of chlorophyll and amino acids, particularly L-theanine, enhancing its umami flavor and reducing bitterness. Culinary-grade matcha is often shaded for a shorter time, leading to lower concentrations of these beneficial compounds and a more bitter taste (Ku et al., 2010). The reduced sunlight during

shading slows the production of catechins that give green tea its bitter flavor. While shading enhances the levels of key compounds like EGCG and L-theanine in ceremonial-grade matcha, it simultaneously limits the synthesis of more bitter catechins, resulting in a smoother, more refined taste (Phuah et al., 2023).

#### 2.1.1 Leaf Selection and Harvesting

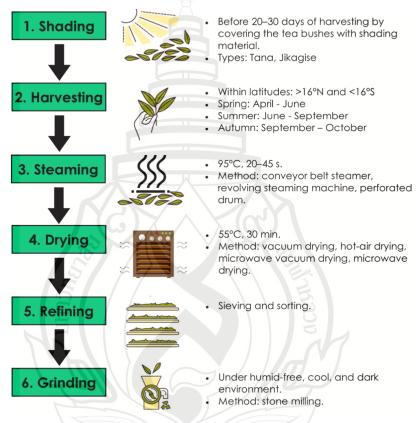
The highest quality matcha is made from the youngest, most tender leaves, which are found at the top of the tea plant (Jakubczyk et al., 2020). Ceremonial-grade matcha is typically made from these tender leaves, which contribute to its vibrant color, smoother texture, and rich umami flavor. Culinary-grade matcha, on the other hand, is usually made from more mature leaves that are lower on the plant (Sano et al., 2018). These older leaves contain more fibrous material, resulting in a coarser texture, less vibrant color, and more bitter taste. The reduced chlorophyll content and higher levels of catechins like epicatechin (EC) in culinary-grade matcha contribute to its stronger, more astringent flavor (Phuah et al., 2023).

#### 2.1.2 Processing and Grinding

The method used to grind the tea leaves into matcha powder also affects the grade. Ceremonial-grade matcha is ground slowly using traditional stone mills, which preserve the delicate flavor and nutritional profile of the tea (Fujioka et al., 2016). The fine grinding process results in a silky smooth powder with a vibrant green color and rich flavor. Culinary-grade matcha may be ground using faster, industrialized methods that generate heat, potentially degrading the taste and quality of the final product. After harvesting, ceremonial-grade matcha undergoes careful processing to remove stems and veins from the tea leaves, leaving behind only the tender leaf matter. This ensures a smoother texture and better taste. Culinary-grade matcha may include stems and veins, contributing to a grittier texture and a less refined taste (Phuah et al., 2023).

The production of high-quality green tea, including matcha and powdered tea, heavily relies on shading cultivation practices to enhance specific morphological and chemical traits of tea plants, *Camellia sinensis* (Sano et al., 2018). A comprehensive study by Sano et al. (2018) assessed the impact of different shading intensities on the structural and chemical properties of new tea shoots, which are crucial for product quality and productivity. The findings revealed that higher shading levels significantly affected the chemical composition of tea shoots, resulting in reduced levels of gallate

catechins, such as epigallocatechin gallate (EGCG), while increasing theanine and caffeine content (Sano et al., 2018). Theanine, an important amino acid responsible for the umami flavor in tea, showed substantial increases under high shading conditions, thereby enhancing the flavor profile. The study also utilized principal component analysis (PCA) to explain the relationships between shading intensity, morphological traits, and chemical components, emphasizing the interconnectedness of chlorophyll content, leaf mass area (LMA), and catechin levels (Sano et al., 2018).



Source Phuah et al. (2023)

Figure 2.1 Matcha Processing Steps and Descriptions

In a study by Fujioka et al. (2016), the differences between leaf-based and powdered green tea were evaluated, focusing on catechin concentration, antioxidant activity, and inhibition of reactive oxygen species (ROS). The researchers found that the powdering process significantly enhanced catechin extraction, with the powdered form yielding over three times the concentration of EGCG compared to traditionally steeped leaf tea (Fujioka et al., 2016).

Additionally, a study by Lee et al. (2014) investigated the effects of different plucking periods on the major constituents of green tea and their antioxidant activity. The analysis demonstrated that the plucking period significantly influences the composition of bioactive compounds (Fujioka et al., 2016). The content of constituents such as theanine, theobromine, and caffeine decreased as the plucking season progressed, while certain catechins like epicatechin (EC), epigallocatechin gallate (EGCG), and epigallocatechin (EGC) increased in older leaves (Fujioka et al., 2016).

# 2.2 Chemical Composition of Green Tea and Matcha

Matcha tea contains high levels of antioxidant substances such as flavonoids, polyphenols, and vitamin C, as well as antioxidant potential, which is determined by when the plant is harvested as well as the temperature used to prepare infusions (Jakubczyk et al., 2020). Accounting for up to 35% of the dry weight of green tea are polyphenols, which include flavonols, flavones, and flavan-3-ols. The commonly known flavan-3-ols, which account for 60-80%, are catechins (Rains et al., 2011). Matcha contains four main catechins, i.e., (–)- epicatechin-3-gallate (ECG), (–)-epigallocatechin-3-gallate (EGCG) and (–)- epigallocatechin (EGC), of which (–)-epigallocatechin-3-gallate (EGCG) is the most active and is present in abundance and matcha is their best source (Bhandari & Sharma, 2019).

The chemical composition of green tea significantly influences its sensory properties and quality. In matcha, the shading process reduces catechin levels, enhancing flavor while amino acids like theanine contribute to its umami taste and are more abundant in ceremonial-grade matcha than in industrial varieties or powdered green teas (Horie et al., 2017). Chlorophyll, which gives matcha its vibrant color, is prevalent in high-quality matcha but can diminish with excessive shading. Additionally, caffeine content varies with leaf quality and production methods. A study by Horie et al. (2017) compares ceremonial-grade matcha, industrial-grade matcha, and powdered green tea by analyzing chemical components such as amino acids and chlorophyll. The research identifies key markers for high-quality

ceremonial-grade matcha, including theanine content over 1.8 g per 100 g dry weight and a specific EGCG to EGC ratio (Horie et al., 2017).

**Source** Thepparkorn and Wongsakil (2012)

Figure 2.2 Chemical Structures of Catechin Compounds in Matcha

However, the study notes challenges in differentiating industrial-grade matcha from powdered green tea based solely on these metrics. Factors like cultivation practices and plucking methods contribute to the variability in chemical composition. High-grade matcha is made from first-crop, hand-plucked leaves, while lower-grade teas often stem from non-shaded or mechanically plucked sources (Horie et al., 2017). The study emphasizes the need for further research into physical properties that can help distinguish tea grades and addresses concerns over potential mislabeling in the market.

A recent study by Meyer et al. (2023) examined the phenolic content, catechin profiles, caffeine levels, and antioxidant properties of different green tea varieties, including matcha, gunpowder, and bagged teas. The focus was particularly on the distinctions between culinary and ceremonial-grade matcha. The research highlighted that catechins, especially epigallocatechin gallate (EGCG) and epigallocatechin (EGC), were the most significant compounds found across all tea types. Among the varieties, gunpowder tea had the highest EGCG concentration at 70.22 mg/g, closely

followed by ceremonial matcha with up to 69.73 mg/g (Meyer et al., 2023). Culinary matcha exhibited intermediate levels of catechins, showing slightly elevated EGC concentrations compared to its ceremonial counterpart. Notably, the study also indicated that the stereoisomers of epi/catechin gallate (E-CG) had a stronger correlation with antioxidant capacities than EGCG, underscoring the role of specific catechins in influencing the health benefits of tea (Meyer et al., 2023).

## 2.3 Antioxidant Properties of Matcha

A recent study by Meyer et al. (2023) analyzed antioxidant activity in teas using the CUPRAC (cupric ion reducing antioxidant capacity) and ORAC (oxygen radical absorbance capacity) assays, which evaluate different mechanisms of antioxidant action. Among the varieties tested, gunpowder and bagged teas demonstrated the highest antioxidant capacities, with Pure Leaf gunpowder green tea achieving an impressive CUPRAC value of 245.5 mmol trolox equivalent antioxidant capacity (TEAC)/100 g. Notably, matcha teas, especially ceremonial-grade varieties, exhibited significantly lower antioxidant capacities (Meyer et al., 2023). In contrast, culinary-grade matcha showcased stronger antioxidant properties, which correlated with its higher total phenolic content (TPC) values. A strong positive relationship was established between TPC and CUPRAC values, highlighting the critical role of phenolic compounds in antioxidant activity. Significantly, the research indicates that gunpowder and bagged teas, despite being the most affordable options, provided superior antioxidant benefits and catechin levels compared to matcha (Meyer et al., 2023).

