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Processing, chemical composition and uses of matcha

tea - a review

BACHELOR'S THESIS

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Author: Žaneta Tejmlová

Supervisor: Ing. Olga Leuner, Ph.D

Declaration

I hereby declare that I have done this thesis entitled “Processing, chemical composition and uses of matcha tea - a review” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 18.4.2024

.....

Žaneta Tejmlová

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Abstract

Matcha is a powdered form of green tea, usually extracted from *Camellia sinensis var. sinensis tea*, which is popular for the sweeter and richer taste of the resulting matcha tea. A key step in obtaining matcha tea is to artificially shade the tea plantation, providing more than 90% even shade, for the last 3 to 4 weeks before hand-picking the tea leaves. After harvesting, the tea leaves are subjected to careful processing and then crushed by one of the appropriate matcha processing methods as the final step to obtain the powdered form of the tea.

During the straining process, chlorophyll accumulates in the matcha tea, which then causes the typically distinctive green colour of the final product, in addition to its antioxidant and anti-inflammatory properties. In terms of chemical composition, matcha is also a very important source of catechins, led by EGCG, which are known for their antioxidant and health-promoting effects. Matcha also contains high levels of L-theanine, an amino acid that promotes relaxation and mental well-being. These compounds, along with caffeine, contribute to matcha's unique flavor profile and stimulating effects.

Due to its chemical composition and tradition, matcha is widely used in the traditional Japanese tea ceremony, and its vibrant colour, earthy flavour and nutritional values make it a versatile ingredient in a variety of dishes, as a garnish or as an effective topping for certain ingredients. It is also used as a health-promoting superfood that fights diabetes and cancer, and finally matcha is used in cosmetic and skin care products for its antioxidant properties that promote skin health and rejuvenation.

Key words: *Camellia sinensis*, shading, EGCG, catechins

Abstrakt

Matcha je prášková forma zeleného čaje, obvykle získávaná z čaje *Camelia sinensis* var. *sinensis*, který je oblíbený pro sladší a bohatší chuť výsledného čaje matcha. Klíčovým krokem při získávání matcha čaje je umělé zastínění čajových plantáží, které poskytuje více než 90% rovnoměrného stínu, a to po dobu posledních 3 až 4 týdnů před ručním sběrem čajových lístků. Po sklizni jsou čajové lístky podrobeny pečlivému zpracování a následně jsou drceny jednou z vhodných metod pro zpracování matcha, jakožto poslední krok pro získání práškové formy čaje.

Během stínění se v matcha čaji hromadí chlorofyl, který pak způsobuje typicky výraznou zelenou barvu výsledného produktu a navíc má antioxidační a protizánětlivé účinky. Co se týče chemického složení, matcha je také velmi významným zdrojem katechinů, v čele s EGCG, které jsou známé svými antioxidačními a zdraví prospěšnými účinky. Matcha také obsahuje vysoké množství L-theaninu, což je aminokyselina, která podporuje relaxaci a duševní pohodu. Tyto sloučeniny spolu s kofeinem přispívají k jedinečnému chuťovému profilu a povzbuzujícím účinkům matcha.

Matcha má díky svému chemickému složení a tradici pak široké využití v tradičním „Japonském čajovém ceremoniálu“ a díky své zářivé barvě, zemité chuti a výživovým hodnotám je univerzální přísadou do různých pokrmů, jakožto přísada nebo efektivní náhrážka některé suroviny. Dále se uplatňuje jakožto zdraví prospěšná superpotravina, která bojuje proti cukrovce a rakovině a nakonec se matcha používá v kosmetických přípravcích a v péči o pleť pro své antioxidační vlastnosti, které podporují zdraví a omlazení pleti.

Klíčová slova: *Camellia sinensis*, stínění, EGCG, katechiny

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List of the abbreviations used in the thesis

MVD – microwave-vacuum drying

SMM – stirred media mill

EGCG - epigallocatechin gallate

EC - epicatechin

ECG – epicatechin gallate

EGC - epigallocatechin

MGT – matcha green tea extract

GTP – green tea polyphenol

1. Introduction

Matcha green tea is one of the most famous tea products, available in powder form. Derived from young tea shoots or leaves, matcha is primarily sourced from *Camellia sinensis* var. *sinensis*, commonly known as the China-type tea plant. However, certain regions find *Camellia sinensis* var. *assamica*, referred to as the Assam-type, more suitable for cultivation (Manikharda et al. 2023).

Originating during the Tang Dynasty in China, matcha found its true refinement and widespread adoption during 12th century in Japan (Huang et al. 2024). Initially, the practice was restricted to temples, where matcha was used to enhance meditation sessions. However, over the following centuries matcha gained popularity among both the elite and the general public, becoming an integral part of social gatherings and ceremonial occasions alike (Dreher 2018).

Matcha differs from other teas not only in its cultivation but also in its processing and preparation methods (Dreher 2018). Unlike conventional tea leaves that are cultivated under direct sunlight, leaves undergo a unique shading process few weeks before harvesting (Xiao et al. 2021). Cultivated primarily in the shade, matcha results in a tea known for its reduced astringency and robust vegetal flavor (Dreher 2018).

The shading technique stimulates the production of chlorophyll and amino acids within the tea leaves and subsequent processing starting with fixing tea leaves prevents fermentation and chlorophyll degradation (Lee et al. 2015; Xiao et al. 2021). Next tea leaves go through drying, refining and grinding processes to transform leaves into powder form (Phuah et al. 2023).

As a result of these techniques, matcha exhibits high levels of chlorophyll and amino acids, contributing to its vibrant green color and smooth, mellow flavor (Xiao et al. 2021). The finest ground matcha with the smallest particle size has been noted to exhibit enhanced antioxidant and anti-inflammatory properties, with heightened content of phenolic compounds (Phuah et al. 2023). Among these compounds, polyphenols such as catechins, with EGCG being the most significant one and phenolic acids, are a very important component of matcha contributing significantly not only to their robust antioxidant potential but to their beneficial

impact on human health as well (Kim et al. 2020). Additionally, matcha tea contains essential amino acids like theanine and gamma-aminobutyric acid (GABA), known for their relaxation and stress-relieving effects. The presence of caffeine further enhances the functional aspects of tea formulations, providing a natural energy boost and cognitive stimulation. Together, these bioactive compounds make matcha tea a valuable product not only as a part of an essential element of Japanese tea ceremonies, but also in various sectors such as cuisine, beverages, health products, and everyday essentials (Devkota et al. 2021; Huang et al. 2024).

2. Aims of the Thesis

The aim of this bachelor thesis was to summarize and organize all the available literature about processing, chemical composition and uses of matcha tea. The emphasis was mainly on the use of shading treatment, grinding methods, significant chemical components and matcha as an enrichment in human diet.

3. Methodology

This bachelor thesis is a literature review. Therefore, the used information is from peer-reviewed books and scientific articles from databases ScienceDirect, Pubmed, Scopus and Google Scholar, mostly with the use of keywords “*Camellia sinensis*”, “shading”, “EGCG” and “catechins”

4. Literature Review

4.1. Green tea

Tea, being the second most consumed beverage in the world, functions not only as a leisurely drink, but also as an integral part of many cultures (Phuah et al. 2023).

Green tea, the most widely consumed type, dominates the tea production while also sets the foundation for processing other varieties (Qin et al. 2024). Based on Statista's data, the global tea consumption stood at approximately 6.3 million tons in 2022 and is projected to reach 7.4 million tons by 2025 (Sun et al. 2024).

Highest production and consumption of green tea occurs in China (Qin et al. 2024). Originating in Southeast China, tea cultivation after then expanded to India, Sri Lanka, and other numerous tropical and subtropical countries. The tea plant is now cultivated in about 30 countries worldwide, thriving in tropical and subtropical regions with sufficient rainfall, good drainage, and slightly acidic soil (Sarma et al. 2023).

Beyond its popularity, the health-related aspects of green tea such as tea polyphenols and caffeine content, are closely linked to human well-being. Moreover, consumers are drawn to green tea significantly due to its aroma which is commonly depicted as tender, faint-scented, chestnut-like, and orchid-like (Qin et al. 2024).

Over 260 volatile compounds have been examined and identified in green tea with the majority forming during processing. Fixation, a crucial step in green tea processing then plays a vital role in shaping its quality (Qin et al. 2024).

Furthermore, we can distinguish two primary tea varieties based on the environment where it is cultivated, biochemical affinities, leaf size and position (Phuah et al. 2023).

Camellia sinensis var. sinensis (China tea) is extensively grown in China, Japan, and Taiwan. India is the second-largest global tea producer after China. The primary tea regions in India are Darjeeling, Nilgiri, Dooars, and Assam contributing over half of India's tea production. *Camellia sinensis var. assamica* (Assam tea) is dominant in south and Southeast Asia, including Malaysia and more recently, Australia (Sarma et al. 2023).

Although *Camellia sinensis var. assamica* can be processed into matcha tea, it is not a popular choice for matcha production. The heightened phenolic content in the product is

favorable for health but also contributes to the development of a bitter taste, making it an unpopular variety (Manikharda et al. 2023).

The preferred and more popular variation for matcha production is *Camellia sinensis* var. *sinensis* attributed to its enhanced sweetness (Phuah et al. 2023).



Figure 1. Left photo: Subglobose capsule ; Middle photo: Dried green tea leaves; Right photo: Graded green tea leaves

4.2. History of matcha

Matcha's history dates back to the Tang Dynasty in China but reached its refined and widely popular form in Japan during the 12th century (Topuz et al. 2014; Huang et al. 2024). At the time matcha was considered as a superior product served only to prominent figures in the society. A Zen priest named Myoan Eisai introduced tea leaf grading and grinding techniques from the Song dynasty to Japan around year 1191. Eisai then initiated a tea plantation in Kyushu and acquainted his friend, Myoe, in Uji. This has brought forth new observations in tea cultivation resulting in the first use of a shading technique named "Tana" established in the 16th century. Two centuries later, a new tea processing method named "Uji green tea processing method" was developed by Soen Nagatani providing a more refined version of the product. This method effectively inhibits the fermentation process and preserves the green color along with getting rid of the grassy taste through the application of steaming. Despite the remarkable advance in technology, both methods are still in use to this day as a crucial step for producing matcha (Phuah et al. 2023).

4.3. Matcha processing

4.3.1. Cultivation of matcha

Matcha is an unfermented, fine-ground Japanese green tea. Put another way, Matcha is a category of green tea (Phuah et al. 2023).

