Determination of Biologically Active Substances in Matcha with Regard to its Digestibility

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Zásady pro vypracování

I. Teoretická část

1. Stručně charakterizovat výrobu zelených čajů, podrobněji se zaměřit na matcha čaj.
2. Charakterizovat biologicky aktivní látky zelených čajů, podrobněji zpracovat jejich polyfenolický profil.

II. Praktická část

1. Připravit matcha čaje dle různého technologického postupu a stanovit u nich vybrané biologicky aktivní látky, včetně antioxidační aktivity, stravitelnosti a analyzovat nestravitelný podíl s důrazem na obsah polyfenolických látek.

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[1] JAKUBCZYK, K., KOCHMAN, J., KWIATKOWSKA, A., KALDUNSKA, J., DEC, K., KAWCZUGA, D. et al.: Antioxidant properties and nutritional composition of matcha green tea. Foods 2020, 9, 483.

[2] WEISS, D.J., ANDERTON, C.R.: Determination of catechins in matcha green tea by micellar electrokinetic chromatography. Journal of Chromatography A 2003, 1011, 173-180.

[3] KOCHMAN, J., JAKUBCZYK, K., ANTONIEWICZ, J., MRUK, H., JANDA, K.: Health benefits and chemical composition of matcha green tea: A review. Molecules 2021, 26, 85.

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ABSTRAKT

Teoretická část zahrnuje charakteristiku zelených čajů, výrobu matcha čaje a stručný popis biologicky aktivních látek s důrazem na polyfenolické látky. V experimentální části A byly za využití metod spektrofotometrických a chromatografických stanoveny polyfenolické látky a hodnoty antioxidačních aktivit vodných výluhů z matcha čaje, připravených při 60, 70 a 80 °C při době luhování 3 a 5 minut. V experimentální části B byla využita gravimetricko-enzymatická metoda pro stanovení hodnoty stravitelnosti technikou *in vitro* a přípravu nestravitelného podílu matcha čaje. V nestráveném podílu čajových listů, stejně jako v nativním vzorku matcha, byly stanoveny polyfenolické látky a hodnoty antioxidačních aktivit. Na základě naměřených dat byl vypočítán retenční faktor, který udává podíl biologicky aktivní látky neuvolněné po simulaci procesu trávení. Výsledky experimentální části A ukazují, že pro jednotlivé stanovované polyfenolické látky je vhodnější jiná teplota extrakce k jejich kvantitativnějšímu vyluhování. Dále nebyl prokázán signifikantní vliv na zvýšení koncentrace polyfenolických látek ve výluhu při delší době extrakce. Všechny vzorky obsahovaly vysoké koncentrace zejména kyseliny kávové, EGCG, EGC, EC a ECG. Hodnoty stravitelnosti matcha čajů se pohybovaly od 69,6 do 79,3 %. Nejvyšších hodnot retence v nestráveném podílu dosáhly kyseliny gallová (6–28%), ellagová (8–19%) a rutin (9–19%). Výsledky měření ukázaly, že i po procesu simulace trávení v podmínkách *in vitro*, nestrávený podíl čajových listů stále obsahuje nezanedbatelné koncentrace polyfenolů a vykazuje zbytkové hodnoty antioxidační aktivity. Tyto polyfenolické látky, převážně vázané na zbytkovou složku vlákniny listů, se tak mohou dostat až do tlustého střeva a mohly by zde působit např. jako antioxidanty či látky s protizánětlivými účinky. Tato diplomová práce tak zvyšuje povědomí o polyfenolickém složení matcha čajů a jeho možných podpůrných zdravotních benefitech.

Klíčová slova: *Camellia sinensis* L., matcha, zelený čaj, polyfenol, katechin, antioxidační aktivita, *in vitro* stravitelnost, HPLC

ABSTRACT

The theoretical part includes the characteristics of green teas, the manufacturing of matcha tea, and a brief description of biologically active substances, emphasizing polyphenolic substances. In experimental part A, polyphenolic substances, and the values of antioxidant activities of aqueous extracts from matcha tea prepared at 60, 70, and 80°C at infusion times of 3 and 5 minutes were determined using spectrophotometric and chromatographic methods. In experimental part B, the enzymatic-gravimetric method was used to determine the value of digestibility by *in vitro* technique and the preparation of the indigestible part of matcha tea. Polyphenolic substances and the values of antioxidant activities were determined in the undigested part of tea leaves, as well as in the native matcha sample. Based on the measured data, a retention factor was calculated, which indicates the portion of biologically active substance not released after the simulation of the digestion process. The results of experimental part A show that for the individual polyphenolic substances determined, a different extraction temperature is more suitable for their more quantitative leaching. Furthermore, there was no significant effect on increasing the concentration of polyphenolic substances in the extract during longer extraction times. All samples contained high concentrations, especially caffeic acid, EGCG, EGC, EC, and ECG. The digestibility values of matcha teas ranged from 69.6 to 79.3%. Gallic acid (6–28%), ellagic acid (8–19%), and rutin (9–19%) reached the highest retention values in the undigested part. The results of the measurements showed that even after the process of simulation of digestion *in vitro*, the undigested part of tea leaves still contains significant concentrations of polyphenols and shows residual values of antioxidant activity. These polyphenolic substances, mainly bound to the residual component of the leaf fiber, can thus reach the large intestine, and can act there, for example, as antioxidants or substances with anti-inflammatory effects. This diploma thesis thus raises awareness of the polyphenolic composition of matcha teas and its possible supportive health benefits.