In a related study by Fujioka et al. (2016), the chemical and functional differences between leaf-based and powdered green tea were examined, with a focus on catechin concentration, antioxidant activity, and inhibition of reactive oxygen species (ROS). Catechins, the primary polyphenolic compounds in green tea, are essential for its antioxidant properties (Fujioka et al., 2016). The study by Lee et al. (2014) emphasized the importance of cis-catechins, particularly EGCG, EGC, and epicatechin gallate (ECG), in influencing antioxidant activity. Various assays,

including ABTS, FRAP, and DPPH, revealed that green teas harvested later in the season had higher antioxidant activity due to increased levels of these catechins. Notably, gallated catechins (EGCG, ECG) correlated most strongly with antioxidant potential, confirming their superior free radical-scavenging capabilities compared to non-gallated catechins (Fujioka et al., 2016).

Additionally, the research explored the structural characteristics of catechins and their impact on antioxidant properties. Gallated catechins, possessing additional hydroxyl groups, exhibited enhanced radical-scavenging activity. The findings indicated a positive correlation between antioxidant activity and the total and ciscatechin content, while trans-catechins and non-gallated catechins demonstrated weaker correlations. This underscores the significance of both catechin concentration and molecular structure in determining antioxidant efficacy (Lee et al., 2014).

#### 2.4 Health Benefits of EGCG in Matcha

Matcha offers various therapeutic benefits, primarily due to its rich polyphenol content and other bioactive compounds. Matcha helps combat oxidative stress by neutralizing reactive oxygen species (ROS), damaging DNA, lipids, and proteins (Bhandari & Sharma, 2019). Its polyphenols and polysaccharides demonstrate significant antioxidant activity, protecting cells from oxidative damage (Farooq & Sehgal, 2018). Epigallocatechin-3-gallate (EGCG), a key compound in matcha, has potent anti-tumor and antioxidant effects, particularly against breast cancer cells, potentially reducing cancer risk (Shroder et al., 2017). Matcha's EGCG protects heart muscles by reducing oxidative stress and inflammation, which helps prevent cardiac damage, especially in patients at risk of heart issues, like smokers. Matcha can help reduce body fat and prevent obesity-related diseases by addressing adipose tissue mass and the secretion of harmful compounds from enlarged fat cells (Zhou et al., 2021). Matcha improves mental well-being, memory, and learning by reducing oxidative stress in the brain, particularly the hippocampus (Unno et al., 2019).

Several studies with a focus on the catechin epigallocatechin-3-gallate or EGCG in matcha have suggested that matcha has anti-proliferative, anti-oxidant, anti-

bacterial, and chemopreventive effects (Bonuccelli et al., 2018). The study by Bonuccelli and colleagues found that the compounds in matcha green tea target cell phosphorylation, effectively downgrading oxygen consumption rate, extracellular acidification rate, impaired cellular metabolic pathways, and signaling pathways. The study concludes that matcha may help to overcome cancer cell resistance to chemotherapy (Bonuccelli et al., 2018). Additionally, a study by Fujiki et al. (2018) reviewed studies on delayed cancer onset, prevention of colorectal adenoma occurrence, inhibition of metastasis of melanoma cells in the lungs of mice, and synergistic enhancement of anticancer activity in human cells with EGCG and anticancer compounds. In combination, the research also focuses on how EGCG decreases and or increases the expression of mRNA and protein in human cancer stem cells. As such, the human stem cells are the target for cancer prevention and treatment with EGCG (Fujiki et al., 2018).

A recent study by Sakurai et al. (2020) investigated the relationship between daily supplementation of matcha and the change in cognitive function. This study was conducted in 12 weeks and focused on elderly individuals within a community. Focusing on the polyphenol EGCG, which has strong antioxidant and anti-inflammatory properties, and is suggested to improve cognitive defects (Sakurai et al., 2020). EGCG can also cross the blood-brain barrier and has neuroprotective effects against amyloid AB (AB) toxicity by inhibiting AB aggression and production (Sakurai et al., 2020). The study concluded that daily supplementation may improve cognitive function, but only in female subjects (Sakurai et al., 2020).

A number of clinical studies in 2022 focused on matcha consumption and its correlation with weight loss and glucose control. El-Elimat and colleagues studied the effects of matcha tea on overweight and obese individuals (El-Elimat et al., 2022). The study focused on weight reduction with a specified low-calorie diet in addition to matcha tea (El-Elimat et al., 2022). Significant reductions in weight, fat mass, decrease in blood glucose and insulin levels were observed. The study concludes that matcha may have a beneficial effect on weight loss as well as anti-inflammatory properties (El-Elimat et al., 2022). In correlation, Wang et al. (2022) focused on matcha green tea and its connection with the gut-liver axis. Chemical analysis shows matcha is rich in EGCG, which can neutralize free radicals as antioxidants and

increase detoxification (Wang et al., 2022). The study concludes that matcha has effects on the diversity and composition of intestinal microbiota which play key roles in the regulation of metabolism (Wang et al., 2022).

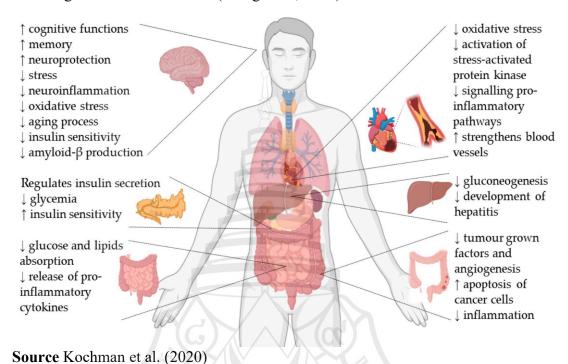


Figure 2.3 Health-Promoting Benefits of Matcha Green Tea

While matcha has numerous health benefits, excessive consumption can cause adverse effects, such as cytotoxicity in liver cells, oxidative DNA damage, goiter, and interference with iron absorption. The presence of caffeine and aluminum in matcha may also pose risks, particularly for individuals with heart conditions, pregnant women, and those with kidney disease. Overconsumption may lead to dehydration, iron deficiency, and other health issues (Bhandari & Sharma, 2019). One of the minerals present in matcha is fluorine, which plays a vital role in the mineralization of hard tissues (Jakubczyk et al., 2020). However, fluorine has a narrow range between beneficial and toxic levels for metabolic functions. Prolonged exposure, even at modest amounts, can lead to fluoride accumulation in the body, potentially disrupting natural physiological processes and causing fluorosis. Consequently, despite the rich phytochemicals and health benefits matcha offers, its consumption should be

moderated, and it should not be considered a primary source of fluoride (Jakubczyk et al., 2020).

## 2.5 Analysis Methods of Chemical Compounds

The quantification of bioactive compounds in green tea, particularly catechins, caffeine, and gallic acid, is essential for understanding its health benefits and ensuring product quality. A study by Theppakorn and Wongsakul (2012) introduced a validated high-performance liquid chromatography (HPLC) method that allows for the rapid and simultaneous analysis of these compounds. The researchers optimized HPLC parameters to enhance separation efficiency and reduce analysis time, making it a reliable method for quality control in green tea. They successfully separated seven key compounds: gallic acid, caffeine, and five catechins, including epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG).

Using a C18 reversed-phase column with a water-acetonitrile mobile phase containing 0.05% trifluoroacetic acid (TFA), the method achieved complete separation of all compounds within a brief seven-minute retention time (Theppakorn & Wongsakul, 2012). The addition of TFA further enhanced peak sharpness and resolution, with limits of detection (LOD) and quantification (LOQ) at 0.2 μg/mL and 1.0 μg/mL, respectively (Theppakorn & Wongsakul, 2012). Validation results showcased high precision, accuracy, and linearity, with recovery rates ranging from 84.7% to 103.7% and relative standard deviations (RSD) below 10%. This method was applied to six Assam green tea samples, revealing variations in bioactive compound levels affected by cultivar, growing conditions, and processing methods (Theppakorn & Wongsakul, 2012).

In a complementary study, Weiss and Anderton (2003) explored the catechin content of matcha green tea using micellar electrokinetic chromatography (MEKC) and found that matcha contains significantly higher levels of EGCG, the most bioactive catechin. Specifically, matcha was reported to have 137 times more EGCG than a popular green tea brand (China Green Tips) when analyzed on a per-gram basis

of dry leaf weight (Weiss & Anderton, 2003). This notable difference is attributed to matcha's shading cultivation method and the consumption of whole powdered leaves, rather than brewed infusions. Furthermore, preparation methods were shown to impact catechin availability, with methanol extraction yielding the highest catechin concentrations. This emphasizes the role of traditional matcha preparation methods in maximizing catechin intake, as the entire leaf powder is consumed compared to steeped green teas (Weiss & Anderton, 2003).