Unlike regular tea cultivated in an open area without the need for any shade, matcha tea is obtained from fresh tea leaves shaded during their growth and subsequently transformed into a powdered form (Huang et al. 2024). Typically, premium matcha is crafted from young tea leaves, resulting in its vibrant green colour, sweet flavor, velvety texture, and abundant antioxidants and amino acids (Phuah et al. 2023)

Given Japan's location in the northern hemisphere, the dormant phase of *C. sinensis* var. *sinensis* typically spans from mid November until early March, with buds beginning to sprout as temperatures gradually rise. However, sudden drops in ground temperature during spring, especially below freezing, pose a risk of significant damage to the tea leaves, potentially resulting in poor-quality leaves (Phuah et al. 2023).

The production of matcha typically involves a sequence of six steps (Fig. 2). Initially, *C. sinensis* var. *sinensis* tea plants in Japan are cultivated under controlled conditions of 11.5–18°C, receiving an annual rainfall of approximately 1500–2000 mm. High-quality tea leaves are primarily harvested from elevated altitudes, typically ranging from 2200 to 3000 m, on hills with slopes of around 0.5–10 degrees. Tea plants are receiving direct sunlight for a minimum of five hours or 11 hours of indirect sunlight. Regarding the cultivation area, it is preferable that the soil in different geographical locations maintains a low pH, excellent aeration, a sandy texture, and is enriched with humus. Significant regions for matcha-producing in Japan are Kawane, Tenryu, Uji, and Nishio (Phuah et al. 2023). The initial processing stages of matcha such as steaming and drying closely resemble those of other green teas (Rezaeian & Zimmermann 2022).

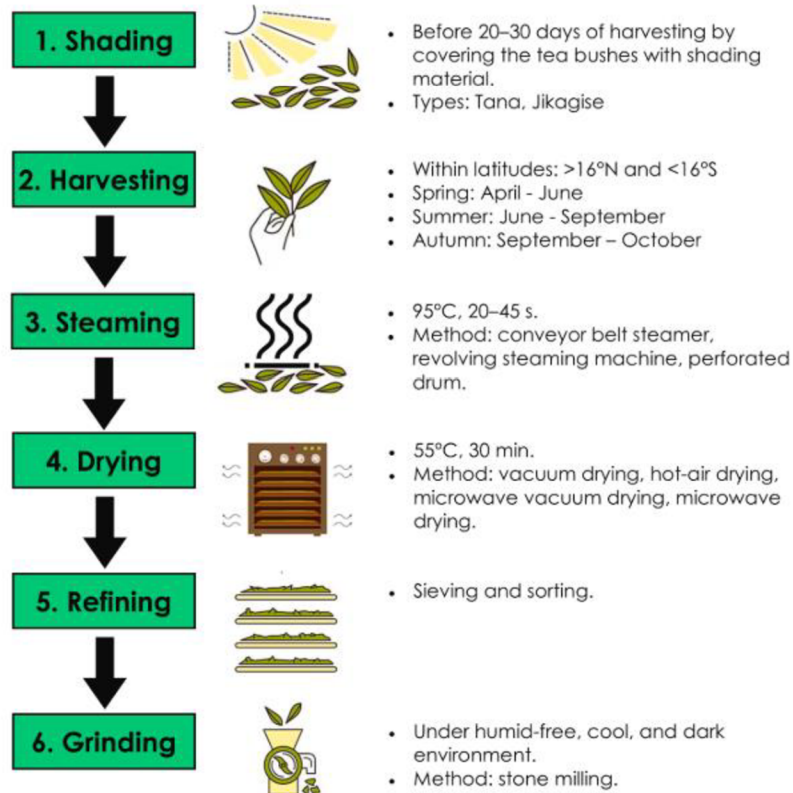


Figure 2. Matcha processing steps and descriptions (Phuah et al. 2023).

4.3.2. Shading

The shading practice, commonly employed to reduce sunlight intensity in the cultivation of perennial crops like coffee, cacao, and tea, serves multiple purposes. By applying shade to tea plants, young shoots are safeguarded against frost and extends the harvesting period, facilitating the production of premium-grade tea (Yang et al. 2012). Furthermore, this process significantly influences critical aspects such as the color and chemical composition of emerging shoots (Manikharda et al. 2023).

To induce growth in the absence of light, shading techniques manipulate light transmission treated as a crucial factor in governing the growth and development of tea. The degree of light intensity is closely tied to triggering the plant's secondary metabolic processes, including the synthesis of phenolic compounds (Manikharda et al. 2023).

These shading practices have the potential to reduce the presence of bitter tasting caused by phenolic compounds in tea while enhancing the aroma. This leads to tea leaves with a more tender texture and reduced fibrous composition, desirable qualities for matcha production. The decreased fiber content facilitates the extraction of cell fluid, aids in the drying process, and

eases the grinding of dried leaves into powder using a stone mill at a reduced speed (Manikharda et al. 2023).

However, the decrease in phenolic content could potentially diminish the bioactivity of the produced tea. Frequent use of shading practices can also induce stress in tea plants and reduce photosynthetic ability (Manikharda et al. 2023). Moreover, prolonged shading might induce tea plants to accelerate their reproductive development, potentially resulting in a reduction in biomass (Yang et al. 2012).

During the cultivation of tea plants, two shading techniques are utilized: ecological and cover shading. Ecological shading utilizes natural approaches such as planting trees in gardens, interplanting with economically valuable forest trees or using tall appropriate tree species to provide shade. On the other hand, cover shading is an artificial technique, primarily achieved through the use of porous covers made from diverse materials. Among the materials used in cover shading are greenhouse covers, sunshade nets made from cereal straw, wheat straw and other suitable artificial materials (Elango et al. 2023).

Ecological shading, achieved through the presence of trees, offers numerous benefits. These include the alteration of microclimate conditions, resulting in reduced radiation, temperature, and wind velocity, while simultaneously increasing relative humidity levels. Natural shading has also a wide array of positive effects on tea plants, enhancing photosynthesis and various physiological processes, ultimately leading to increased growth and yield (Elango et al. 2023). While this method may be ideal alternative for cultivating other crops that thrive in everyday shade, it is not suitable for matcha production due to its distinct cultivation requirements and shading needs (Yang et al. 2012; Sonobe et al. 2018; Phuah et al. 2023; Elango et al. 2023).

Tea for matcha production is grown under artificial shade on average for 3 weeks as the final stage of cultivation before harvesting. Gyokuro and Tencha, acknowledged as Japan's finest teas, are sourced from leaves cultivated under ceiling-shelf covering. While field-cultivated tea plants of the Yabukita variety, which is highly esteemed as Japan's most favored tea cultivar, were shaded using canopies, exploring various levels of shading. (Yang et al. 2012). Another two shading methods were described and used in a scientific paper by Phuah et al. (2023) in creating matcha tea, Tana and Jikagise. Large-scale plantations mostly employ Tana shading, utilizing woven wire mesh, while Jikagise shading is applied in small-scale

settings using cloth (Phuah et al. 2023). Plantations are usually covered to provide 70-95% shade to achieve required attributes (Sonobe et al. 2018).

In experiment by Huang et al. (2024) were designated experimental plots, shielded from direct sunlight. The area was spanned of approximately 40 m² each, and used plots were randomly replicated three times. To establish the shaded setting, a shading net with approximately 90% coverage was positioned roughly 15 cm² above the topmost leaves of the tea plants. This shading routine began when the new shoots had reached the stage of one bud and two leaves and lasted for a period of 21 days (Huang et al. 2024).

4.3.3. Harvest

Following the shading period, new shoots consisting of one bud and five leaves are harvested typically in three primary harvesting seasons: autumn, spring, and summer (Phuah et al. 2023; Huang et al. 2024) In general, harvested leaves usually consist of three to five leaves along with a single bud (Yang et al. 2012; Phuah et al. 2023).

Tea plantations pick young tea shoots containing satisfactory number of leaves either manually or mechanically (Phuah et al. 2023). Recently there is a decline in the number of proficient tea harvesters in China, leading to inconsistency in the quality of the fresh tea leaves picked by unskilled workers. However, existing equipment fails to meet the stringent standards for sorting premium tea raw materials (Gan et al. 2023). So even though mechanical plucking is quicker method, hand-plucked tea leaves are still utilized for producing higher-quality tea (Phuah et al. 2023).

4.3.4. Drying

Following harvest, fixation stands as a crucial step with a significant impact on its overall quality. Shaded green tea processing methods primarily fall into two categories based on fixation techniques: pan-fired green tea and steamed green tea (Tan et al. 2019; Qin et al. 2024). The usage of one of the methods is influenced by consumer preferences, cultural inclinations, and cost-effectiveness. Green teas processed through steaming exhibit a greenish colour, along with vegetal notes, a grassy flavor, and a hint of bitterness, compared to pan-fired green tea varieties (Adhikary et al. 2023).

Tea leaves go through immediate steaming or pan firing to prevent fermentation of unfermented leaves and preserve freshness (Lee et al. 2015; Phuah et al. 2023). This process

effectively halts the enzymatic activity of polyphenol oxidase (PPO), preventing any oxidation (Lee et al. 2015). Typically, in steam-fixing the temperature is maintained around 100°C. Leaves undergo a process where they are conveyed through a rotating drum, and hot steam is introduced for up to 2 minutes before the leaves are extracted once more. The crucial determinant at this stage is the quantity of steam utilized, as an excess can damage the leaves while too little may trigger fermentation prematurely. The steam treatment proves advantageous in preserving the antioxidant properties, as well as the appearance and texture of the final product, while also reducing processing time (Singh et al. 2014).

Freshly plucked tea leaves that go through pan-firing are processed at a temperature of $250 \pm 10^\circ\text{C}$ for a duration of 8 to 10 minutes, following leaf standards. Throughout the process, the leaves are gently tossed intermittently to prevent any surface burning, until they reached a dehydrated and dry appearance (Adhikary et al. 2023).

Drying is utilized to hinder microbial growth, mitigate unwanted chemical reactions, and additionally decrease enzyme activity (Liu et al. 2023). This method offers the benefits of uniform heating, deep penetration, and prevents the leaves from becoming scorched (Qin et al. 2024). Tea leaves go through the process of several cycles of steaming and cooling to achieve thorough drying and approximately half of the total moisture content is reduced during the drying phase (Lee et al. 2015; Phuah et al. 2023).

For example, in research by Manikharda et al. (2023) for matcha produce, leaves were initially heated in a rotary dryer at 80°C for 10 minutes, followed by a 6-minute cooling period at room temperature. Subsequently, to reduce moisture content, the leaves go through drying in a bed dryer at 80°C for 30 minutes (Manikharda et al. 2023). Drying is utilized to hinder microbial growth, decrease enzyme activity, and mitigate unwanted chemical reactions (Liu et al. 2023). Approximately half of the total moisture content is reduced during the drying phase (Phuah et al. 2023).