Keywords: *Camellia sinensis* L., matcha, green tea, polyphenol, catechin, antioxidant activity, *in vitro* digestibility, HPLC

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I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

CONTENTS

INTRODUCTION

Tea is one of the most consumed beverages all over the world. The history of tea began approximately 4000 years ago in ancient China. According to the myth, tea was discovered accidentally when the Chinese Emperor Shennon was boiling water and a few tea leaves fell inside. After he tasted it, he was surprised by its delicious smell and flavor. From China, tea started to spread to India and Japan first, then it reached the western world. Nowadays, drinking tea is becoming more and more popular because of the well-known health benefits that come with its consumption [1].

There are a lot of different kinds of tea. To be defined as tea, it has to be made of the plant called *Camellia sinensis* L. These are black tea, white tea, green tea, oolong tea, and pu-erh tea. Teas known as herbal teas, such as mint, chamomile, or fruit teas are not technically teas, as they do not come from *Camellia sinensis* L. This work is going to focus on green tea, especially matcha tea, as a special kind of green tea [2]. The difference between the various types of teas appears due to different times of harvest, growing conditions, production processes, and others. Green tea is high in polyphenols like catechins and phenolic acids, as well as antioxidants, chlorophyll, vitamins, amino acids, minerals, and other nutrients. Because of its richness of bioactive substances, it has many positive effects on the human body. For instance, we can highlight the reduction of the level of blood pressure, cholesterol, sugar, and fat in the blood, improving liver detoxification, as well as helping oneself with maintaining body weight. It has antibiotic effects and works against viruses and fungi, while it also protects the denture from dental flaws. Many medical studies have proven that green tea shows also antitumor efficacies. It can stop the growth of tumor cells and prevents the spread of metastases [3]. While green tea, in general, is usually bagged or loose, matcha is in the form of soft powder. Therefore, when you drink matcha tea you consume the whole tea leaves, not only leaves brewed in hot water. This is the reason why matcha teas contain a higher number of biologically active substances than normal green tea and might have a bigger impact on the human body.

Since matcha tea may be consumed directly in the form of all leaf parts, there is still little information on the digestibility values and releasing ability of biologically active substances during digestibility. Therefore, a two-step *in vitro* digestion process with pepsin and pancreatin under 37°C was applied. It was aimed at measuring the antioxidant activity and contents of individual phenolic acids, and flavonoids in native and undigested parts

of matcha teas using high-performance liquid chromatography and spectrophotometric method.

I. THEORY

1 GREEN TEA PRODUCTION

Green tea is obtained from the plant *Camellia sinensis* L. This plant requires a humid and warm climate, lots of rain, drained soil, and diffused light. The quality of tea depends on the climate, altitude, type of soil, and the processing techniques. From the same tea leaves, it is possible to produce variable types of teas, according to the different types of processing. Fresh tea leaves are harvested either by mechanical or hand plucking. Mechanical plucking is faster, easier, and more efficient. However, hand plucking has a higher uniformity. Teas of the highest quality are mostly hand-plucked. After plucking, the tea leaves are moved to tea factories, and the manufacturing process starts [4].

Figure 1 *Camellia sinensis* L. [5]

1.1 Green tea manufacturing

Various types of green tea are manufactured a little bit differently but the most important steps are described below.

1.1.1 Plucking

As already mentioned above, plucking can be done by hand or mechanically. The picker uses his thumb and forefinger and picks the shoots that usually include the terminal bud with two or three adjacent leaves. Plucking by hand is the most expensive and the most difficult way of picking. Therefore, the green teas of the highest quality are often hand-plucked. Tea harvesting seasons can be either three or four. It depends on the altitude and latitude [6][7].

1.1.2 Fixing

To inhibit fermentation, prevent oxidation and maintain its green color, it is necessary to deactivate the enzymes. The degradation of enzymes causes the typical green liquor. This is done by applying high heat. The two main fixing methods for green tea are steaming and pan-frying. Steaming, mainly used in Japan, results in higher polyphenolic content, antioxidant bioactivity, and more color than pan-frying. This method was developed in China where the leaves are directly exposed to a high heat source on a dry pan [6].

1.1.3 Rolling, shaping, and drying

The next step after fixing is rolling. This causes the release of leaf moisture, disruption of cell walls, and shaping of the final product. Rolling can last from ten minutes to one hour. It depends on the age of the leaves. Older leaves need a longer duration of rolling compared to young leaves [6][7].