A study by Fujioka et al. (2016) found that techniques such as high-performance liquid chromatography (HPLC) and tandem mass spectrometry (LC-MS/MS) confirmed the efficient release of catechins due to reduced particle size and increased surface area. Furthermore, scanning electron microscopy (SEM) revealed notable differences in particle morphology and size between leaf and powdered tea, with the fine particles produced by a ceramic mill being crucial in enhancing the extraction of bioactive compounds (Fujioka et al., 2016). Additionally, research by Koláčková et al. (2019) underscores that preparation methods, including solvent choice, can significantly affect the extraction and bioavailability of these health-promoting compounds.

#### **CHAPTER 3**

#### RESEARCH METHODOLOGY

# 3.1 Sample Collection

The matcha samples were purchased from reputable tea shops, which include Matcha Maru, Osha Ocha, Matchalabo, Matchaten, and Okucha Matcha in Bangkok, Thailand. The selection criteria for the shops were based on their popularity within the matcha community. All shops have FDA approval, and all matcha products are imported from various parts of Japan. Information on brand, location, grade, cultivar, harvest, and price can be found in Table 1.

Table 3.1 Matcha Powder Samples

Brand	Product	Grade	Cultivar	Harvest	Price THB/g	
Uji	15	Z/\/				
Matcha maru	Hikari	Ceremonial	Hikari	1st	₿55	
	(Single Origin)					
Matcha maru	Tenarai	Premium	Yabukita/Samid ori	1st & 2nd	<b>₿14</b>	
Matchaten	Classic	Culinary	Yabukita/Samid	3rd	₿5	
			ori			
Shizuoka						
Osha Ocha	Yabukita (JAS Organic)	Ceremonial	Yabukita	1st	в16	
Osha Ocha	Yabukita	Premium	Yabukita	1 st	<b>B</b> 10	
Matchalabo Tsuko		Culinary	Yabukita/Samid ori	2nd	в4	
Kagoshima						
Matchamaru	Kagoshima 2.0	Ceremonial	Asanoka/Sakimi	1st	₿20	
	(Organic)		dori			
Matchamaru	Kagoshima 3.0	Premium	Asanoka/Sakimi	1st	<b>₿14</b>	
	(Organic)		dori			
Okucha Matcha	Shibushi	Culinary	Okumidori/Yabu	2nd	₿8	
			kita			

Matcha powders were obtained in various grades, ranging from ceremonial, premium, and culinary grade. To ensure a standard regarding cultivation and processing methods, matcha powders from the same cultivars were selected, focusing on different grade matcha in various regions of Japan. The locations include Uji, Shizuoka, and Kagoshima. These locations are recognized and well-known for matcha production and maintain a standard of cultivation and quality.

Uji, located in Kyoto Prefecture, is celebrated as the historical birthplace of Japanese matcha and is renowned for producing the highest quality ceremonial-grade tea (Ashardiono & Cassim, 2015). The region experiences a humid subtropical climate with distinct seasons, characterized by cool winters and warm, rainy summers. The morning mists from the Uji River are crucial in maintaining moisture and shielding the tea leaves from excessive sunlight, enhancing their flavor and color (Ashardiono & Cassim, 2015). The soil in Uji is rich in minerals and well-drained, making it ideal for cultivating premium tea. Although its production volume is smaller than in other regions, Uji continues to set the standard for hand-crafted, artisanal matcha (Ashardiono & Cassim, 2015).

Shizuoka Prefecture, renowned as Japan's largest tea-producing region, is responsible for approximately 40% of the country's total tea output (Sugawara, 2013). While it is primarily known for its sencha, Shizuoka also cultivates matcha, especially on the Makinohara plateau, where shaded tea-growing techniques are employed. The region benefits from a moderate oceanic climate characterized by ample rainfall and a diverse landscape of both mountainous and coastal terrains (Sugawara, 2013). The notable temperature variations in Shizuoka contribute to the development of complex flavors in the tea leaves. Additionally, the volcanic, nutrient-rich soil and well-drained topography create an ideal environment for tea cultivation, solidifying Shizuoka's position as a key player in Japan's tea industry (Sugawara, 2013).

Kagoshima Prefecture, situated in the southernmost region of mainland Japan, ranks as the country's second-largest tea-producing area and is a leader in the large-scale production of high-quality matcha (Pulkus & Moreno, 2024). The prefecture enjoys a warm, subtropical climate characterized by abundant sunshine and significant rainfall, factors that facilitate accelerated tea growth. Additionally, the volcanic soil, enriched by the nearby Sakurajima, is both fertile and well-draining,

creating ideal conditions for tea cultivation (Pulkus & Moreno, 2024). Kagoshima stands at the forefront of mechanized and organic tea farming, significantly contributing to matcha production for both domestic and international markets. While it may lack the historical prestige of Uji, the prefecture plays an essential role in contemporary matcha production through its innovative agricultural practices (Pulkus & Moreno, 2024).

To ensure homogeneity, the sample of leaf tea ground following ISO1572 and stored in well-sealed containers protected from light (International Organization for Standardization, 2005).

# 3.2 Matcha Extracts and Standard Preparation

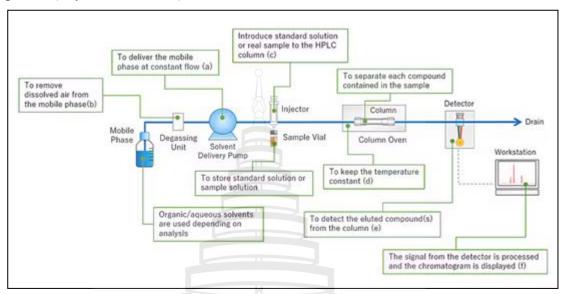
Three replicates were extracted for each of the tea products using a method adapted from Mae Fah Luang Standard Operating Protocol. Briefly, weigh approximately 0.8 grams of finely ground matcha sample and record the exact weight into a 100 ml beaker. Heat 50 ml of distilled water to 80°C and add to the sample, stirring for 10 minutes. Filter through filter paper and repeat the extraction. Collect the filtrate and adjust the volume to 100 ml in a Volumetric flask (if not yet analyzed, store at 4-6 °C).

The standard solution of EGCG was created by dissolving the compound in a small amount of methanol, resulting in a stock solution at a concentration of 1000  $\mu$ g/ml. Before conducting HPLC analysis, working standard solutions were prepared by diluting the mixed stock solution with methanol to create 1 ppm, 5 ppm, 10 ppm, 50 ppm, and 100 ppm standard solutions.

# 3.3 HPLC Analysis

To analyze the chemical components for this study, high-performance liquid chromatography or HPLC will be used. High-Performance Liquid Chromatography (HPLC) is an advanced analytical technique used to separate, identify, and quantify components in a mixture (Snyder et al., 2012). It operates by passing a liquid sample

through a column packed with a solid stationary phase under high pressure, allowing components to separate based on their interaction with the stationary and mobile phases (Snyder et al., 2012).



Source SHIMADZU (2024)

Figure 3.1 HPLC Machine Components

The key components of an HPLC system include:

#### 3.3.1 Mobile Phase

A liquid solvent that carries the sample through the column.

#### 3.3.2 Stationary Phase

A solid or liquid-coated material within the column that interacts with the sample components.

#### 3.3.3 **Pump**

Delivers the mobile phase under high pressure to achieve efficient separation.

#### 3.3.4 Injector

Introduces the sample into the system.

#### 3.3.5 Detector

Identifies and quantifies the separated components, often using UV-Vis, fluorescence, or mass spectrometry detectors.

HPLC is widely employed in various fields, including pharmaceuticals, food analysis, environmental monitoring, and biochemistry, due to its high sensitivity, precision, and ability to handle complex mixtures (Snyder et al., 2012).

#### 3.4 Instrumentation

HPLC analysis of standards and samples was conducted, and the HPLC conditions are as follows, adapted from Theppakorn & Wongsakul (2012).

- 3.4.1 High-performance liquid chromatography (HPLC), ACQUITY Arc System, WATERS, US
  - 3.4.2 Column: Cortecs C18 2.7um 4.6\*50mm Column
  - 3.4.3 Injection volume: 5 µl
  - 3.4.4 Flow rate: 2.0 ml/min
  - 3.4.5 Column temperature: 30 °C
  - 3.4.6 Sample temperature: 10 °C
  - 3.4.7 Run time: 12 min
  - 3.4.8 Detector wavelength: 210 nm for EGCG
  - 3.4.9 Mobile phase: 0.05% TFA in water (A), acetonitrile (B)

Table 3. 2 HPLC Gradient Table

Time (min)	Flow rate	%Solvent A	%Solvent B
0	2.0	95	5
2	2.0	95	5
5	2.0	90	10
6	2.0	85	15
8	2.0	85	15
8.5	2.0	95	5
12	2.0	95	5

#### 3.5 Data Collection

The methods of data collection and HPLC analysis were obtained following the ISO 14502-2:2005(E). A brief description is as follows: once the flow rate of the mobile phase and temperature are stable, condition the column with a blank gradient run. Then, inject into the column 5 µl of each of the mixed standard solutions, followed by an equal volume of the diluted sample extract. Repeat the injection of the mixed working standard solutions at regular intervals. Collect data using the data collection/integration system for all peaks in the mixed standards and test sample solutions. Each sample will be analyzed three times to ensure the precision of the HPLC system. After each batch of analysis, thoroughly flush the chromatographic system and column with 50 % (volume fraction) acetonitrile and replace column sealing plugs if disconnected for storage (International Organization for Standardization, 2005).