Raw materials are commonly subjected to sun-drying or industrial air-drying. However, to obtain the premium dried products available in the market, it is necessary to utilize more expensive methods such as freeze-drying and microwave-drying (Liu et al. 2023). Microwave and steam fixation methods facilitate quick heat transfer and thorough penetration, enhancing the greenish colour of the tea, as well as its liquor colour and the appearance of the infused leaves. However, they often yield an inferior aroma and an unpleasant taste. Hot-air fixation has a short heating time, fosters the development of a pleasant aroma and a smooth taste, but it

can lead to swift moisture loss from the leaf edges, resulting in charred edges and a yellowish coloration (Yu et al. 2023).

A relatively recent addition to drying techniques is microwave-vacuum drying (MVD) (Ando & Nei 2024). In contrast to conventional drying methods, microwave vacuum drying represents one of the emerging techniques in process of drying. MVD combines the benefits of both microwave and vacuum drying. As a result of that, this method is able to effectively preserve nutrients and provide relatively higher sensory quality (Liu et al. 2023). Studies have also demonstrated the benefits of MVD, such as its high drying velocity, reduced surface colour alteration and effective preservation of antioxidant activity (Ando & Nei 2024).

Microwave irradiation when using MVD causes water molecules within the product to become excited, generating heat and facilitating moisture transfer within the product. Because the boiling point of water decreases under lower pressures, the supplied microwave energy is effectively utilized for water evaporation. This mechanism of water transfer grants MVD the advantages of a high drying rate and prevention of excessive increases in sample temperature, which typically result in quality degradation (Ando & Nei 2024).

Of all the drying techniques, microwave vacuum drying is regarded as the most effective method in matcha production compared to other alternatives (Phuah et al. 2023). The leaves that have been fully dried are referred to as "Aracha," representing raw form of tea and are ready for further processing (Phuah et al. 2023).

4.3.5. Refining

Following steaming and drying, Aracha goes through a sieving process to eliminate impurities, yellowish leaves, and undesired elements (Phuah et al. 2023)

Sorting, being the follow up refining process, is based on physical attributes such as color, shape, and size of the tea leaves. The refined final product of this process is known as tencha - a steamed and dried form of tea. (Phuah et al. 2023; Liu et al. 2024)

Within the spectrum of tea varieties, only a few undergo the sorting process, where stems are separated from leaves, with examples including Tencha, Lu'an Guapian and Dahongpao (Xiao et al. 2021; Phuah et al. 2023). Stem-leaf separation is particularly vital in the production of tencha, aimed at achieving a matcha of exquisite greenness and fineness. In the cultivation of high-grade tencha, stem-leaf separation is meticulously managed throughout the entire process, starting from manual plucking of fresh leaves. Subsequently, stems are meticulously

separated from leaves multiple times using machinery, with a secondary manual inspection to ensure precision (Xiao et al. 2021).

. The separation of stems from leaves serves the purpose of achieving a fine particle size, a critical standard parameter in assessing powder quality. This fine particle size plays a pivotal role in determining the market value of matcha (Xiao et al. 2021).

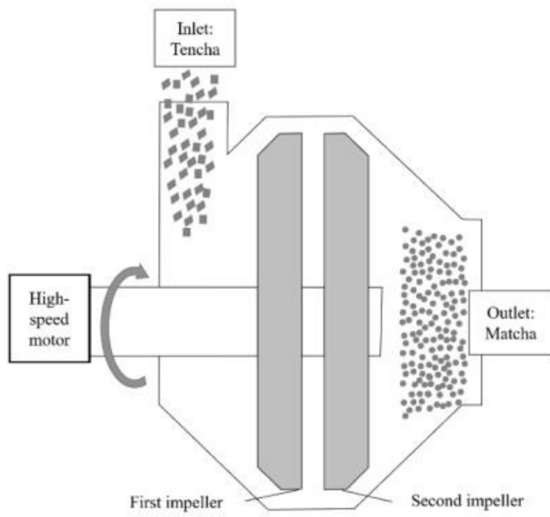
4.3.6. Grinding

The grinding process is a laborious and time-consuming procedure with a low yield (Phuah et al. 2023). To achieve the powder form, a variety of grinding methods can be used. Depending on type of milling technique, matcha changes its internal structure, resulting in different particle dimensions and appearance (Huang et al. 2022). The alteration in the physical characteristics of particles is directly linked to the reduction in particle size caused by particle breakage, leading to a cascade of effects. For instance, as particle size decreases, the mechanical attributes of matcha particles, such as particle collision velocity, the frequency of particle collisions, and the external force acting on the particle, go through changes. These aspects represent the three primary factors influencing the breakage of individual particles (Jiang et al. 2023). Moreover, grinding also affects the airiness of the tea, as well as the properties of its froth. These changes in matcha's chemical and physical anatomy suggest that the flavour and aroma are also influenced (Huang et al. 2022).

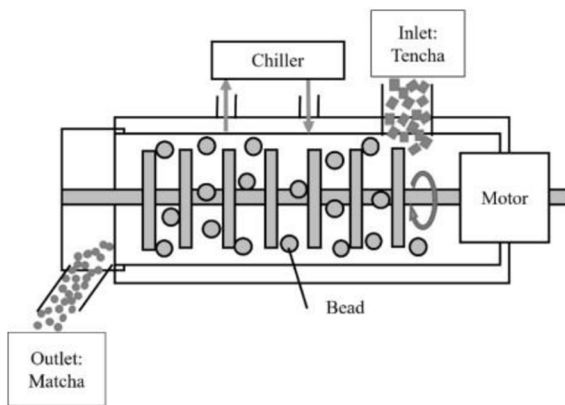
Research has shown that matcha samples with a smaller particle size have enhanced antioxidant and anti-allergenic activities (Phuah et al. 2023).

The current focus of research in matcha production lies in unravelling its bioactive components. Among the crucial processes, grinding stands out for its profound impact on the physical, chemical, and nutritional aspects of matcha, encompassing fluidity, color, taste, and aroma. The method and degree of grinding are crucial determinants of matcha quality, yet scholarly attention dedicated to this process remains limited (Jiang et al. 2023).

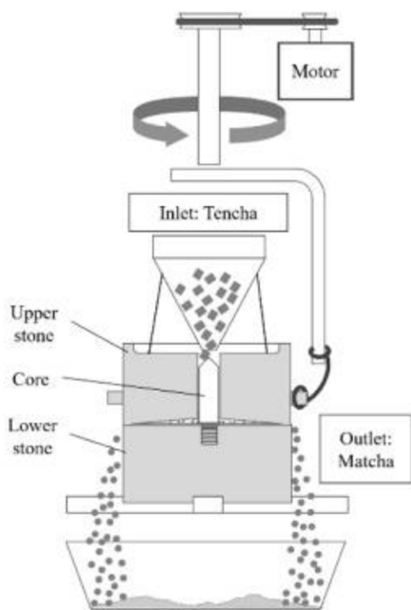
In the research by Huang et al. 2022 three milling techniques were examined– cyclone -, bead -, and stone – milling (Fig. 3), and their impact on the physical properties, non-volatile and volatile compositions, as well as the sensory characteristics of the matcha samples (Huang et al. 2022).



a)



b)



c)

Figure 3. Cyclone – (a), bead –(b), and stone – (c) milling techniques (Huang et al. 2022).

Cyclone milling employs the rapid rotation of an impeller to propel the tencha across a grinding ring, completing the process in less than 1 minute. Conversely, bead milling involves the rotation of a mechanical shaft to stir beads that aid in breaking down the tencha over a duration of 20 minutes. Stone milling, deemed more traditional, operates by crushing the tencha between two stone beds for up to 3 minutes. Throughout all three milling methods, the temperature was maintained at approximately 40 ± 5 °C (Huang et al. 2022).

While stone milling, a traditional approach, it falls short for large-scale production (Jiang et al. 2023).

Based on the microscopic images, was already evident that the cyclone-milled matcha exhibited a smaller size compared to both bead- and stone-milled matcha. This was further confirmed by the particle size distribution analysis, which indicated a lower mean value for cyclone-milled matcha. Additionally, a visual inspection revealed that the surface of stone-milled matcha was more uneven compared to cyclone- and bead-milled matcha. It was reported lower roundness and surface irregularities in stone-milled matcha due to the higher shearing force involved in the milling process. That finding led to conclusion that the milling process significantly influences the surface area and properties of matcha particles (Huang et al. 2022).

Other techniques employed in matcha preparation is ball and jet milling (Jiang et al. 2023).

Ball mill

Ball milling, known for its efficiency in particle size reduction, is widely used in large-scale matcha production in China (Shi et al. 2022). Ball milling stands out as one of the most employed methods for finely ground green tea leaves into powder. During the ball milling process, green tea is subjected to various mechanical forces such as impact, shearing and rolling, among others. These forces not only result in mechanical pulverization, but also trigger mechanochemical effects through energy conversion (Zhao et al. 2023). Mechanical pulverization influences parameters like particle size and specific surface area, while mechanochemical effects induce alterations in the functional constituents of green tea powder. These changes may include oxidation or degradation of the functional components during the ball milling process (Zhao et al. 2023).

So even though this method is considered as a traditional approach to remove agglomeration, it often results in irregular powder shapes and the incorporation of impurities (Wang et al. 2024).

A simulation of ball mill processing for matcha was conducted utilizing the established scaling model to explore the variations in the mechanical and physical attributes of matcha particles (Jiang et al. 2023). In the simulation model, there's a negative correlation between the size of matcha particles and their collision velocity. However, it's evident that the collision velocity doesn't exhibit continuous growth. Instead, as milling time prolongs, the average collision velocity of the particles tends to stabilize at a consistent value, alongside the stabilization of particle size distribution within a certain range (Jiang et al. 2023).

After 6 hours of ball milling time, a substantial increase in the number of smaller particles adhering to the surface of larger particles was observed. By the 10th hour of ball milling, large particles were scarcely visible, and the particles took on a flaky appearance with smooth surfaces. With ball milling durations surpassing 10 hours, a notable decrease in particle size and significant enhancement in uniformity were noted. The overall structure exhibited clustering with large aggregates attributed to the high specific surface area and interparticle interaction (Zhao et al. 2023).

The observation also revealed a notable escalation in the cell breakage rate over time. This indicates a significant level of damage inflicted on the tissues and cells of matcha throughout the process (Jiang et al. 2023).

The thermal effects of ball milling can degrade active components, posing challenges to maintain nutritional and functional qualities. The time-intensive nature of ball milling, requiring over 4 hours to achieve the desired quality, raises concerns. Based on mentioned disadvantages, it makes sense to look for more efficient method with high energy density and low process temperature (Jiang et al. 2023).