After rolling shaping follows. Tea leaves can be shaped into different forms. Those can be, for example, either flat, compressed, round, ground powder, or flaky. The size of the leaf pieces determines the grade of the tea. Young leaves and full buds have higher grades than tea dust that is usually used to produce tea bags [6][7].

Leaves after shaping go through the process of drying. The main methods used are pan-drying, baking, sun-drying, and basket drying. The time of drying depends on the method applied. It can be from twenty minutes to overnight. Pan-dried tea products keep a better aroma compared to the sun or air-dried teas [6].

1.1.4 Green tea quality

The major impacts on green tea quality are climate, soil, and altitude. Even though the yield of tea decreases with the increasing altitude, the quality of tea gets better [8]. Green tea requires adequate sunlight and proper shading [7].

Other important effects are cultural practices, processing technology, fertilization, and plucking methods. The height of the tea plant, the number of leaves it produces, and the duration of the harvest of each type of green tea differ depending on the weather [7].

1.2 Matcha tea manufacturing

Matcha tea is a special type of green tea. It also comes from the plant *Camellia sinensis* L. but the growth, harvest, production, and substances contained are significantly different.

Matcha tea has its origin in China but it became a part of Japanese culture around 800 years ago. The Japanese improved matcha manufacturing hence matcha tea from Japan is becoming more and more popular in Europe and the United States [9]. In Japan, it is grown and produced in two major regions named: Uji in Kyoto and Nishio in Aichi Prefecture [10]. Matcha tea is unfermented, steamed, and dried in the form of powder where the whole tea leaves are ground. Therefore, it contains all of the nutrients, vitamins, and minerals while it has a greater impact on the human organism since no important compounds are lost during the process. The highest grade of matcha is said to be from three Japanese cultivators: okumidori, yabukita, and samidori. These three cultivators have a history that spans back many hundreds of years [11].

1.2.1 Matcha cultivation

The most important part of the production process is shading. Two to six weeks before harvest the tea leaves are completely shaded by rice straws or bamboo mats. The harvest is usually in May and only new tea leaves are picked. The reduction of sunlight causes the reduction of photosynthesis and leads to a higher amount of chlorophyll and amino acids in the final product. Chlorophyll is responsible for the typical bright green color of matcha, and the number of amino acids leads to its umami flavor. The final product is mouth-watery and sweet without any traces of bitterness [10][12].

1.2.2 Matcha manufacturing

After the harvest, tea leaves are steamed for around 20 seconds at a very high temperature. This process stops the enzymatic reactions and prevents the loss of green color and nutrients. When the steaming process is finished, tea leaves go to the blower, where they are blown and dried. After drying, tea leaves are sorted by grade and cleaned from stems and veins. The part that remains is called tencha [11][12][13].

1.2.3 Matcha grinding

The final step of matcha production is grinding. This used to be done manually in the past. Today large granite wheels are used. The grinding process is quite slow as it usually takes one hour to grind 30 to 40 g of tea leaves. The final particles are about 4 μm. The final powder is vacuum packed and put in the freezer to prevent oxidation and flavor loss [12][13][14].

1.2.4 Matcha preparation

The water temperature is crucial to preparing matcha tea. The water should be between 70 to 80°C. In case the water is too hot, it will result in a bitter taste [15]. Another way of preparing matcha is by cold brewing. This can be done by mixing matcha powder with water between 20 – 40°C even ice can be added. Matcha as a cold drink prepared according to this method may be consumed immediately or may be stored in a fridge for up to two days. As an alternative, water can be replaced with coconut water [16].

Another important step is the mixing technique. In Japan, there is typically a bamboo whisk used to mix matcha powder and water. In European countries, it is possible to see alternative ways, for example, a shaker or mixer [15].

1.3 Other Japanese green teas

There are roughly twenty different types of green tea in Japan. The differences are based on altitudes, regions, production processes, and how they are grown. These aspects lead to the different tastes, caffeine levels, health benefits, etc. The most important Japanese green teas are mentioned below [17].

Sencha is the most consumed and the most exported tea in Japan. The youngest tea leaves are used for this kind of tea. Therefore, it contains a lot of nutrients. In contrast to matcha tea, sencha tea is sun-grown. The leaves are like dried grass as they are thin and long. Their smell is very refreshing. After brewing, it has a nice yellow color [18].

Tencha tea is grown in the shade and all the stems and veins are removed. This tea is used for matcha production. It has the shape of little green flakes [19].

Gyokuro tea is also grown in shade approximately around three weeks before harvesting. It is one of the priciest teas. It is a tea of high-quality and richer in caffeine. This tea can also be used for matcha production [18].

Bancha tea is grown in sunlight. The leaves used are older. Therefore, it contains a less amount of caffeine and the flavor is more robust. The harvest takes place later than the sencha harvest [19].

Genmaicha tea is blended with roasted brown rice. Sencha, gyokuro, bancha, and sometimes also matcha can be used for genmaicha. The ratio of pure tea and brown rice is 1:1. The addition of rice reduces the bitter flavor, and the final product is having a nutty and sesame smell and flavor [19].