# 3.6 Data Analysis

All experimental data were expressed as mean and standard deviation and analyzed by one-way ANOVA followed by Tukey's post hoc test at p < 0.05 using the SPSS program (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) Lee et al. (2014).

#### **CHAPTER 4**

#### **RESULTS**

## 4.1 HPLC Analysis Results

High-Performance Liquid Chromatography (HPLC) was used to quantify the levels of epigallocatechin gallate (EGCG) in matcha powder samples across three grade categories: ceremonial, premium, and culinary. A total of 27 samples (n=27) were analyzed using a standardized HPLC protocol with UV detection at 210 nm and an injection volume of 5  $\mu$ l. The retention time for EGCG was consistently observed around 4.5 minutes across all samples. Calibration standards ranging from 1 ppm to 100 ppm were used to generate a standard curve (Figure 4.1).

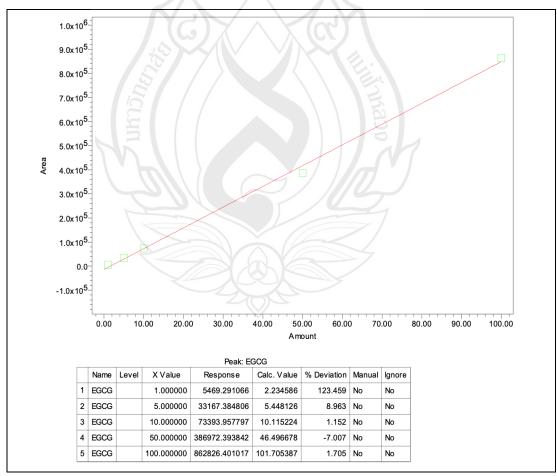


Figure 4.1 Calibration Plot

The ceremonial-grade samples (U1, K1, S1) showed average EGCG concentrations ranging from 0.85 to 22.99 mg/g, while the premium-grade samples (U2, K2, S2) demonstrated a broader and higher range, averaging from 2.52 to 35.55 mg/g. Culinary-grade matcha (U3, K3, S3) showed mid-level concentrations between 1.21 and 33.63 mg/g. Despite the high variability in the premium group, the overall highest values were consistently found in premium-grade samples, particularly from the Kagoshima and Shizuoka regions. However, due to the variability within each group, these apparent differences did not reach statistical significance.

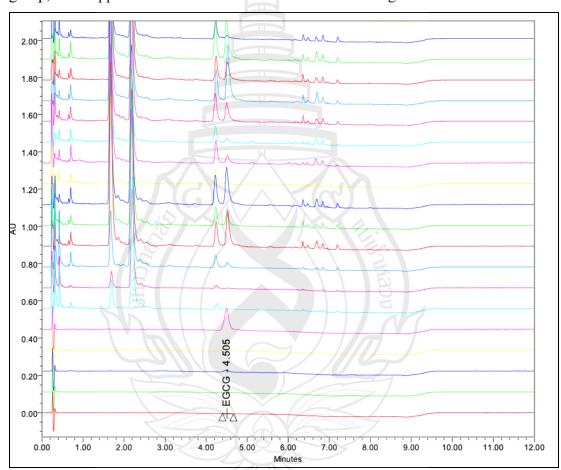


Figure 4.2 Peak Summary Report

 Table 4.1 Result Set Report

-	Sample Name	Vial	Inj	Name	Retention	Area	% Area	Height	Amount	Units
	- map - r mine				Time (min)		, 0 . 11 04		unt	
1	EGCG 1ppm	1:A,2	1	EGCG	4.505	5469	100	816	1	ppm
2	EGCG 5ppm	1:A,3	1	EGCG	4.505	33167	100	4436	5	ppm
3	EGCG 10ppm	1:A,4	1	EGCG	4.493	73392	100	9836	10	ppm
4	EGCG 50ppm	1:A,5	1	EGCG	4.487	386972	100	51303	50	ppm
5	EGCG 100ppm	1:A,6	1	EGCG	4.497	862826	100	114533	100	ppm
6	U1-1	1:A,7	1	EGCG	4.536	18796	100	3470	3.8	ppm
7	U1-2	1:A,8	1	EGCG	4.517	12938	100	2374	3.1	ppm
8	U1-3	1:B,1	1	EGCG	4.514	14698	100	26649	18.7	ppm
9	U2-1	1:B,2	1	EGCG	4.52	1012926	100	186885	119.1	ppm
10	U2-2	1:B,3	1	EGCG	4.506	324586	100	60612	39.3	ppm
11	U2-3	1:B,4	1	EGCG	4.499	1060670	100	193823	124.7	ppm
12	U3-1	1:B,5	1	EGCG	4.514	69996	100	12953	9.7	ppm
13	U3-2	1:B,6	1	EGCG	4.516	211209	100	38687	26.1	ppm
14	U3-3	1:B,7	1	EGCG	4.499	106369	100	19409	13.9	ppm
15	S1-1	1:B,8	1	EGCG	4.5	523344	100	96228	62.3	ppm
16	S1-2	1:C,1	1	EGCG	4.524	1564000	100	291467	183.1	ppm
17	S1-3	1:C,2	(1)	EGCG	4.519	486015	100	92383	58	ppm
18	S2-1	1:C,3	1	EGCG	4.498	1159348	100	214012	136.1	ppm
19	S2-2	1:C,4	1	EGCG	4.508	67992	87.67	13839	9.5	ppm
20	S2-3	1:C,5	1	EGCG	4.515	2398496	100	438384	279.9	ppm
21	S3-1	1:C,6	1	EGCG	4.512	872605	2 100	164391	102.8	ppm
22	S3-2	1:C,7	1	EGCG	4.492	2300198	100	414427	268.5	ppm
23	S3-3	1:C,8	1	EGCG	4.498	1173325	100	214504	137.7	ppm
24	K1-1	1:D,1	1	EGCG	4.515	13080	44.19	3498	3.1	ppm
25	K1-2	1:D,2	1	EGCG	4.513	151939	95.77	29118	19.2	ppm
26	K1-3	1:D,3	1	EGCG	4.499	187431	97.67	35698	23.3	ppm
27	K2-1	1:D,4	1	EGCG	4.499	155834	100	29817	19.7	ppm
28	K2-2	1:D,5	1	EGCG	4.509	286511	100	53607	34.8	ppm
29	K2-3	1:D,6	1	EGCG	4.51	550445	100	101825	65.5	ppm
30	K3-1	1:D,7	1	EGCG	4.498	511350	100	96745	60.9	ppm
31	K3-2	1:D,8	1	EGCG	4.49	297432	100	57202	36.1	ppm
32	K3-3	1:E,1	1	EGCG	4.494	514134	100	96327	61.3	ppm
Mean					4.506					
Sid. Dev.					0.011					
%RSD					0.25					

# 4.2 Statistical Analysis Results

A one-way ANOVA was performed to determine whether EGCG concentrations differed significantly across ceremonial, premium, and culinary grades of matcha powders analyzed by HPLC (n=27, p=.211). The results showed that Premium-grade matcha had the highest mean EGCG level (average = 19.47 mg/g, SD = 12.92), followed by Culinary-grade (average = 13.51 mg/g, SD = 15.16), while Ceremonial-grade matcha had the lowest mean EGCG content (average = 8.19 mg/g, SD = 10.97) (Table 4.2).

Table 4.2 Average EGCG Concentrations in Different Grade Matcha Powders

Matcha Grade	Average ECGC (mg/g)
Ceremonial	$8.19 \pm 10.97$
Premium	$19.47 \pm 12.92$
Culinary	$13.51 \pm 15.16$

Levene's test for homogeneity of variances was not significant, validating the assumption of equal variances across groups. The one-way ANOVA did not reveal any statistically significant differences between the groups. Additional statistical tests, including Welch and Brown-Forsythe, corroborated the non-significant outcome. Tukey's HSD post hoc analysis found no statistically significant pairwise differences among the groups.

#### **CHAPTER 5**

#### **DISCUSSION AND CONCLUSION**

### 5.1 Discussion

The findings of this study contrast with the perception that more expensive matcha contains higher levels of health-promoting catechins such as EGCG. While traditional cultivation practices, such as extended shading and first-harvest leaf selection, are often thought to enhance quality (Ku et al., 2010; Sano et al., 2018), this research revealed that ceremonial-grade matcha had the lowest mean EGCG content among the samples tested.