Jet mill

Jet milling stands out as an advanced technique for powder processing, renowned for its cost-effectiveness, high efficiency, and non-polluting (Palaniandy et al. 2009; Wang et al. 2024). The jet mill offers numerous benefits over other high-energy mills, such as the capability to generate micron-sized particles with a precise size distribution and the capacity to grind materials sensitive to heat (Palaniandy et al. 2009). Its main disadvantage is its high energy consumption, with only 2% of the energy supplied being used to form new surfaces, leading to an increase in total energy consumption as the particles become smaller (Palaniandy et al. 2009).

However, in the research by Jiang et al. (2023) there's consensus with research by Palaniandy et al. (2009) and Wang et al. (2024) that jet milling offers advantages like minimal temperature rise and facing challenges of higher energy usage, but article differs in statement of creating even higher dust pollution than other methods, while producing matcha (Jiang et al. 2023).

Stirred media mill

Another grinding technique, stirred media mill emerges as a potential solution, offering advantages in energy consumption and particle size distribution (Jiang et al. 2023).

SMM introduce an innovative grinding technique, utilizing grinding media such as zirconia beads or stainless-steel balls to diminish particle size through collision, compression, and shear effects. The experimental configuration encompassed an XCFB model stirred media mill, comprising a grinding chamber, cooling water unit, and material collection system. The pivotal component of this setup was the grinding chamber, which employed a highly efficient large-area jacketed water circulation system for cooling. Within the SMM, the agitator generates concentrated energy in the grinding chamber, leading to expedited achievement of the desired particle size (Jiang et al. 2023).

According to earlier studies, the stirred media mill has consistently proven to be more proficient in achieving finer grinding results than conventional ball milling techniques, when grinding finer powders like matcha. Stirred media mills offer energy-saving benefits at comparable grinding levels while providing a wider particle size distribution, attributed to the high-density frictional force within the mill, resulting in finer particle production. These findings indicate that the stirred media mill utilized in this investigation is more adept at disrupting tissues and cell walls compared to traditional ball milling methods (Jiang et al. 2023).

Nevertheless, there remains a scarcity of research on employing SMM for the production of high-quality matcha with desirable characteristics. Further exploration is essential to scrutinize the impact of SMM processing on the physicochemical properties, nutritional quality, and aroma profiles of matcha (Jiang et al. 2023).

In the research by Huang et. al (2022) the particle size, distribution and shape were determined based on the matcha samples milled using the three different methods (Huang et al. 2022). Target particle size while producing matcha is below 18 μm , which all the referred grinding methods meet (Palaniandy et al. 2009; Huang et al. 2022; Zhao et al. 2023; Jiang et al.

2023). However, the top-quality tea leaves are finely ground into a powder with a particle size ranging from 5 to 10 μm to create matcha.

The mean, median, and d90 values (where 90% of the particles fall below this value) for the particle size distribution were as follows:

Table 1. Particle size distribution of matcha, using different milling methods

Methods	Particle size (μm)	Duration (min)
Cyclone-milled	8.93 ¹ ; 7.42 ² ; 16.92 ³	1
Bead-milled	19.57 ¹ ; 9.03 ² ; 35.53 ³	20
Stone-milled	15.87 ¹ ; 8.65 ² ; 27.56 ³	3
Ball - milled	17.9 ² ; 12.7 ² ; 10.6 ²	1080; 1440; 1800
Jet - milled	4.75 ²	30
Stirred media mill	14.1 ²	140

Footnote: ¹ : mean; ² : median; ³ : d90

References: (Palaniandy et al. 2009; Huang et al. 2022; Zhao et al. 2023; Jiang et al. 2023)

The mean particle size values recorded for stone-milled matcha fell within the range 15.0 to 18.3 μm . Cyclone-milled matcha exhibited a narrower distribution curve compared to bead- and stone-milled matcha. Moreover, the mean value was closer to the median value for cyclone-milled matcha, indicating a particle size distribution that closely followed a normal distribution and was more uniform in size. Furthermore, bead- and stone-milled matcha showed particles with larger diameters in the range of 50–200 μm , which were absent in cyclone-milled matcha. This suggests that matcha particles from cyclone milling were more uniformly sized (Huang et al. 2022). During the grinding process, there was a notable rise in the cell breakage rate from 8.81% to 92.65% after 140 minutes, indicating substantial harm to the tissues and cells of matcha. In contrast, a prior investigation, recorded a cell wall breakage ratio of 90.24% for tea powder after 8 hours of ball milling (Jiang et al. 2023).

Based on the comparison of methods in Table 1, the fastest grinding method with the smallest mean particle size distribution have been observed in cyclone milling and the widest particle size distribution is obtained using bead milled technique, resulting in finer particle production.

To measure dynamic behaviour of particles, the discrete element method (DEM) was introduced. Within DEM, individual particle motion within a granular system is tracked using an explicit central difference scheme. This method meticulously observes interactions among elements (particle-particle, particle-grinding medium, and particle-wall) at every contact point within the system (Zhao et al. 2024)

After its drying and milling processes are completed, only finely ground powder is left. Matcha powder is typically characterised by a more vibrant colour compared to regular green tea powder, accompanied by a finer and smoother texture (Phuah et al. 2023). While regular tea is only made into a decoction, matcha is becoming a mixable additive with water (Devkota et al. 2021).

4.4. Chemical composition of matcha tea

Matcha tea comprises approximately 60-70% insoluble components, including fat-soluble vitamins, insoluble dietary fibers, chlorophylls, and proteins. Meanwhile, soluble components make up 30-40% of its composition, consisting of polyphenols, water-soluble vitamins, caffeine, water-soluble dietary fibers, amino acids, saponin, and minerals. The distinctive cultivation and harvesting methods contribute to higher concentrations of bioactive compounds in matcha compared to other green teas (Rubel Mozumder et al. 2021; Sokary et al. 2023).

Matcha contains phenolic acids, flavanols, amino acids and caffeine in a similar compositional profile to typical green tea. However, the concentration of these constituents is significantly higher in matcha (Phuah et al. 2023). The quality of matcha, is determined by the harmonious combination of various components such as theanine, catechins and caffeine which also act as key components to help relax. (Sokary et al. 2023; Phuah et al. 2023). Furthermore, matcha is recognised for its antioxidant and anti-inflammatory properties, largely due to epigallocatechin gallate (EGCG). Numerous experimental studies have documented the beneficial effects associated with matcha consumption (Phuah et al. 2023).

4.4.1. Phenolic compounds

The heightened curiosity surrounding the composition of matcha stems from its remarkable antioxidant activity, which correlates directly with its elevated phenolic content (Lorenzo & Munekata 2016).

Phenolic compounds are synthesized by plants as a response to various ecological and physiological stresses, including pathogen attacks, insect infestations, UV radiation exposure, and physical damage. These compounds are characterized by an aromatic ring structure with one or more hydroxyl groups attached (Khoddami et al. 2013; Phuah et al. 2023).

Plant phenolics are broadly classified into two categories: simple phenols and polyphenols, depending on the number of phenol units present in the molecule. Thus, the diverse array of plant phenolics includes simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids, and flavonoids (Khoddami et al. 2013)

Polyphenols

The health benefits of green tea are due to its abundance of natural antioxidants, particularly polyphenols. These make up to 30% of its dry weight. These polyphenols are thought to possess potent antioxidant properties similar to vitamins like C and E, carotene, and tocopherol. The concentration of these beneficial compounds in tea drinks varies depending on factors such as tea type, leaf quantity, brewing temperature, and duration (Kochman et al. 2020).

It was reported a total polyphenolic content in matcha tea ranging from 169 to 273 mg/g. However, it was noted as a lower polyphenol level in matcha compared to other green teas, possibly attributed to the shading of tea plants, which inhibits polyphenol synthesis. On the other hand, green teas boast substantially higher catechin content compared to black teas, with levels ranging from 5.46 to 7.44 mg/g, in contrast to 0–3.47 mg/g in black tea (Kochman et al. 2020).

Phenolic acids

Phenolic acids are another prominent class of phenolics in matcha, typically in the form of esters, glycosides or amides, although rarely in free form. The diversity of phenolic acids is due to variations in the number and position of hydroxyl groups on the aromatic ring. These compounds are derived from two parent structures: hydroxycinnamic and hydroxybenzoic acids. Hydroxycinnamic acid derivatives include ferulic, caffeic, p-coumaric and sinapic acids, while hydroxybenzoic acid derivatives include gallic, vanillic, syringic and protocatechuic acids (Khoddami et al. 2013).

In tea leaves, this subclass tends to be less prominent, with concentrations of compounds below those typically observed for flavanols (Lorenzo & Munekata 2016).

Chlorogenic acid was identified as the primary phenolic acid in matcha. Studies reported that chlorogenic acid levels are ranging from 2640 - 2910 $\mu\text{g/g}$ in water-extracted matcha samples. Additionally, their study revealed the presence of other phenolic acids such as sinapic acid (96–592 $\mu\text{g/g}$), gallic acid, ellagic acid, protocatechuic acid, ferulic acid, p-hydroxybenzoic acid, and caffeic acid. The detection of considerable amounts of tannic acids in matcha suggests that the tea leaves may not have been shaded adequately (Phuah et al. 2023).

Flavonoids

Flavonoids represent a diverse group of phenolic compounds found abundantly across various plant tissues. This extensive family encompasses flavones, flavonols, iso-flavonols, anthocyanins, anthocyanidins, proanthocyanidins, and catechins (Khoddami et al. 2013; Phuah et al. 2023). Derived from the aromatic amino acids phenylalanine and tyrosine, all flavonoids share a characteristic three-ringed structure. Their structural diversity stems from intricate modifications involving hydroxylation, prenylation, alkylation, and glycosylation reactions, which impart distinctive properties to the basic molecule (Khoddami et al. 2013).

Within matcha, flavanols emerge as the most prevalent flavonoids and are led by EGCG, the primary flavonoid in matcha (Phuah et al. 2023).

Catechins

Catechins are the most abundant phenolic compounds in matcha (Fig.4), accounting for approximately 80% of the total polyphenols present (Sugimoto et al. 2021). Shielded from sunlight before harvest, matcha tea exhibits lower catechin levels than green teas sourced from leaves exposed to sunlight. However, once dissolved in water, matcha releases approximately three times more catechins than loose-leaf green tea (Sokary et al. 2023).



Figure 4. Chemical structure of catechin (Luo et al. 2023).