Hojicha tea is a very specific one because it's a roasted tea. It contains less amount of caffeine compared to other teas. It is frequently used for iced tea preparation [19].

2 BIOACTIVE SUBSTANCES IN GREEN TEA

Green tea contains various types of components. Around 15–20% of proteins are present. Those are formed from amino acids such as valine, serine, arginine, glycine, leucine, glutamic acid and aspartic acids, tryptophan, tyrosine, etc. Green tea is also rich in mineral and trace elements. The most common ones are phosphorus, chromium, calcium, zinc, copper, magnesium, manganese, iron, cobalt, fluorine, and selenium. Also, vitamins B_2, B_3, C, E, and K can be present. It is a source of lipids and sterols, besides linoleic and α-linoleic acids as representatives of fatty acids. Green tea is also full of xanthine bases, such as caffeine, theophylline, and pigments, especially chlorophyll and carotenoids [20][21].

Regarding strong antioxidants, a very important group present in green teas is the group of polyphenols. This includes phenolic acids, flavanoids (such as flavonols and catechins), tannins, and lignans. Phenolic acids include two main groups of hydroxybenzoic and hydroxycinnamic derivates [22]. Flavanoids are the most common and diverse type of polyphenols. Typical flavanoids measured in green teas are catechins. Those are catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG) [21].

2.1 Profile of biologically active substances in green teas

The number of polyphenols and other substances present in matcha tea depends on the time and temperature of the infusion. Studies have shown that the highest number of polyphenols appeared when tea was brewed between 5–10 minutes at 80 – 90°C [23].

2.1.1 Flavanoids

2.1.1.1 Catechins (Flavanols)

As mentioned above catechins are flavanoids of green tea. The basic structure of catechin is also called “flavan-3-ol” and consists of a dihydropyran heterocycle with a hydroxyl group on the third carbon and two benzene rings. Epigallocatechin (EGC) is a flavan-3-ol containing a benzopyran-3,5,7-triol linked to a 3,4,5-hydroxyphenyl moiety. Epigallocatechin-3-gallate (EGCG) is an ester of epigallocatechin and gallic acid [24]. EGCG is the most studied catechin of all because it is the most common one. Out of all catechins, EGCG accounts for 65% and one cup of green tea might contain up to 200 mg of it [25].

Catechins show significant antioxidant properties. Therefore, it can be very helpful in protecting against diseases caused by free radicals, such as cardiovascular and neurodegenerative diseases and cancer. In various studies, green tea showed protection against ischemic damage, Parkinson's and Alzheimer's diseases, as well as anti-diabetic effects. Catechins also dispose of anti-mutagenic, anti-angiogenic, and hypocholesterolemic effects in addition to showing anti-inflammatory, anti-aging, and anti-bacterial activity [25]. Catechins also help to fix DNA damage that was caused by UV radiation [21].

Catechins can scavenge free radicals. The phenolic hydroxyl groups on the B-ring of epicatechin and epigallocatechin, as well as the B- and D- rings of epicatechin-3-gallate and epigallocatechin-3-gallate, induce this. Catechins are stronger antioxidants than vitamins C and E. The strongest antioxidant is epicatechin-3-gallate, followed by epigallocatechin-3-gallate, epigallocatechin, and epicatechin. Catechin shows the least effects as a free radical scavenger [25].

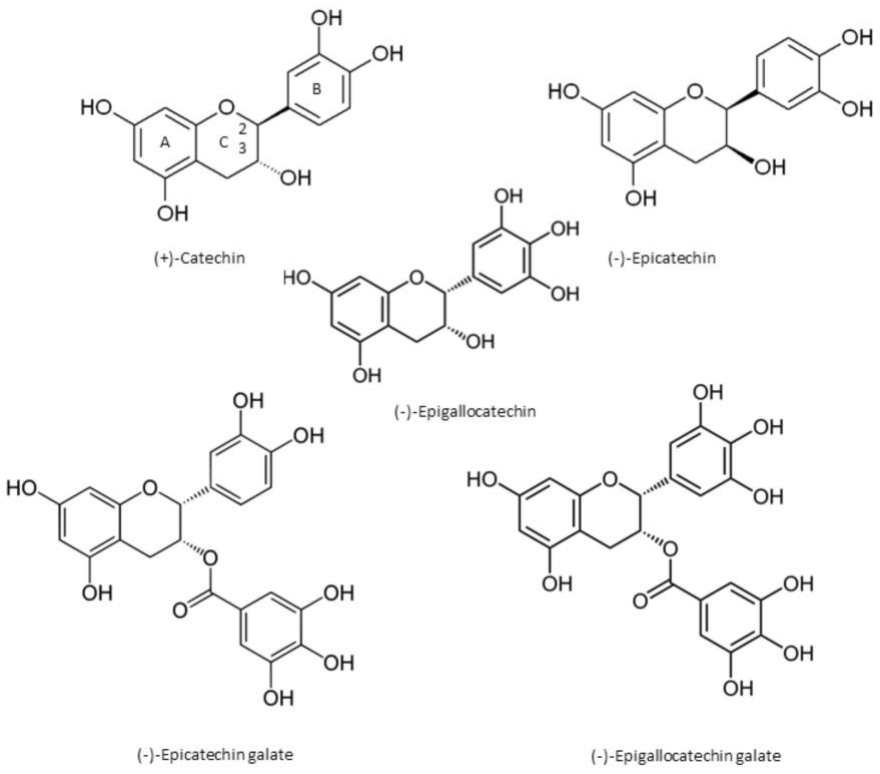

Figure 2 Catechins structure [26]