This aligns with studies by Meyer et al. (2023) and Horie et al. (2017), which showed that while ceremonial-grade matcha may excel in sensory attributes like color and taste due to higher theanine and chlorophyll concentrations, these characteristics do not necessarily correlate with elevated catechin levels. Meyer et al. (2023) specifically found that culinary and premium-grade matcha samples often displayed higher total phenolic content and antioxidant capacity, particularly due to increased levels of EGC and EGCG.

Furthermore, Sano et al. (2018) demonstrated that excessive shading can decrease the biosynthesis of gallated catechins such as EGCG, even as it improves amino acid profiles. This may help explain why ceremonial-grade matcha, while smoother and richer in umami, does not consistently exhibit higher catechin levels. Fujioka et al. (2016) and Weiss and Anderton (2003) emphasized the impact of processing methods and particle size on catechin bioavailability, which can vary across commercial matcha products regardless of grade.

One notable limitation of this study is the sample size, which comprised only 27 matcha powder samples across three grade categories (ceremonial, premium, and culinary). The high variability observed within and across matcha grades further underscores the need for broader and more representative sampling. Factors such as batch differences, geographic origin, cultivation practices, processing methods, and

storage conditions can significantly influence catechin concentrations. For instance, samples from Kagoshima displayed notably higher variability in EGCG content than those from Uji, suggesting that regional and brand differences may play a larger role than grade designation alone.

The observed variability is further reflected in the large standard deviations relative to the mean EGCG values across all grades, most prominently within the ceremonial group where the standard deviation (10.97 mg/g) exceeded the mean (8.19 mg/g). Such high dispersion indicates a wide range of EGCG concentrations within single grade categories, likely driven by heterogeneity in agricultural and processing factors. Shading duration, leaf maturity at harvest, fertilization practices, and post-harvest handling have all been shown to influence catechin biosynthesis (Sano et al., 2018), potentially contributing to the inconsistent EGCG content even among products marketed under the same grade label. This degree of variability not only complicates the interpretation of mean differences between grades but also diminishes the statistical power to detect significant group differences without larger and more controlled sample sets.

To enhance the reliability and generalizability of findings, future research should incorporate a larger sample size encompassing multiple batches, brands, and geographical regions. Including longitudinal data—analyzing matcha samples across different harvest seasons and years—would further account for seasonal variations. Expanding the analysis to include additional bioactive compounds (such as other catechins, L-theanine, and caffeine) would also provide a more comprehensive understanding of matcha's nutritional and functional properties. Ultimately, replication and broader sampling are crucial to establish robust conclusions about the relationship between matcha grade and its health-promoting compounds. These steps will ensure that findings are not only statistically significant but also biologically and commercially relevant, guiding both consumers and producers in making informed decisions about matcha quality and value.

This study provides insights with direct relevance to both consumers and industry stakeholders. Contrary to the belief that ceremonial-grade matcha offers superior health benefits, the findings indicate that premium-grade matcha may contain higher levels of EGCG, a key bioactive compound associated with antioxidant, anti-

inflammatory, and metabolic benefits. For consumers, this highlights the importance of informed purchasing decisions, recognizing that higher price points or ceremonial labeling may not necessarily guarantee superior nutritional quality. Premium-grade matcha, often less expensive than ceremonial-grade, may offer a better cost-to-benefit ratio for those prioritizing health benefits over ceremonial qualities such as flavor and appearance. Additionally, consumers should consider preparation methods, including temperature and water quality, to maximize EGCG extraction.

For industry stakeholders, these findings suggest a need to reconsider product positioning strategies. Rather than focusing solely on ceremonial designations, producers may benefit from shifting their marketing emphasis toward transparent reporting of bioactive content, such as EGCG levels. Establishing standardized testing protocols for catechin content could further enhance quality control and help substantiate health claims, building consumer trust. Educational initiatives targeting consumers, including information about factors that affect matcha quality, such as shading practices, harvest time, and processing methods, could also help promote premium products that balance both sensory appeal and health benefits. Moreover, the industry could explore innovative formulations or blends with verified high EGCG content to cater to the growing health-conscious consumer segment. These strategies not only align with consumer interests but also contribute to maintaining a competitive advantage in the global matcha market.

The results suggest that EGCG content is influenced by a complex interplay of factors beyond grade alone, including cultivar selection, shading duration, harvest timing, and post-processing techniques. The assumption that price or visual quality equates to nutritional superiority should therefore be reconsidered. These findings also reflect the antioxidant behavior and catechin profiles observed in prior literature and stress the importance of accurate compositional analysis in labeling and marketing.

#### 5.2 Conclusion

The results do not support the hypothesis that ceremonial-grade matcha contains significantly higher EGCG levels than premium and culinary grades. Ceremonial-grade matcha exhibited the lowest EGCG content among the three categories. Despite traditional perceptions and higher market prices associated with ceremonial-grade matcha, this study demonstrates that higher EGCG content may be more consistently present in premium-grade matcha. These findings align with research by Meyer et al. (2023) and Sano et al. (2018), which indicated that shading and harvesting practices influence catechin content variably, and that ceremonial-grade matcha may prioritize sensory qualities (e.g., umami, color) over catechin concentration. Consumers seeking EGCG-related health benefits should therefore not rely solely on grade or price as indicators of nutritional potency.

This study underscores the importance of empirical chemical analysis in evaluating functional food claims and provides insight into the correlation between matcha grade and health-related compounds. Future studies should include larger sample sizes, with focus on cultivation processes within specific regions, and expand the analysis to additional catechins and bioactive components to further validate these findings.

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# **APPENDIX A**

# **HPLC LAB RESULTS**



# Result Set Report

Sample Set Name:

Matcha EGCG Test 1332025 Matcha EGCG Test 1332025 Processed By: System/Administrator

32

Sample Set Method:

Printed By: System

System Node: System Name: PDA

Desktop-vh9bkcg

Result Set ID:

# of Results:

Acquired By: System

Sample Set Start Date: 3/13/2025 3:36:26 PM ICT Sample Set Finish Date: 3/13/2025 11:21:50 PM ICT

Result Set Date:

Sample Set Table

							10 001 1 0010			
	Sample Name	Sample Type	Vial	lnj#	Run Time (Minutes)	Injection Volume (ul)	Acquistion Method Set	Sample Weight	Processed Channel Descr.	Dilut
1	EGCG 1ppm	Standard	1:A,2	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
2	EGCG 5ppm	Standard	1:A,3	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.000
3	EGCG 100ppm	Standard	1:A,6	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
4	U1-1	Unknow n	1:A,7	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
5	U1-2	Unknow n	1:A,8	. 1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
6	U1-3	Unknow n	1:B,1	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
7	S1-3	Unknow n	1:C,2	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
8	S2-1	Unknow n	1:C,3	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
9	U2-1	Unknow n	1:B,2	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
10	U2-2	Unknow n	1:B,3	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
11	U2-3	Unknow n	1:B,4	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
12	U3-1	Unknow n	1:B,5	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
13	U3-2	Unknow n	1:B,6	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
14	U3-3	Unknow n	1:B,7	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
15	S1-1	Unknow n	1:B,8	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
16	S1-2	Unknow n	1:C,1	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
17	EGCG 10ppm	Standard	1:A,4	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
18	EGCG 50ppm	Standard	1:A,5	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
19	S3-1	Unknow n	1:C,6	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
20	S3-2	Unknow n	1:C,7	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
21	K3-2	Unknow n	1:D,8	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
22	K3-3	Unknow n	1:E,1	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
23	S3-3	Unknow n	1:C,8	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
24	K1-1	Unknow n	1:D,1	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
25	K1-2	Unknow n	1:D,2	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00
26	K1-3	Unknow n	1:D,3	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00

Reported by User: System Report Method: Result Set Report Report Method ID: 1013

Page: 1 of 35

Project Name: EGCG\_Flavanols Date Printed: 3/14/2025 11:16:20 AM Asia/Bangkok

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	Sample Name	Sample Type	Vial	# Íul	Inj# Run Time (Minutes)	Injection Volume (ul)	Acquistion Method Set	Sample Weight	Processed Channel Descr.	Diluti
27	K2-1	Unknown	1:D,4	1	12.00	9.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.00(
28	K2-2	Unknown	1:D,5	1	12.00	9.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.000
29	K2-3	Unknown	1:D,6	1	12.00	9.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.000
30	K3-1	Unknown	1:D,7	-	12.00	9.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.000
31	S2-2	Unknown	1:C,4	-	12.00	9.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.000
32	S2-3	Unknown	1:C,5	1	12.00	5.00	EGCE_Gradient12 min_13032025	1.00000	2998 Ch1 210nm@2.4nm	1.000