Catechins are a very important component of matcha contributing significantly to their robust antioxidant potential. It is also known for its beneficial impact on human health such as

notable reduction in fasting blood glucose levels, neuroprotection, inhibition of fat accumulation and alleviation of hypersensitivity reactions (Kim et al. 2020; Kochman et al. 2020).

Catechins derived from tea are known for their remarkable ability to neutralize free radicals and enhance the detoxification function of enzymes like glutathione peroxidase, catalase, and glutathione reductase. They even surpass glutathione, vitamin C, and flavonoids in antioxidant potency, highlighting their pivotal role in cellular redox balance and making matcha a significant dietary source of catechins (Kochman et al. 2020).

Green tea contains four primary catechins, namely (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG), with EGCG being the most abundant and active (Fig.5). These polyphenols exhibit superior ability to counteract free radicals compared to vitamin C alone, (Kochman et al. 2020; Huang et al. 2022; Luo et al. 2023).

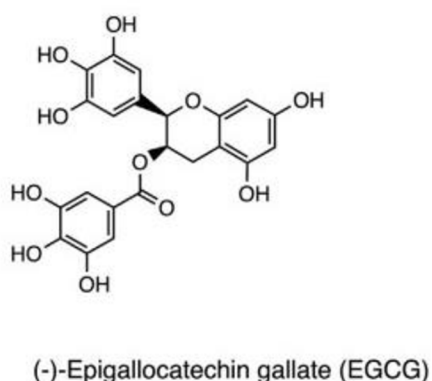


Figure 5. Chemical structure of Epigallocatechin gallate (EGCG) (Luo et al. 2023).

Rutin

Rutin emerges as the key flavonoid, followed by myricetin, quercetin, luteolin, and kaempferol (Phuah et al. 2023). Rutin is a polyphenolic compound abundant in matcha green tea that exerts potent antioxidant effects. It also interacts synergistically with ascorbic acid, enhancing their protective roles in cardiovascular health by fortifying blood vessels. Additionally, its antidiabetic and anti-inflammatory properties offer preventive measures against diabetes-related complications and conditions originating from oxidative stress and inflammation, including neurodegenerative disorders. Research highlights matcha's exceptional richness in rutin, surpassing even renowned sources like buckwheat. Contrasting rutin levels

between matcha (1968.8 mg/L) and buckwheat (62.30 mg/100 g), they suggest matcha's potential as a superior dietary source of this beneficial compound (Kochman et al. 2020).

Quercetin

Quercetin, a potent phytochemical renowned for its antioxidant properties, also demonstrates neuroprotective effect. Moreover, it exhibits a regulatory role in carbohydrate metabolism, modulating glucose absorption in the gastrointestinal tract, enhancing insulin secretion, and improving tissue sensitivity to insulin. Furthermore, when combined with (-)-epigallocatechin gallate (EGCG), quercetin's anticarcinogenic effects are potentiated. The content of quercetin in matcha's aqueous extract is 1.2 mg/ml, slightly higher than that in traditional green tea (1.1 mg/ml). However reported quercetin levels in alcoholic extracts of matcha is reaching up to 17.2 µg/g (Kochman et al. 2020).

4.4.2. Caffeine

As a central element in tea beverages, caffeine content in matcha tea, contributes significantly to its distinctive taste and desirable properties, while also enhancing their antioxidant capacity. Variations in caffeine levels can be related to factors such as plucking time, tea type and environmental conditions during growth, as well as brewing techniques (Kochman et al. 2020). Moreover, matcha's elevated caffeine levels stem from the greater caffeine content found in the buds and young leaves of tea plants, compared to their mature leaves (Sokary et al. 2023). With its antioxidant properties, caffeine combats reactive oxygen species, boosts antioxidant enzyme activity and increases total glutathione levels, which may reduce oxidative stress-related disorders. Furthermore, caffeine has anti-inflammatory properties by potentially suppressing the release of pro-inflammatory cytokines (Kochman et al. 2020).

Matcha, known for its higher caffeine content than other green teas, has a distinctive aroma and flavour profile (Kochman et al. 2020). Caffeine plays a role mainly in the perceived bitterness of green tea infusions (Huang et al. 2024). While green teas typically contain 11.3-24.67 mg/g of caffeine, matcha stands out with concentrations ranging from 18.9 to 44.4 mg/g. In comparison, most coffee beans contain approximately 10.0-12.0 mg of caffeine per gram (Kochman et al. 2020).

4.4.3. Chlorophyll

Chlorophylls, lipophilic phytochemicals, play a significant role in the metabolic processes of tea leaves. While various types of chlorophyll exist, chlorophyll-a and -b are typically found in plant tissues, with chlorophyll-a concentration being double or triple that of chlorophyll-b (Phuah et al. 2023). Research has indicated high levels of chlorophyll-a (2733.33 $\mu\text{g/g}$) and chlorophyll-b (1467.50 $\mu\text{g/g}$) in matcha, surpassing other tea samples with the highest chlorophyll content reported in matcha at 3580 $\mu\text{g/g}$ (Phuah et al. 2023; Huang et al. 2024).

The chlorophyll content in tea leaves heightens due to the cultivation method involving shading (Kochman et al. 2020). Chlorophyll also enhances the attractive green colour of green tea which is a factor that can influence consumer choice. (Huang et al. 2024).

Its derivatives are renowned for their potent antioxidant and anti-inflammatory properties. Determination of the content of bioactive compounds, including chlorophyll, in Tencha type tea leaves specifically for matcha production was carried out. The chlorophyll content in Tencha leaves surpassed that of conventional green tea, measuring 5.65 mg/g and 4.33 mg/g, respectively (Kochman et al. 2020).

4.4.4. Amino acids

Typically, matcha has a higher abundance of bound amino acids compared to free amino acids. Glutamic acid predominates among the bound amino acids, while theanine is the major free amino acid in matcha. Studies indicate that the levels of free amino acids in regular green tea samples surpass those found in matcha, including amino acids like theanine, arginine, and glutamine. Free amino acids constituted approximately 4% of the plant's dry weight (Phuah et al. 2023).

The content of free amino acids in tea, such as theanine, glutamate, threonine and aspartate, collectively enhance the 'umami' flavour of green tea, a characteristic attributed to the abundant amino acid content of matcha (Sokary et al. 2023; Huang et al. 2024). Overall, these constituents play a pivotal role in shaping the flavor and overall character of matcha (Huang et al. 2024).

Theanine

The primary amino acid detected was theanine (Huang et al. 2022). Elevated theanine content gives matcha its distinctive non-bitter taste and, when combined with caffeine, imparts the tea's characteristic umami flavor. The synergy between theanine and caffeine may enhance

focus, alertness, and more effectively than either compound alone, while also mitigating stress. Some studies revealed a theanine content of 6.1 mg/l in matcha tea infusions, whereas other found levels as high as 44.65 mg/g (Kochman et al. 2020; Huang et al. 2022).

4.4.5. Organic acids

Six different organic acids were identified, with citric acid exhibiting the highest concentration (Huang et al. 2022).

Organic acids are multifunctional, contributing to various aspects such as acidification (e.g., tartaric, malic, citric, and ascorbic acids), antioxidation (malic, citric, and tartaric acids), preservation (sorbic and benzoic acids), and improvement of sensory qualities (malic, citric, acetic, and tartaric acids) upon their integration into food products (Das et al. 2019).

The organic acid profiles of matcha, as assessed through high-performance liquid chromatography (HPLC) and gas chromatography (GC). GC analysis revealed oxalic acid as the most abundant organic acid in matcha, succeeded by citric and malic acids. Conversely, in a separate investigation, HPLC analysis identified lactic acid as the predominant organic acid in matcha, followed by citric acid, propionic acid, and acetic acid (Phuah et al. 2023).

4.4.6. Vitamins

Vitamins play a crucial role in supporting various physiological functions and metabolic processes. Among them, vitamins B, C, and E constitute approximately 5% of the dry weight of matcha (Phuah et al. 2023).

Content of Vitamin C

Vitamin C stands out as a potent exogenous antioxidant renowned for bolstering the body's immune defenses, essential for daily intake in sufficient quantities. Matcha tea infusions boast vitamin C concentrations ranging from 32.12 to 44.8 mg/l, varying with infusion temperature and tea type. Notably, the vitamin C content in matcha has been reported to be twice as high as that found in regular green tea (Kochman et al. 2020). Research by Phuah et al. (2023) indicates a concentration ranging from 1.35 to 1.53 mg/g, while in Kochman et al. (2020) is concentration ranging from 1.63 to 3.98 mg/g, contingent on product type and source (Kochman et al. 2020; Phuah et al. 2023).

4.4.7. Minerals

Minerals constitute nearly 5% of the total dry weight of matcha, with their analysis commonly conducted using inductively coupled plasma mass spectrometry or flame atomic absorption spectrometry, coupled with electrothermal atomic absorption spectrometry (Phuah et al. 2023).

Essential minerals have drawn significant public interest due to their potential effects on human health. However, it's crucial to monitor their dietary intake carefully to determine their safety levels (Koláčková et al. 2020).

Potassium emerges as the most abundant mineral in matcha, followed by magnesium, manganese, calcium, iron, zinc, sodium, selenium, and mercury. While heavy metals like mercury, cadmium, lead, and chromium are detected in matcha, they remain below the maximum residue levels established by regulatory authorities, particularly for mercury (Phuah et al. 2023). Nonetheless, further investigations are warranted to assess the presence of trace levels of toxic minerals in matcha, despite its well-established health-promoting attributes (Koláčková et al. 2020; Phuah et al. 2023)

Table 2. Comparison of the main chemical components of regular green tea (*Camelia sinensis* var. *sinensis*) and matcha tea

Composition (mg/g)	Species	
	<i>Camelia sinensis</i> (var. <i>sinensis</i>)	Matcha tea
Catechin	0.30 – 0.42	0.80 – 2.89
EC	0.80 – 1.11	0.95 – 4.40
ECG	2.55 – 4.45	7.45 – 8.30
EGC	1.53 – 4.14	11.50
EGCG	22.72 – 31,19	9.55 – 70.20
Caffeine	3.17 – 3.85	14.4–34.1
Theanine	29.56 – 33.39	>17

Footnote: EC, (-)-epicatechin; ECG, (-)-epicatechin gallate; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate

References: (Xu et al. 2021; Devkota et al. 2021; Phuah et al. 2023)

4.4.8. Influence of harvest on chemical composition

Tea plants harvested in May tend to have higher levels of minerals, volatile compounds and antioxidants. Conversely, those harvested in July have higher levels of phenolics, crude fibre and moisture. Across the three plucking seasons - autumn, spring and summer - studies have shown that tea leaves from the second and third harvests have higher total polyphenol and flavonoid content than those from the first harvest. In addition, their free radical scavenging activities exceed those of the first harvest, highlighting the importance of leaf maturity in determining matcha quality. Premium matcha is derived from young tea leaves to reduce astringency, resulting in matcha with high caffeine but low EC and EGC content (Phuah et al. 2023).