Another important feature of catechins is metal chelation. Catechins have the ability to bind metal ions like copper and iron. This feature supports the antioxidant activity by inhibiting free radical formation catalyzed by metal. Other mechanisms of antioxidant effects are enzyme inhibition, enzyme induction, and inhibition of transcription factor activation [27].

Compared to leaf or bagged green tea, matcha tea consumption increases EGCG and other polyphenols intake [28]. This is probably caused by the powder form of matcha. Therefore, the antioxidant activity of matcha may be higher compared to other teas.

2.1.1.2 Flavonols

Regarding green and black teas, we can find flavonols such as myricetin, quercetin, and kaempferol. Quercetin is the most important flavonol, followed by kaempferol and myricetin. Green tea has a higher level of flavonols than black tea. The amount of flavonols is influenced by the production process as well. It was proven that the extraction from ground tea leaves contains a significantly higher amount of flavonols compared to the extraction of whole tea leaves [29]. Flavonols have shown high biological activity, and antioxidant ability and have been reported as anti-HIV-1 compounds [30].

The concentration of quercetin in aqueous solutions was only a little bit higher in matcha tea than in "normal" green tea [31].

2.1.1.3 Flavones

Rutin is a glycoside that combines quercetin and the disaccharide rutinose. It belongs to the group of polyphenols. Except for green tea, rutin is found in buckwheat, asparagus, and citrus leaves. Rutin provides antioxidant and anti-inflammatory qualities, as well as immune system support [23].

Compared to other beverages, matcha tea is the richest source of rutin. Matcha contains almost 50 times higher amounts of rutin than green tea. Usually, matcha contains between 1220 – 1970 mg/l of rutin, and green tea only 37.0 mg/l. Compared to buckwheat which is considered an important source of rutin it contains around 62 mg per 100 grams. Rutin and vitamin C show a synergistic effect. This means that they both positively support the circulatory system and show antioxidant properties [23].

2.1.2 Phenolic acids

Phenolic acids are compounds that contain one carboxylic acid group. They are found in plant foods such as fruits, seeds, and vegetable leaves. They are usually divided into two groups: hydroxybenzoic and hydroxycinnamic acids. Hydroxycinnamic acids are derived from cinnamic acid. The most typical ones are caffeic, sinapic, ferulic, and *p*-coumaric acids.

Hydroxybenzoic acids are derived from benzoic acid. The most common ones are syringic, protocatechuic, *p*-hydroxybenzoic, gallic, and vanillic acids [32].

Phenolic acids have many positive effects on the human organism. These are anticarcinogenic, antimicrobial, antimutagenic, and anti-inflammatory effects. They have also shown significant antioxidant properties [22].

2.1.3 Lignans

Lignans are a big group that belongs to polyphenols. They are phytoestrogens that support the regulation of estrogen production in the human body. In the intestine, lignans are transformed into enterolignans, enterodiol, and enterolactone by the bacteria. Enterolactone decreases the risk of prostate, colon, and breast cancers, as well as cardiovascular diseases. Lignans help to promote healthy body weight and reduce the risk of diabetes in addition to insulin resistance [30].

2.1.4 Xanthine alkaloids

There are three xanthine alkaloids present in green tea: caffeine, theobromine, and theophylline. All of them are derived from methylxanthines. These compounds have an influence on various parts of human organism such as respiratory, renal, cardiovascular, gastrointestinal, and central nervous system. They are consumed on daily basis as they are occurring in various foods, such as tea, coffee, or chocolate. Their concentrations are dependent on the genotype of the plant, and it has an impact on the taste of food and beverages [33].

The amount of caffeine (1,3,7-trimethylxanthine) depends on the production process and part of the tea plant used. Teas containing more tea buds usually have a higher level of caffeine than teas containing stems and mature leaves. The caffeine level of tea leaves differs from 1.4 to 4.5% by weight. The amount of caffeine is also higher in matcha tea compared to other green teas [34]. Caffeine is beneficial for human organisms. It stimulates the digestive and cardiovascular systems. Also, it has a positive effect on the nervous system. It may increase focus and improve mood as well as alertness. Another benefit of caffeine is that it burns fat because it increases energy. Therefore, it is used in supplements for weight loss. Caffeine has protective features against heart diseases and anti-inflammatory properties. Of course, it should be consumed in moderation [30].