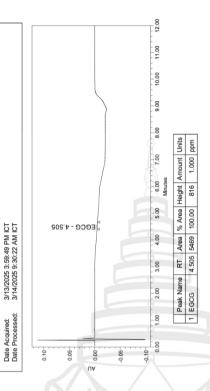
System Matche EGCG Test 1332025 EGCE Gradient12 min 13032025 EGCE Gradient12 min 13032025 298 Ch1 210nm@2.4mm 2998 Ch1 210nm@2.4mm

Acquired By:
Sample Set Name:
Acq. Method Set:
Processing Method:
Channel Name:
Proc. Chnl. Descr.:
2

5.00 ul 12.0 Minutes EGCG 1ppm Standard 1:A,2

Sample Name: Sample Type: Vial: Injection #: Injection Volume: Run Time:

SAMPLE INFORMATION

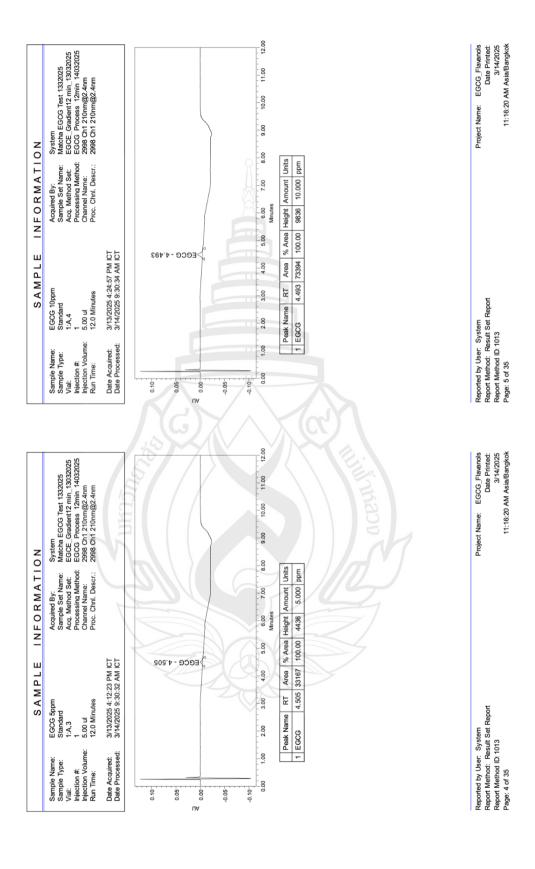


Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 2 of 35

Project Name: EGCG\_Flavenols
Date Printed:
3/14/2025
11:16:20 AM Asia/Bangkok

Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 3 of 35

Project Name: EGCG\_Flavanols
Date Printed:
3/14/2025
11:16:20 AM Asia/Bangkok



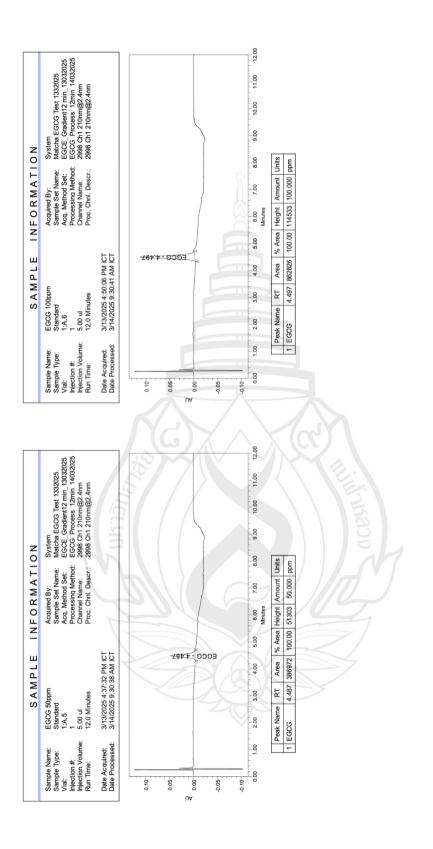
Project Name: EGCG\_Flavanols 3/14/2025 11:16:20 AM Asia/Bangkok Date Printed:

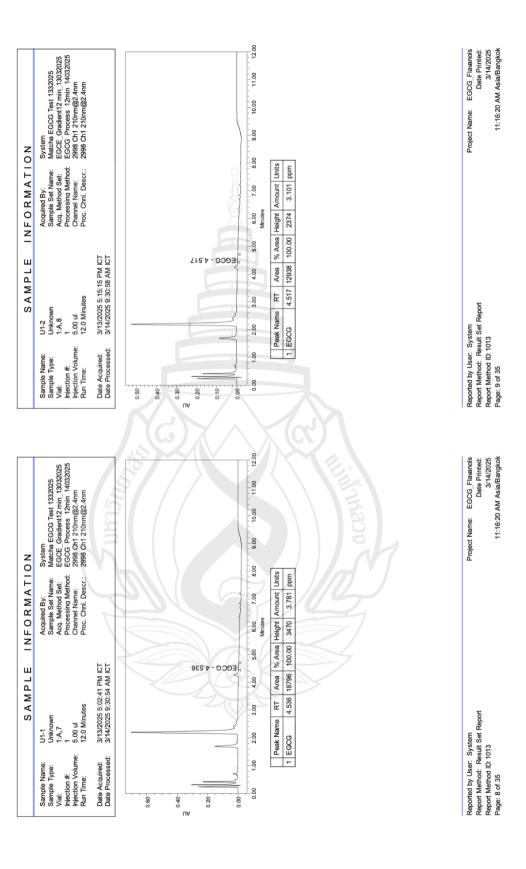
Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 7 of 35

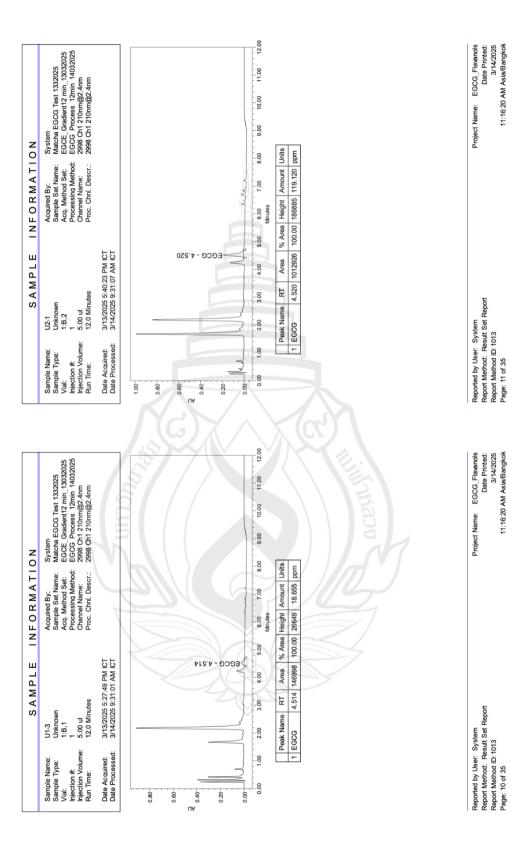
3/14/2025 11:16:20 AM Asia/Bangkok Project Name: EGCG\_Flavanols

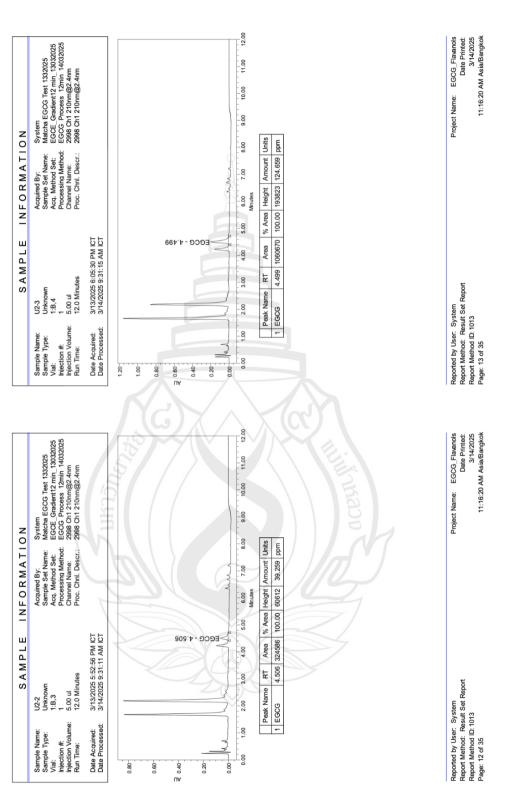
Date Printed:

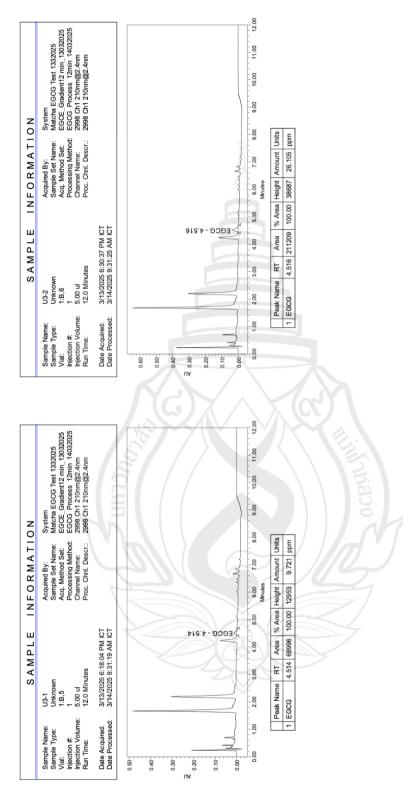
Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 6 of 35