4.4.9. Influence of shading on chemical composition

Achieving a vibrant green colour in matcha is indicative of high quality, signalling that the tea leaves have undergone shading (Phuah et al. 2023). During artificial shading researchers identified that the decreased light intensity promoted the accumulation of chlorophyll in tea leaves (Elango et al. 2023). Higher content of chlorophyll is a crucial aspect in enhancing the visual appeal of tea leaves along with overall quality assessment and infusion (Sonobe et al. 2018).

Shading not only affects the physical characteristics of matcha, but also enriches its functional constituents, including theanine, chlorophyll, oxalic acid, coniferyl alcohol, myricitrin, GC, and malvidin 3-O-galactoside (Fig. 6). In addition, the ratio of chlorophyll b to chlorophyll a and chlorophyll a to carotenoids increases after shading. However, shading results in reduced levels of EGCG, EGC, EC, ECG, GCG, CG, anthocyanins and proanthocyanidins due to down-regulation of gene expression related to bioflavonoid synthesis. In addition, light intensity affects amino acid content, with weak light exposure during shading resulting in increased levels of various amino acids. The shading process, coupled with adequate nitrogen supply, promotes the production of theanine, volatile metabolites, crude fibre and mineral accumulation in tea leaves (Phuah et al. 2023; Manikharda et al. 2023).

The biosynthesis of tea catechins, regulated by enzymes like phenylalanine-ammonia-lyase (PAL) and others such as flavanone-3-hydrolase, dihydroflavanol-4-reductase, and leucoanthocyanidins, shows higher expression levels in young leaves compared to mature ones. PAL activity tends to decrease in shaded plants but increases with temperature rises. The

temperature around shaded tea plants is typically lower than in unshaded areas (Manikharda et al. 2023).

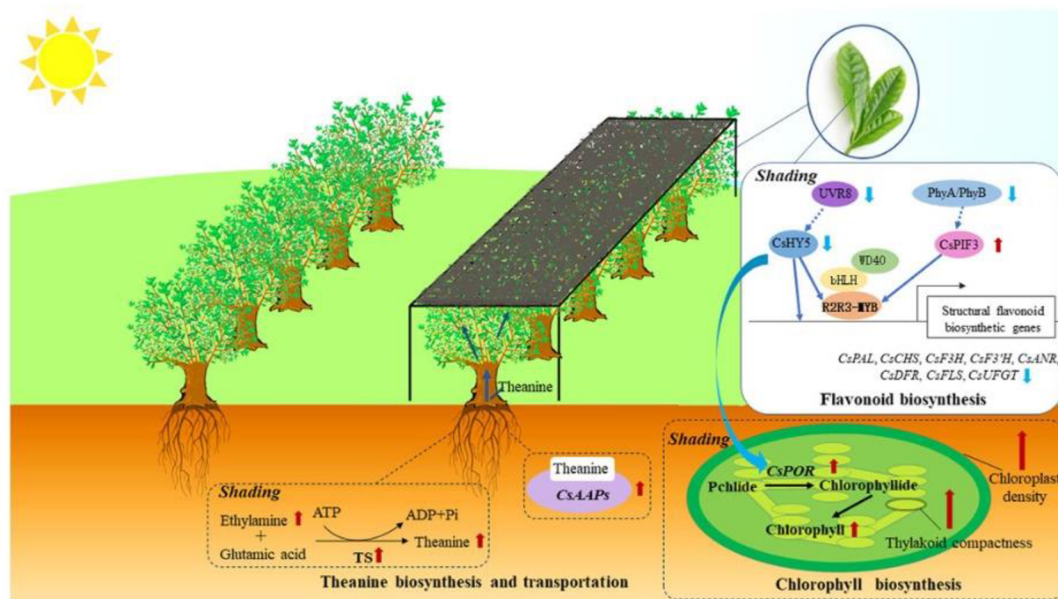


Figure 6. The impact of shading on the production of flavonoids, theanine, and chlorophyll (Ye et al. 2023).

4.4.10. Influence of grinding on chemical composition

The milling process has been identified as a crucial step in improving the extraction yield and bioactivities of functional compounds, especially EGCG. In particular, finer matcha samples have shown enhanced antioxidant and anti-inflammatory activities (Phuah et al. 2023).

Furthermore, studies have shown that matcha samples with particle sizes as small as 2 μm exhibit enhanced antioxidant and anti-allergenic activities. This phenomenon is attributed to the increased availability of phenolic compounds facilitated by the finer particle size, which aids the extraction process (Phuah et al. 2023).

In addition, grinding methods and duration also influence the microbial content of matcha and induces the tissue damage, thereby enhancing enzymatic activity and the production of aromatic alcohols in matcha (Tan et al. 2019; Phuah et al. 2023). Future research investigating the effect of grinding on matcha processing has significant potential for further insights (Phuah et al. 2023).

A study conducted by Huang et al. 2022 showed the major non-volatile compounds like sugars, organic acids, amino acids, and polyphenols in matcha aimed to identify the variations

in flavour profile resulting from the different milling processes. Generally, cyclone-milled matcha, characterized by the smallest diameter, exhibited the highest levels of sugars (sucrose, glucose, galactose, fructose), organic acids, caffeine, and amino acids. Increased concentrations of soluble carbohydrates with a decrease in particle size were also observed in green tea extracts. This phenomenon may be attributed to the greater exposed surface area, facilitating enhanced extraction of these compounds during brewing (Huang et al. 2022).

In contrast to the trends observed for sugars, organic acids, caffeine, and amino acids, the concentrations of polyphenols varied. Bead-milled matcha (673.010 mg/l) and cyclone-milled matcha (668.579 mg/l) exhibited higher polyphenol contents than stone-milled matcha (646.697 mg/l). The lower polyphenol content in stone-milled matcha may be attributed to the degradation of these sensitive compounds during the harsher stone-milling process, which involves higher shearing force. (Huang et al. 2022).

Non-volatile components played a significant role in the positive correlation of non-volatile and potential compounds contributing to the flavour. Cyclone-milled matcha, rich in sugars, organic acids, and amino acids, clustered within this sector. In contrast, compounds potentially contributing to flavour were dispersed throughout the chart. Bead-milled matcha stood out due to its elevated levels of compounds associated with floral characteristics, like geraniol and β -damascenone, while stone-milled matcha exhibited higher concentrations of compounds linked to roasted attributes, such as pyrazines (Huang et al. 2022).

Other evident correlation was between polyphenols, caffeine, sugars, and organic acids with astringent and bitter tastes. Theanine ensured a rich taste, whereas the other amino acids showed a closer association with the umami taste (Huang et al. 2022).

Distinct variations in physical and chemical properties were noted among the three types of milled matcha. Matcha with smaller particle sizes exhibited a greater release of various taste components but also experienced a more significant loss of polyphenol compounds. These differences in chemical release among the differently milled matcha types also translated into variations in sensory performance. This indicates that the milling processes impacted chemical release, thereby influencing matcha quality in terms of rich taste and aroma (Huang et al. 2022).

4.4.11. Influence of matcha variety on chemical composition

Theanine levels in matcha are influenced by the tea plant variety. For example, *C. sinensis* var. *sinensis* has been reported to contain higher levels of theanine than *C. sinensis* var.

assamica. Among the commonly cultivated varieties, Asahi and Samidori stand out as superior varieties for the production of high-quality matcha. Longjing 43 green tea has the highest free amino acid content, followed by Asahi, Kyomidori and Yabukita. In terms of caffeine content, Yabukita ranks highest, followed by Longjing 43, Kyomidori and Asahi. In addition, Asahi has the highest concentrations of GC, EGC, EC, catechins, EGCG, GCG, CG and ECG, while Longjing 43 and Kyomidori have lower levels. Kyomidori has particularly high levels of theanine, chlorophyll a and chlorophyll b. In addition to these cultivars, Asatsuyu, Okuyutaka and Sofu are also used in matcha production. Notably, Asatsuyu contains the highest oxalic acid content compared to Okuyutaka and Sofu. Thus, the choice of cultivar has a significant impact on the flavour profile, physical attributes, market price, and grade of matcha (Phuah et al. 2023).

Table 3. Chemical composition of main components in different matcha varieties (%)

Components	Okumidori	Longjing 43	Zhongcha 108	E'cha 1
C	0.20 ± 0.01	0.06 ± 0.003	0.09 ± 0.01	0.02 ± 0.002
EC	0.32 ± 0.03	0.35 ± 0.01	0.28 ± 0.01	0.39 ± 0.04
ECG	0.69 ± 0.05	1.21 ± 0.04	0.93 ± 0.05	0.95 ± 0.11
EGC	0.90 ± 0.05	0.69 ± 0.02	0.67 ± 0.03	1.23 ± 0.13
EGCG	4.03 ± 0.14	4.31 ± 0.17	4.76 ± 0.27	4.80 ± 0.57
Caffeine	3.04 ± 0.18	2.91 ± 0.09	2.83 ± 0.10	2.80 ± 0.19

Footnote: All values are shown as mean ± SD

References: (Huang et al. 2024)

4.4.12. Influence of storage on chemical composition

Storage temperature plays a crucial role in maintaining the quality of matcha. Research shows that storing matcha at 80°C for two months results in a significant decrease in brightness. Furthermore, exposure to temperatures between 45-80°C leads to a reduction in moisture content within the first two days of storage, accompanied by a significant deterioration in bioactive compounds and a decrease in antioxidant activity (Phuah et al. 2023).

Conversely, when matcha is stored at room temperature (25°C), changes in moisture content, total phenolic content, and ABTS and DPPH free radical scavenging activities remain

negligible for up to four weeks. However, stability varies for different compounds; EGC, ECG and rutin remain stable for up to three weeks, while total flavonoid content gradually decreases after seven days (Phuah et al. 2023).

Catechin is highly sensitive to storage conditions, leading to a decline in its physiological effects. Degradation of catechin and theaflavin is influenced by factors like pH, temperature, oxygen levels, antioxidant concentration, metal ions, and EGCG concentration. While numerous studies have explored matcha stability concerning oxygen presence and pH changes, research on the impact of temperature fluctuations remains limited to certain flavonoids (Kim et al. 2020).

In addition, caffeine degradation begins on the fourth day of storage at room temperature (Phuah et al. 2023).