Theobromine (3,7-dimethylxanthine) is a bitter compound occurring in cacao, chocolate, and *Camellia sinensis* L. Theophylline (1,3-dimethylxanthine) is found in cocoa, mate (*Ilex paraguariensis*), and tea leaves as well [33].

2.1.5 Protein content and amino acids

One of the most important free amino acids is L-theanine. Out of all free amino acids contained in green tea, L-theanine is representing nearly 50%. The amount of L-theanine depends on the cultivation and production process. Studies say that the highest amounts are contained in black tea [30].

Matcha contains a higher number of proteins and amino acids. Amino acids occur in a bound form, but the free ones are more important as they support the umami taste [35]. L-theanine is the most abundant amino acid. Shade growing leads to L-theanine not being broken down. L-theanine also decreases bitterness. Together with caffeine, they can improve vigilance and focus [34].

Other amino acids available in green tea are arginine, glutamine, asparagine, serine, valine, glycine, leucine, glutamic acid, aspartic acid, tryptophan, and tyrosine [30].

2.1.6 Vitamins

Green tea contains vitamins C, E, B_2, and folic acid. Vitamins C and E show antioxidant and radical scavenging effects. The content of vitamin C is twice as high in matcha as in ordinary green tea [36]. Vitamin C also prevents the flu and has a hypoglycemic effect. Vitamin B maintains healthy skin and mucus membranes, folic acid prevents arterial sclerosis and fetal neurol tube defects [37].

2.1.7 Mineral and trace elements

Tea is full of macro-, micro- and trace elements. There are calcium, sodium, potassium, manganese, magnesium, zinc, cobalt, copper, iron, chromium, and nickel. Green tea is not as rich in trace elements as black tea. What is known is that the plant *Camellia sinensis* also cumulates aluminum at a high level [30].

When consuming matcha tea the whole tea leaves are consumed. It is necessary to pay attention to the release of mineral and trace elements from the digestible and indigestible parts as well. The content of mineral and trace elements in matcha depends on the cultivation process and the geographical origin [38].

2.1.8 Tea polysaccharides

Tea polysaccharides are soluble and insoluble fibers with a high molecular weight. It consists of more than ten monosaccharides that are connected by glycosidic linkages. Polysaccharides present in tea decrease the blood sugar level and slow down the absorption of glucose. Therefore, it might be useful for people with diabetes [30].

Matcha tea contains up to 30% fiber. There are present both fibers which are soluble and insoluble. Insoluble fiber consists of lignins (which belong to the group of polyphenols), cellulose, and insoluble parts of hemicelluloses. The fiber content has an impact on digestibility. The digestibility is increasing with lower fiber content [36].

2.1.9 Saponins

Tea saponins are triterpene glycosides. They are found in seeds, flowers, leaves, roots, and stems. They are natural surfactants. Saponins help improve the bioavailability of pollutants in contaminated soils [39]. In the human body, they show anti-fungal, anti-allergic, anti-inflammatory, and anti-obese activities [40].

2.1.10 Pigments

Chlorophyll is responsible for the unique green color of matcha tea. Due to growth in the shade, matcha tea contains a higher level of chlorophyll compared to other teas [34].

While ordinary green tea contains around 1.2 mg/g of chlorophyll, in matcha tea only the concentration of chlorophyll *a* is approximately 4 mg/g. Concerning chlorophyll *b* in matcha, its concentration is lower. It can occur in an amount up to 1.4 mg/g [36].

2.2 Potential health effects of matcha green tea

As mentioned above, matcha green tea displays many possible positive effects on the human organism. Its consumption might prevent cancer, or it can be consumed as a support during cancer therapy. EGCG is the anticancer compound in matcha. Studies *in vivo* have shown that EGCG can suppress carcinogenesis at all stages [41]. It is related to the inhibition of cancer cells' growth and causing their apoptosis. EGCG is a strong antioxidant and interferes with the inflammatory processes. Vitamin C and phenolic acids are also known for their anti-cancer features [34].

Inflammation is related to the production of reactive oxygen species. The component that is associated with anti-inflammatory effects is EGCG. It scavenges free radicals and helps to regulate the inflammatory condition and response [34].

Rutin and EGCG also show cardioprotective effects. These compounds decrease oxidative stress, strengthen the blood vessels, and inhibit the activation of kinase protein [34].

According to many studies performed on traditional green tea, it shows antiviral properties. Not enough studies were done on matcha tea. However, matcha has the potential to prevent and inhibit infectious diseases. Catechins and quercetin show possible mechanisms against Covid-19 infection [34]. Flavonoids especially ECG and EGCG decrease the propagation of HIV retrovirus due to the inhibition of reverse transcriptase, which is an enzyme that allows the virus to establish itself in a host cell [42].

EGCG, phenolic acids, and quercetin support the metabolism of carbohydrates. This is done by inhibition of starch digestion, inhibition of gluconeogenesis, improvement of insulin sensitivity, regulation of insulin release, and inhibition of glucose absorption [34].