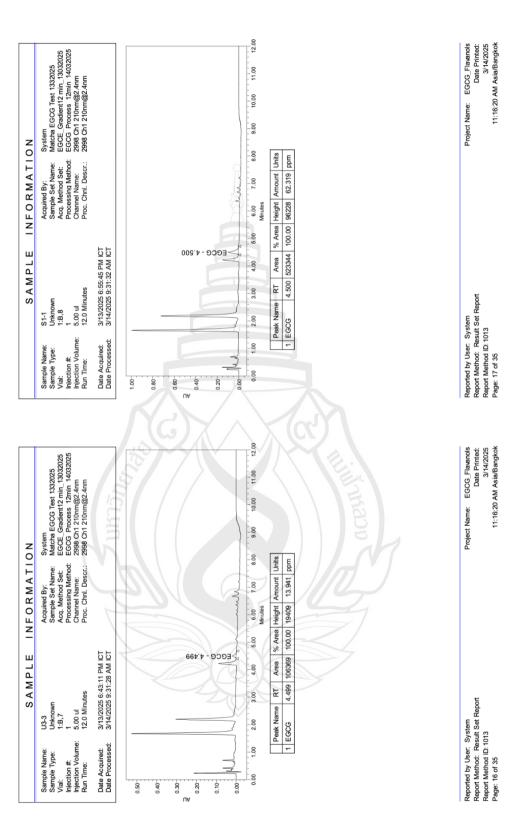


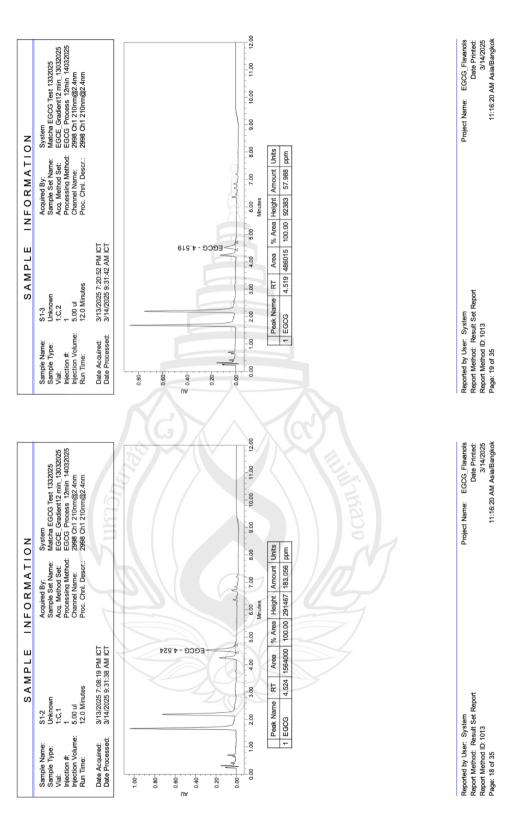


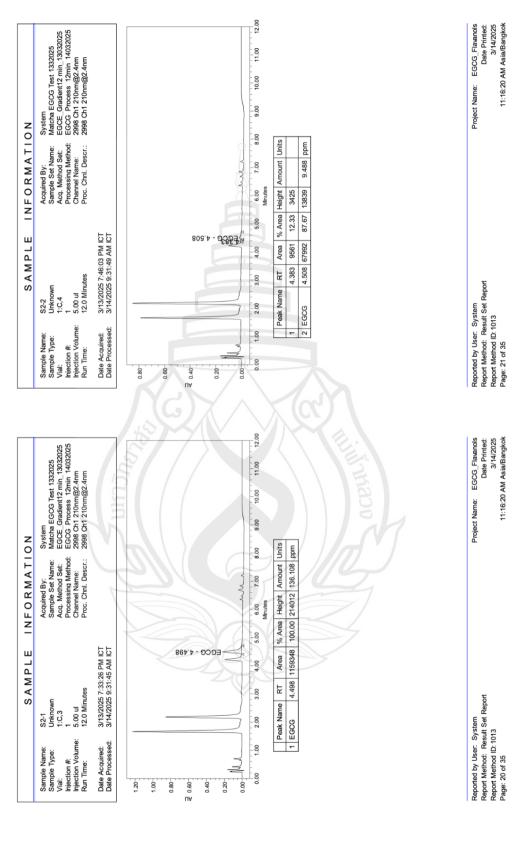
Project Name: EGCG\_Flavanols Date Printed: 3/14/2025 11:16:20 AM Asia/Bangkok Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 14 of 35

Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 15 of 35

3/14/2025 11:16:20 AM Asia/Bangkok Project Name: EGCG\_Flavanols Date Printed:







# Sample Name: Sample Type: Vial: Injection #: Injection Volume: Run Time: Date Acquired: Date Processed: Acquired By: System Sample Set Name. Bridhe EGCG Test 1332025 Acq. Method Set. EGCE Gradient12 min. 13032025 Processing Method. EGCG Process 12min 14032025 Channel Name. 2998 Ch1 210nm@2.4mm Proc. Chirl. Descr.: 2998 Ch1 210nm@2.4mm INFORMATION SAMPLE 3/13/2025 7:58:37 PM ICT 3/14/2025 9:31:54 AM ICT 5.00 ul 12.0 Minutes S2-3 Unknown 1:C,5 Sample Name: Sample Type: Vial: Injection #: Injection Volume: Run Time: Date Acquired: Date Processed:

System Matche EGCG Test 1332025 EGCE, Gradient12 min. 13032025 EGCB, Gradient12 min. 14032025 2998 Ch1 Z10nm@2.4mn 2998 Ch1 Z10nm@2.4mn

Acquired By:
Sample Set Name:
Acq. Method Set:
Processing Method:
Channel Name:
Proc. Chnl. Descr.: 2

3/13/2025 8:11:10 PM ICT 3/14/2025 9:31:59 AM ICT

5.00 ul 12.0 Minutes

S3-1 Unknown 1:C,6

INFORMATION

SAMPLE



0.00 0.00

0.20

₩ 0.60

0.40

1.20 1.00 0.80



Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 22 of 35

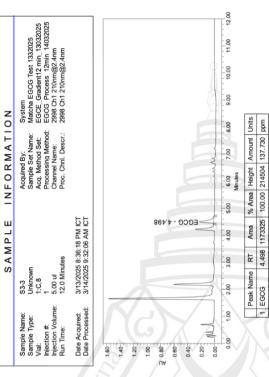
Project Name: EGCG\_Flavanols Date Printed:

3/14/2025 11:16:20 AM Asia/Bangkok

Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 23 of 35

3/14/2025 11:16:20 AM Asia/Bangkok Project Name: EGCG\_Flavanols Date Printed:





12.00

11.00

10.00

9.00

8.00

7.00 6.00 Minutes 5.00

4.00

3.00

2.00

8

JUN 0.00 0.00

0.40

EGCG - 4.492

₩ 0.80 1.00

09.0

RT Area % Area Height Amount Units 4.492 2300198 100.00 414427 268.470 ppm

Peak Name RT

1 EGCG

Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 25 of 35

Date Printed: 3/14/2025 11:16:20 AM Asia/Bangkok Project Name: EGCG\_Flavanols

Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 24 of 35

Date Printed: 3/14/2025 11:16:20 AM Asia/Bangkok Project Name: EGCG\_Flavanols



System Matche EGCG Test 1332025 Matche EGCG Test 133022025 EGCG Process 12min 14032025 EGCG Process 12min 14032025 2998 Ch1 210nm@2.4mm

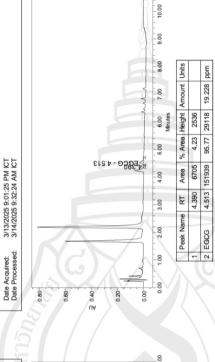
Acquired By:
Sample Set Name:
Acq. Method Set:
Processing Method:
Channel Name:
2
Proc. Chnl. Descr.:

5.00 ul 12.0 Minutes

K1-2 Unknown 1:D,2

INFORMATION

SAMPLE



12.00

11.00

	12.00				
	11.00				
	10.00				
	9.00				
	8.00	Units		mdd	
	7.00	Height Amount		3.118	
	6.00 Minutes	Height	3855	3498	740
212.4 - 425.4	5.00	% Area	43.33	44.19	12.49
777 4884g	4.00	Area	12826	13080	3696
	3.00	RT	4.391	4.515	4.647
	2.00	Peak Name		EGCG	
	-0.		-	7	က