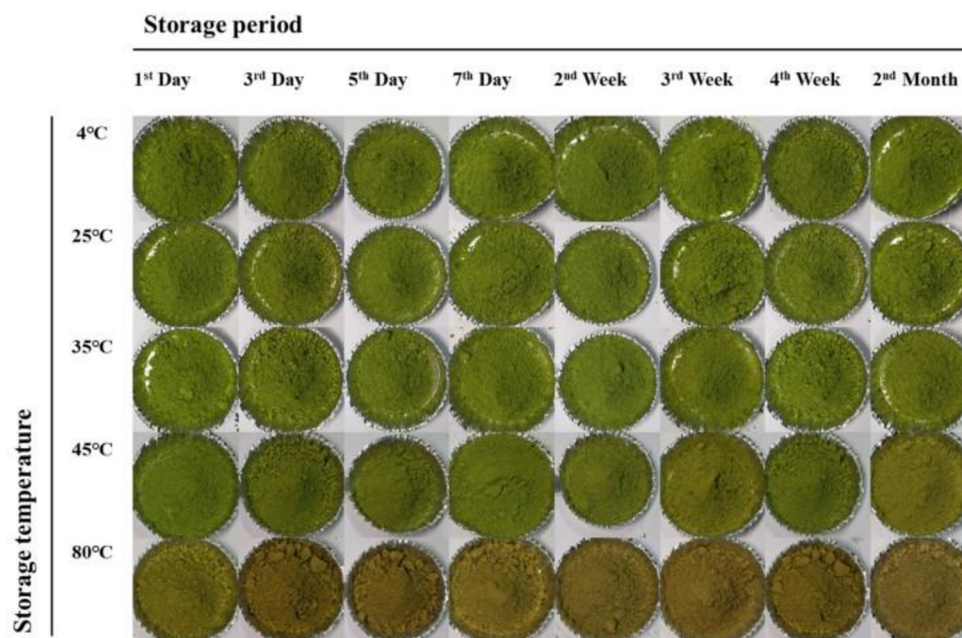


Figure 7. The alteration of matcha colour over a two-month storage period under varied temperature conditions (Kim et al. 2020)

4.4.13. Influence of matcha preparation on chemical composition

Using boiling water to brew green tea is common practice, but it's not the norm for matcha. Studies show that tea extracted at 80°C tends to have higher levels of phenolics, flavonoids and vitamin C compared to tea extracted at higher temperatures of 90-96°C, because during thermal processing in aqueous systems, tea catechins experience degradation and epimerization processes (Wang et al. 2008; Phuah et al. 2023). Furthermore, higher extraction

temperatures result in an increased release of caffeine and gallate-type catechins, while theanine levels tend to decrease. Interestingly, first- and second-picked tea leaves exhibit the highest iron-reducing and free radical scavenging activities when extracted at 90°C, while similar effects are observed for second- and third-picked leaves when extracted at 80°C. In matcha, leaching of calcium, magnesium and manganese is most pronounced at 70°C for five minutes, while optimal conditions for potassium and zinc leaching are suggested to be 10 minutes of infusion at 20°C. Overall, matcha tea brewed at 80°C and extracted for 20 minutes has the highest total phenolic content and antioxidant activity per 100 ml/g (Phuah et al. 2023).

4.4.14. Influence of pH levels and water quality on chemical composition

Water quality and pH play an important role in influencing the antioxidant activity and the stability of tea catechins (Wang et al. 2008; Phuah et al. 2023). Optimal catechin release and stability were observed in extractions using water with a pH below 6. Furthermore, matcha prepared with ultrapure, reverse osmosis and distilled water showed higher free radical scavenging activity than those prepared with deionised, tap and activated carbon adsorbed water. Interestingly, the addition of milk to Matcha slightly reduced its antioxidant activity (Phuah et al. 2023).

4.5. Uses of matcha tea

Matcha stands out among superfoods for its many health benefits, ranging from anti-aging and weight loss to detoxification and muscle building (Dreher 2018).

Matcha finds its application in traditional tea ceremonies, food processing and so on (Sokary et al. 2023). Recently, there has been a surge in demand for matcha, stemming from its unique taste, striking colour, and potential revitalizing capabilities. This has led to more media exposure (Fig. 8) and its subsequent widespread availability (Huang et al. 2022). Top-grade matcha typically has a vibrant green hue, smooth flavour profile, and rich nutritional content, all of which are intricately linked to its component types and concentrations. It is characterised by elevated levels of amino acids and chlorophyll, coupled with lower levels of catechins. As a result, a high concentration of phenolic ammonia and reduced greenness in matcha are considered less desirable. However, due to the prevalence of lower quality tencha raw materials, much of the matcha available on the market tends to have a yellowish hue and an astringent taste (Xiao et al. 2021).

In Japan, the main sources of matcha come from varieties like the 'Uji,' 'Chaoru,' and 'Yabunhok' series. Meanwhile in China a previous study pointed out that among eight cultivars 'Longjing 43' demonstrated the most favorable attributes in terms of appearance, seaweed-like fragrance, and umami taste in tencha. Another thorough evaluation involving cluster analysis of 11 quality parameters across 36 tea cultivars revealed that matcha produced from 'Zhongcha 102,' 'Zhongcha 108,' and 'Fuding Dabaicha' displayed superior color and taste quality (Huang et al. 2024)

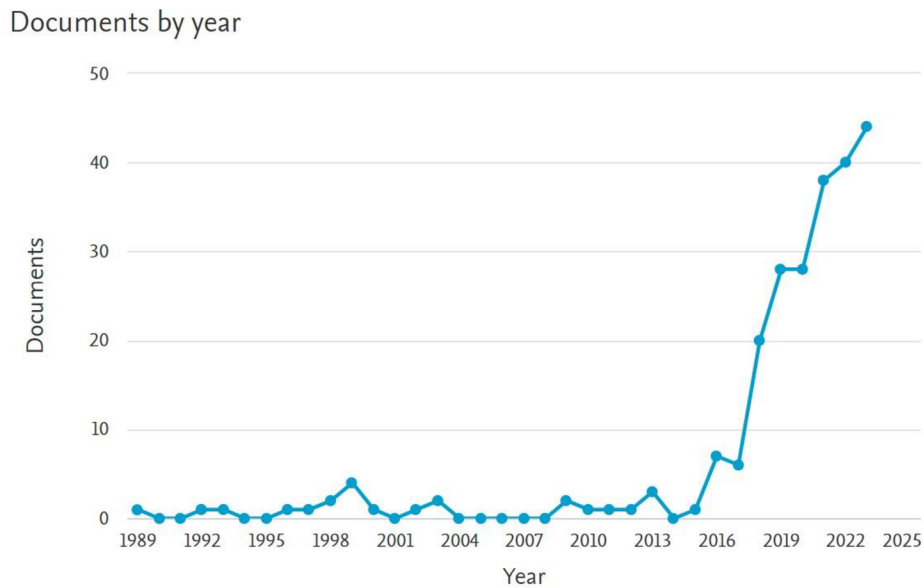


Figure 8. Growing interest in matcha tea over the past years (Scopus 2024)

4.5.1. Ceremonial purposes

Matcha, once a revered ceremonial beverage among Zen Buddhists in ancient China, found its way to Japan through cultural exchanges (Topuz et al. 2014). While its consumption has waned in China over the centuries, matcha remains a staple in Japanese culture (Topuz et al. 2014; Dreher 2018).

Today, the traditional preparation of matcha involves whisking it vigorously in hot water using a special bamboo whisk known as a "chasen," typically done in a ceremonial bowl called a "chawan." (Topuz et al. 2014).

The tea ceremony stands as a cornerstone of both traditional and contemporary culture, practiced and cherished for centuries. Its enduring appeal not only attracts visitors from around the world seeking an authentic cultural experience but also teaches invaluable lessons in

patience and mindfulness as participants meticulously prepare and enjoy tea together (Say et al. 2022).

The Japanese have long celebrated matcha through a time-honored tradition called "Chanoyu" (Fig. 9) or the Japanese tea ceremony. This ritual, steeped in history and tradition, offers a deeply meaningful way to enjoy matcha (Dreher 2018)

In the traditional Japanese tea ceremony, matcha is more than just a beverage—it's a deeply ingrained cultural practice (Say et al. 2022.; Dreher 2018). Before the ceremony itself begins, the tatami-floored room must be tidy (Say et al. 2022). Before entering the teahouse, guests ritually cleanse themselves by washing their hands and mouths (Say et al. 2022; Dreher 2018). This step has symbolical meaning, representing cleansing from the dust of the outside world (Say et al. 2022). Once inside, they kneel on tatami mats arranged by the host and take a silk cloth *Fukusa* that symbolize their spirit. Next, the host starts to make tea by scooping three portions of matcha and pouring a small amount of hot water (Say et al. 2022.; Dreher 2018). Each bowl of matcha is carefully selected and served to the guests. After savoring the tea, there's a moment of silent reflection. As the ceremony concludes, guests observe the host's careful cleaning of the utensils. With the departure of the host, guests bid farewell and leave, carrying with them the peaceful essence of the tea ceremony (Dreher 2018).



Figure 9. A 1895 print of chanoyu ceremony in Japan (Dreher 2018)

4.5.2. Culinary application

Once limited to traditional ceremonies in past years, nowadays you can find matcha being used in a great deal of different products, other than tea. Ranging from ingredients in various products including drinks, beverages, snacks, and numerous other consumer goods (Devkota et al. 2021; Ye et al. 2023).

When blended with warm water, not quite reaching the boiling point, a small mound of the green powder, slightly granular in texture, undergoes a remarkable metamorphosis (Dreher 2018). On the other hand throughout the baking process, the profiles of moisture and temperature undergo continuous changes (Wang et al. 2008).

Additionally, there is a growing demand for convenience products that offer added nutritional value. For example, utilizing green tea powder in bakery items has the potential to mitigate the perceived bitterness or astringency often associated with green tea (Phongnarisorn et al. 2018). Several research studies have explored the incorporation of matcha into bakery goods, revealing impacts on various properties including physicochemical attributes, color, texture, and shelf life. Research have indicated favourable sensory acceptance of cakes enriched with up to 20% green tea, replacing wheat flour (Wang et al. 2008; Phongnarisorn et al. 2018).

Matcha used in the preparation of biscuits with varying amounts of matcha green tea powder (MGTP) was investigated in the research by Phongnarisorn et al. (2018). Using high-performance liquid chromatography with diode-array detection (HPLC-DAD), the catechins and caffeine content in both the dough and biscuits were quantified. Results revealed a notable decrease in most catechins post-baking, with a maximum reduction of 19% in total catechins. Particularly, EGC exhibited the highest losses, reaching 31% in biscuits enriched with the highest MGTP level (6 g per 100 g of flour). Conversely, there was a marked increase in GCG levels by up to 40% in the same biscuits, suggesting potential epimerization of EGC to GCG during baking. Caffeine content also declined during baking by a maximum of 24%. The highly enriched biscuits (6 g MGTP per 100 g of flour) contained approximately 20–23 mg of total catechins per biscuit, equivalent to about 9% of the content in a standard green tea infusion (Phongnarisorn et al. 2018).