EGCG seems to have anti-obesity and anti-diabetic properties. The mechanisms of these features are likely to be connected to the modulation of the balance of energy, metabolism of lipids and carbohydrates, endocrine systems, or food intake [42].

EGCG and caffeine are beneficial for the central nervous system. EGCG improves cognitive function and mental clarity, inhibits free radicals, and lowers amyloid-β production in the brain. Caffeine prevents brain aging, inhibits neuroinflammation, and overturns oxidative processes [34]. As one of the reasons for Alzheimer's and Parkinson's disease development is oxidative stress, the regular intake of matcha tea might prevent the occurrence of neurodegenerative diseases [43].

II. ANALYSIS

3 OBJECTIVES OF THE DIPLOMA THESIS

This diploma thesis is aimed at determining the polyphenolic profiles and the antioxidant activity of five different samples of matcha tea (*Camellia sinensis* L.) under defined leaching conditions and after simulation of *in vitro* digestion.

The subjects of the theoretical part were the description of tea manufacturing and the differences between classical green tea and matcha tea. Furthermore, the most typical polyphenols and other substances appearing in matcha tea were described.

In the experimental part of this work, the matcha tea samples, chemicals, and equipment are described in detail. Concerning laboratory analysis, the objective was divided into two parts:

a) to measure TPC, antioxidant activity values, and phenolic profiles of matcha tea under different leaching conditions,

b) to determine the digestibility value of matcha tea, to prepare the undigested part of matcha tea samples by applying *in vitro* digestion process, as well as to measure TPC contents, antioxidant activity values, and phenolic profiles in both the native and undigested part of tea leaves.

4 METHOD SECTION

4.1 Devices

- Micropipettes with adjustable volume
- Analytical balance Kern ABT 200 – 4NM
- Ultrasonic bath PS 04000A (Notus, Slovakia)
- Laboratory oven Venticell, BMT a.s., MMM-Group
- Magnetic stirrer IKA (Verkon, Prague, Czech Republic)
- UV/VIS spectrophotometer Lambda 25 (Perkin Elmer, USA)
- HPLC Dionex Ultimate 3000 (Thermo Fisher Scientific, Waltham, USA)
- Syringe filters with nylon membrane NY 0,22 μm (Chromservis s.r.o.)
- High-speed micro-centrifuge (DLAB D 3024, HPST s.r.o., Czech Republic)
- *In vitro* incubator Daisy (Ankom Technology, Macedon, USA)
- Thermo shaker TS-100 (Biosan, Vilnius, Lithuania)

4.2 Chemicals and reagents

- Redistilled water (Aqua osmotic, Tišnov, Czech Republic)
- Methanol (Ing. Petr Švec – Penta s.r.o.)
- Standard of trolox (Merck, Darmstadt, Germany)
- Standard of gallic acid (Merck, Darmstadt, Germany)
- ABTS (Merck, Darmstadt, Germany)
- DPPH (Merck, Darmstadt, Germany)
- Na_2CO_3 (Ing. Petr Lukeš)
- Folin-Ciocaulteu reagent (Merck, Darmstadt, Germany)
- $K_2S_2O_8$ (Ing. Petr Lukeš)
- CH_3COONa (Ing. Petr Lukeš)
- CH_3COOH (Ing. Petr Lukeš)

- 0,1M HCl (Ing. Petr Lukeš)
- Pepsin (E.C. 3.4.23.1) with an activity of 0.7 FIG-U/mg (Merck, Darmstadt, Germany)
- Pancreatin – enzyme mixture of pancreatin with activities of 350 FIG-U/g protease, 7500 FIG-U/g amylase, and 6000 FIG-U/g lipase (Merck, Darmstadt, Germany)
- KH_2PO_4 (Ing. Petr Lukeš)
- $NA_2HPO_4.12H_2O$ (Ing. Petr Lukeš)

4.3 Samples of matcha teas

Five different commercial matcha tea samples were purchased online from food stores in the Czech Republic. All matcha samples had an expiration date of either the 2022 or 2023 years.

Samples were identified as follows:

Sample 1 – ORGANIS Matcha Tea Premium, 100% powder of tea leaves (*Camellia sinensis*), country of origin China. The information on individual nutrients that was provided on the product label is presented in Table 1.

Table 1 Nutrition information - Sample 1

Nutrition information (100 g):	
Energy	1545kJ/369kcal
Fats	1 g
of which saturated fatty acids	0.8 g
Saccharides:	63 g
of which sugars	1 g
Proteins:	26 g
Salt:	0 g

Figure 3 ORGANIS Matcha Tea Premium

Sample 2 – ALLNATURE Matcha Tea Premium, 100% tea powder made of *Camellia sinensis*, country of origin China. The nutrition information is displayed in Table 2.