1.00

0.00

0.40

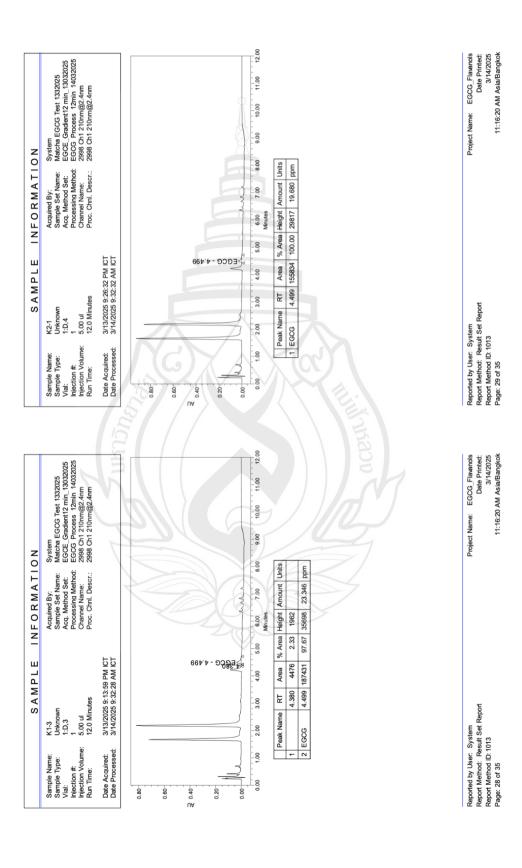
0.20

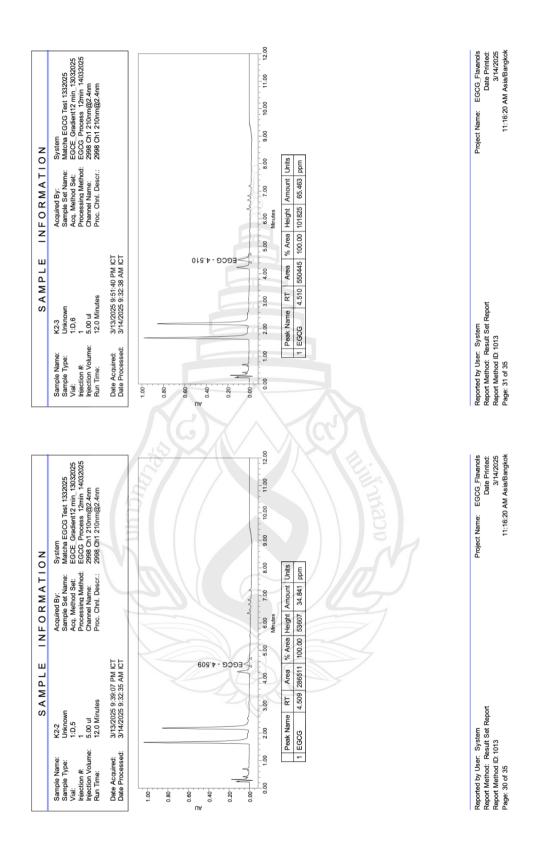
0.80 -09.0 Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 27 of 35

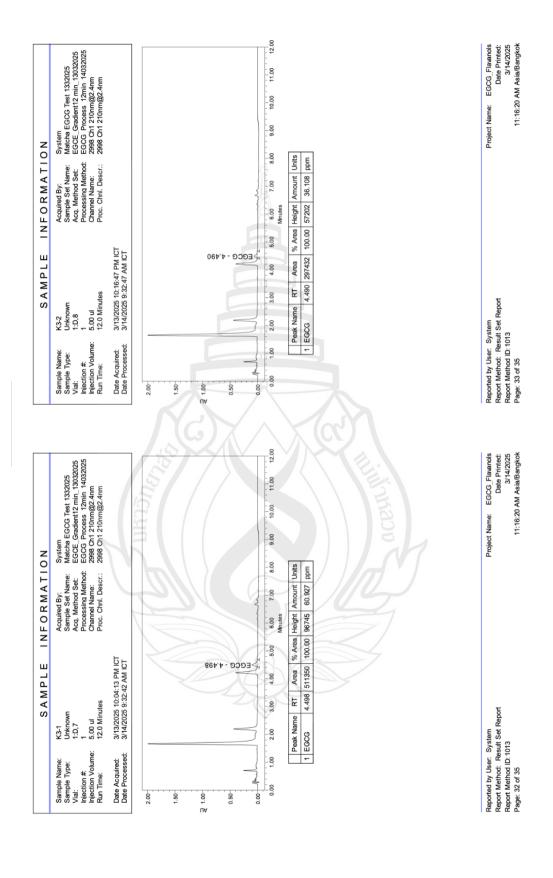
Project Name: EGCG, Flavanols
Date Printed:
3/14/2025
11:16:20 AM Asia/Bangkok

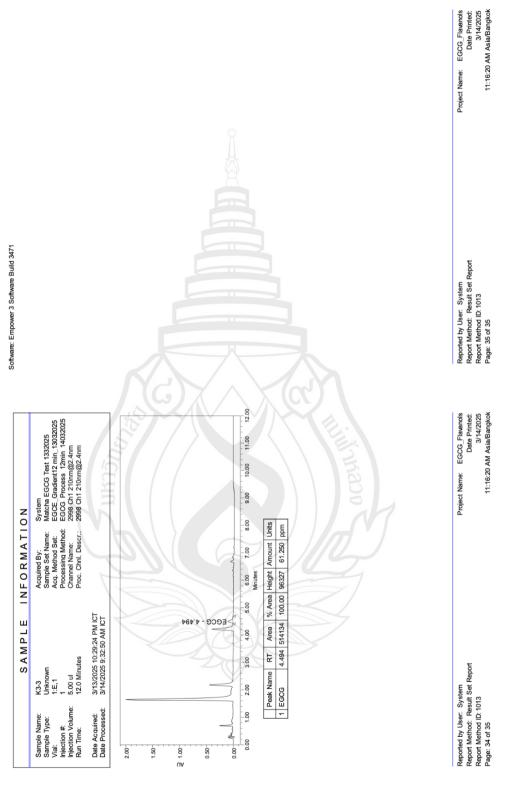
Reported by User: System Report Method: Result Set Report Report Method ID: 1013 Page: 26 of 35

Project Name: EGCG\_Flavanols Date Printed: 3/14/2025 11:16:20 AM Asia/Bangkok









# **APPENDIX B**

# STATISTICAL ANALYSIS RESULTS

#### Oneway

#### Descriptives

Amou	ınt

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1	9	8.1922	10.97406	3.65802	2432	16.6276	.77	22.99
2	9	19.4678	12.91999	4.30666	9.5366	29.3990	2.52	35.55
3	9	13.5089	15.15608	5.05203	1.8589	25.1589	1.21	33.63
Total	27	13.7230	13.45826	2.59004	8.3991	19.0469	.77	35.55

#### Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Amount	Based on Mean	1.176	2	24	.326
	Based on Median	.240	2	24	.788
	Based on Median and with adjusted df	.240	2	20.404	.789
	Based on trimmed mean	1.048	2	24	.366

#### **ANOVA**

#### Amount

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	572.740	2	286.370	1.662	.211
Within Groups	4136.504	24	172.354		
Total	4709.244	26			13

#### ANOVA Effect Sinesa,b

05%	Confidence	Intorval	

		Point Estimate	Lower	Upper
Amount	Eta-squared	.122	.000	.329
	Epsilon-squared	.048	083	.273
	Omega-squared Fixed- effect	.047	080	.266
	Omega-squared Random-effect	.024	038	.153

- a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.
- b. Negative but less biased estimates are retained, not rounded to zero.

# **Robust Tests of Equality of Means**

#### Amount

	Statistica	df1	df2	Sig.
Welch	1.915	2	15.733	.180
Brown-Forsythe	1.662	2	22.483	.212

a. Asymptotically F distributed.

#### **Post Hoc Tests**

# **Multiple Comparisons**

Dependent Variable: Amount

Tukey HSD

(I) Group	(J) Group	Mean Difference (I- J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-11.27556	6.18878	.184	-26.7307	4.1796
	3	-5.31667	6.18878	.671	-20.7718	10.1385
2	1	11.27556	6.18878	.184	-4.1796	26.7307
	3	5.95889	6.18878	.607	-9.4963	21.4140
3	1	5.31667	6.18878	.671	-10.1385	20.7718
	2	-5.95889	6.18878	.607	-21.4140	9.4963

# **Homogeneous Subsets**

#### **Amount**

Tukey HSD<sup>a</sup>

		Subset for alpha = 0.05	
Group	N		
1	9	8.1922	
3	9	13.5089	
2	9	19.4678	
Sig.		.184	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

#### Means Plots