The overall acceptability response surface illustrates those biscuits with lower levels of matcha green tea powder (MGTP) and reduced sugar content garnered the highest acceptability ratings. Interestingly, augmenting the sugar content in the biscuit formulation did not notably impact the acceptability of the enriched biscuits. This pattern was mirrored in the response for

appearance, wherein biscuits with darker green and brown colour tended to receive lower acceptability scores (Phongnarisorn et al. 2018).

Studies suggest that traditional matcha preparation, simply mixed with water, retains its nutritional value better than matcha added to other products. While food processing may reduce its nutritional value, Matcha remains a healthy alternative or addition to such products (Wang et al. 2008; Dietz et al. 2017; Phongnarisorn et al. 2018).

4.5.3. Medicinal and health application

In recent times, there has been a growing interest in plant-derived healthy foods, nutraceuticals, functional foods, and food supplements. These products are increasingly recognized for their potential role in maintaining health and preventing and treating various diseases, including matcha (Devkota et al. 2021). In a separate investigation, the effects of matcha extract, residues, and the entirety of matcha were examined. It was revealed that the residues, containing predominantly water-insoluble fibers, play a significant role in matcha's health-enhancing properties (Sokary et al. 2023).

Meta-analyses have shown that caffeine intake may aid in weight and fat loss and enhance muscle strength and power. Recent systematic reviews have highlighted the combined effects of theanine and caffeine in enhancing cognitive function, supported by individual studies demonstrating the cognitive benefits of theanine, caffeine, and catechins (Sokary et al. 2023).

The presence of catechins in green tea has demonstrated promising implications in combating cancer, diabetes, and cardiovascular ailments. Research revealed a notable 25% reduction in carbohydrate absorption following the consumption of green tea extract containing 300 mg of EGCG and 100 mg of ECG alongside a carbohydrate-rich meal. This suggests that green tea might inhibit the absorption of glucose into the bloodstream, potentially reducing the risk of diabetes development (Phongnarisorn et al. 2018).

Matcha and instant tea as two prevalent elements found in tea, were made into the formulation of a unique cornstarch-tea diet designed specifically for individuals managing diabetes (Zhang et al. 2018, 2020; Silva et al. 2022).

Cornstarch, comprising approximately 50% resistant starch, undergoes slower digestion compared to other starch types, resulting in a gradual release of glucose over time. This reduced digestion potentially lowers the absorption of blood glucose from the small intestine in diabetic mice. EGCG in instant tea and matcha can inhibit pancreatic lipase activity, thereby reducing

lipid absorption, downregulate the expression of glucose transporter proteins to decrease glucose absorption, and enhance insulin synthesis for improved glucose utilization. Thus, the observed trend of reduced blood glucose levels with a cornstarch-tea diet may stem from the diminished digestion and absorption of energy in the gastrointestinal tract (Zhang et al. 2020).

Supplementing with instant tea and matcha demonstrated positive effects in regulating blood glucose levels and gut microbiota, counteracting the alterations induced by alloxan injection. The pathway influenced by the combination of cornstarch and tea involves the regulation of bacterial groups rather than individual species. This indicates that the combination of cornstarch and tea could successfully serve as a functional food supplement for individuals with diabetes (Zhang et al. 2020).

Another use of matcha that research has highlighted is GTP's potential protective effect against various types of cancer cells and its usefulness in the treatment of cardiovascular disease. MGTE (matcha green tea extract) exhibit a cytotoxic effect on WERI-Rb1 cells, inhibiting their growth while leaving ARPE-19 cells unaffected. Moreover, MGTE induced apoptosis and led to an accumulation of cells in the sub G0/G1 phase, indicating DNA fragmentation. The expression of proapoptosis genes implicated MGTE as a pro-apoptotic agent. These findings suggest the therapeutic potential of MGTE in retinoblastoma by influencing cancer cell metabolic reprogramming. Further investigation is warranted to understand its effects on different retinoblastoma cell lines, as they may exhibit varying inhibition mechanisms (Sayuti et al. 2023).

A study by Kurauchi et al. 2019 investigated how matcha tea powder, extracts, and fractions impact anxiety levels, using the elevated plus maze (EPM) in healthy male mice. Additionally, the focus extended to understanding the influence of matcha on the brain's dopaminergic and serotonergic systems, particularly exploring the role of dopamine D1 and serotonin 5-HT1A receptors in mediating its anxiolytic effects (Kurauchi et al. 2019).

The anxiolytic properties of matcha tea powder through meticulous experiments involving mice and the elevated plus maze (EPM) test discovered that the activation of dopamine D1 and serotonin 5-HT1A receptors plays a significant role in these effects. Additionally robust and synergistic anxiolytic effects in the less water-soluble components of matcha were observed, which are typically challenging to extract from regular green tea (Kurauchi et al. 2019).

Regular consumption of matcha may positively impact both physical and mental health due to its potential to prevent diseases and support cognitive function, including in human samples (Kochman et al. 2020). The study conducted by Dietz et al. (2017) examined how the consumption of two average servings of matcha tea, either in its pure form or incorporated into a solid product (a bar), affected mood and cognitive performance in healthy young people. During each session, participants were given one of the four test products: matcha tea, matcha tea bars (each containing 4 g of matcha tea powder), placebo tea and placebo bars. Cognitive assessments were conducted at two time points: before consumption and 60 minutes after consumption. During these assessments, participants underwent a series of cognitive tests to evaluate attention, information processing, working memory, and episodic memory (Dietz et al. 2017).

The primary hypothesis regarding cognitive performance centred on the anticipated synergistic effects of L-theanine and caffeine, both constituents of matcha tea. These effects were expected to manifest in improved vigilance and sustained attention tasks, driven by the modulation of neuronal activity within brain regions linked to executive control and attentional functions. It was also expected that the caffeine component in matcha tea would affect performance on prolonged, demanding tasks through pathways beyond increased alertness. However, the findings did not support the anticipated mood-enhancing effects of matcha tea consumption (Dietz et al. 2017).

Previous research also has demonstrated cognitive improvements in the absence of mood changes, even with high doses of caffeine. However, the study revealed minimal differences attributable to the presence of matcha tea across most cognitive factors, with notable distinctions observed between the two formats tested. Specifically, the beverage form outperformed the bar format, particularly evident in tasks assessing accuracy in "Delayed picture recognition" and "Spatial working memory" (Dietz et al. 2017).

4.5.4. Cosmetics

Matcha also exerts its use in a variety of beauty and skincare products (Ye et al. 2023).

The skin faces numerous damaging elements, primarily oxygenated molecules commonly referred to as "free radicals" (Korać & Khambholja 2011). UVB wavelengths between 280 and 320 nm pose a significant risk, with repeated exposure leading to various skin diseases, including melanoma and non-melanoma skin cancer (Wu & Sato 2003; Saeed et al. 2017). Moreover, UVA wavelengths ranging from 320 to 400 nm contribute to the aging of

skin cells. Collagen, the primary structural protein in the skin, experiences photopolymerization under UV irradiation, altering the arrangement of collagen fibrils (Saeed et al. 2017)

To support the skin's natural repair and rejuvenation process, it requires a robust selection of potent ingredients. The antioxidant feature of a tea signifies its ability to protect the human body against the harmful effects of free radicals and to mitigate the onset of degenerative conditions stemming from prolonged oxidative stress (Korać & Khambholja 2011).

Topical application or oral consumption of green tea polyphenols (GTP) can effectively inhibit skin carcinogenesis induced by chemical carcinogens or UV radiation. Application of GTP and its principal component, EGCG, has shown promise in preventing UVB-induced inflammatory responses, immunosuppression, and oxidative stress, all of which are key biomarkers for several skin conditions (Wu & Sato 2003; Saeed et al. 2017). Catechins play also a crucial role in maintaining skin elasticity by inhibiting the activity of collagenase and elastase enzymes, which are responsible for breaking down collagen and elastin in the skin (Saeed et al. 2017).

In animal models, pre-treatment with topical GTP and EGCG prior to UVB exposure has been found to protect against both local and systemic immune suppression, with associated reductions in the infiltration of inflammatory cells. This protective effect is linked to the inhibition of UVB-induced production of the immunosuppressive cytokine interleukin (IL)-10, along with enhanced IL-12 production (Wu & Sato 2003).

Similar antioxidant and anti-inflammatory effects have been observed in human skin upon treatment with EGCG, leading to reduced UVB-induced erythema, oxidative stress, and inflammatory cell infiltration. Moreover, studies have shown that GTP treatment in human skin can prevent the formation of UVB-induced cyclobutane pyrimidine dimers, which are implicated in UVB-induced immune suppression and the development of skin cancer (Wu & Sato 2003).

The evidence from in vitro, animal, and human studies collectively suggests that green tea polyphenols possess photoprotective properties and hold potential as pharmacological agents for preventing solar UVB light-induced skin disorders, including photoaging, melanoma, and nonmelanoma skin cancers (Wu & Sato 2003).

5. Conclusions

In conclusion, the study of matcha tea throughout this thesis has provided valuable insights into its processing, chemical composition and diverse applications. From its origins in Japan to its cultivation and processing methods, matcha is a unique tea with a rich history and cultural significance. The shade grown tencha leaves are carefully processed to produce a tea powder with distinctive characteristics, including a vibrant green colour and a robust flavour profile.

Chemically, matcha tea is rich in catechins, particularly EGCG, which contribute to its powerful antioxidant properties. These properties have been linked to a range of health benefits, including the reduction of fasting blood glucose levels, neuroprotection and inhibition of fat accumulation. In addition, matcha contains significant amounts of amino acids, including theanine, which contribute to its unique taste and potential health benefits.

The uses of matcha extend beyond traditional tea ceremonies to include a wide range of culinary, functional food and cosmetic applications. Its versatility as an ingredient in various recipes highlights its adaptability and growing popularity in contemporary cuisine. Furthermore, its antioxidant and anti-inflammatory properties make it a promising candidate for potential therapeutic applications in health and wellness products.

Overall, this review highlights the importance of matcha tea not only as a beverage, but also as a multifaceted ingredient with diverse cultural, nutritional and functional significance. As research in this field continues to evolve, further exploration of matcha's properties and applications holds the promise of uncovering additional benefits and expanding its utility in various fields.

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