Table 2 Nutrition information - Sample 2

Nutrition information (100 g):	
Energy	1545kJ/369kcal
Fats	1 g
of which saturated fatty acids	0.8 g
Saccharides:	63 g
of which sugars	1 g
Proteins:	26 g
Salt:	0 g

Figure 4 ALLNATURE Matcha Tea Premium

Sample 3 – IMBIO Matcha Tea, 100% powder prepared from tea leaves (*Camellia sinensis*), country of origin China. No nutrition information was available for this sample.

Figure 5 IMBIO Matcha Tea

Sample 4 – ISWARI Bio Matcha Tea, 100% of BIO matcha tea powder, country of origin Japan. The nutrition information is displayed in Table 3.

Table 3 Nutrition information - Sample 4

Nutrition information (100 g):	
Energy	1357kJ/324kcal
Fats	3.1 g
of which saturated fatty acids	0.8 g
Saccharides:	37 g
of which sugars	0.9 g
Fiber	32 g
Proteins:	21 g
Salt:	0.03 g
Vitamin C	163 mg
Magnesium	198 mg
Caffeine	3.2 g

Figure 6 ISWARI Bio Matcha Tea

Sample 5 – NATU Matcha Tea Bio, 100% of BIO matcha tea powder, country of origin Japan. The nutrition information is displayed in Table 4.

Table 4 Nutrition information - Sample 5

Nutrition information (100 g):	
Energy	1155kJ/275kcal
Fats	3.5 g
of which saturated fatty acids	0.7 g
Saccharides:	33 g
of which sugars	1.8 g
Fiber	31.2 g
Proteins:	27.3 g
Salt:	0.01 g
Vitamin E	19 mg
Vitamin C	196 mg
Potassium	2150 mg

Figure 7 NATU Matcha Tea

All of them were obtained as loose-leaf tea, which was in the original 70–250 g packs. Samples were kept out of the sunlight in an air-conditioned laboratory (20–22°C) no longer than four months prior to all analysis.

4.4 Sample preparation for leaching ability measurements

Five different matcha tea samples were used to prepare matcha infusions under three different temperatures. Half a gram (0.5 g) of powder matcha teas were weighed and transferred into 50 ml beakers marked with a line. Then, 50 ml of redistilled water of a temperature about 60, 70, and 80°C were added and the mixture was allowed to infuse for 3 and 5 minutes while being stirred in a blender. Finally, samples were filtered through a paper filter (type KA4), and the obtained filtrate was used for TPC and antioxidant activity analysis. In the case of phenolic profile measured using HPLC, the samples were filtered through a NY syringe filter (0.22 μm).

4.5 *In vitro* digestion process

Firstly, the dry matter and ash contents of powdered form of matcha samples were determined according to ISOs 1573 [44] and 1575 [45], respectively.

For the determination of digestibility values, the *in vitro* method that simulates the process of digestion was applied. The digestion method was provided according to two sequential steps: gastric (when pepsin as an enzyme was used) and intestinal (similarly, a mixture of pancreatin was applied). Measurement was carried out in a Daisy incubator. For the determination, the bags of the F57 type were used. The bags were submerged in acetone and left to dry before the beginning. In each bag, approximately 0.5 g of matcha tea was weighted. All of the bags were carefully sealed. To simulate gastric digestion, there were

also 1.7 l of 0.1M HCl added as well as 3 g of pepsin while the sample bags were incubated at 37°C for 2 hours. For simulating intestinal digestion, both 32.49 g of $Na_2HPO_4.12\ H_2O$ and 3.09 g of KH_2PO_4 were dissolved in 1.7 l of redistilled water. After, the samples were incubated at 37°C for 24 hours in a phosphate buffer containing 3 g of pancreatic enzymes. After incubation, samples were washed with redistilled water and then dried on filter paper at 105°C for 24 hours. Finally, the samples were burnt in a muffle furnace at 550°C for 5.5 hours, then cooled and weighed. The *in vitro* digestibility values (%) were calculated according to the equations 1–7:

$$Digestibility\ (\%) = 100 - \left(\frac{DMR - AR}{m_2 \times DM \times OM}\right) \times 100 \quad (1)$$

$$DMR = m_3 - m_1 \times c_1 \quad (2)$$

$$AR = m_4 - m_1 \times c_1 \quad (3)$$

$$DM = \frac{DW \times m_s}{100} \quad (4)$$

$$OM = \frac{DW - A}{100} \quad (5)$$

$$c_1 = \frac{m_s}{m_1} \quad (6)$$

$$c_2 = \frac{m_p}{m_1} \quad (7)$$

where:

DMR is the weight of the sample without the sack after digestion and drying (g), DM is the dry weight of the sample (g), DW is dry weight of the sample (%), AR is ash weight of the sample with the sack (g), OM is organic matter content in dry matter of the sample (g), A is ash content in the sample (%), m_s is the weight of the sample for dry matter determination (g), c_1 is the correction of the sack weight after incubation (g), c_2 is the correction of the sack weight after combustion (g), m_p is the weight of ash from the empty correction sack (g), m_1 is the weight of the empty bag (g), m_2 is the weight of sample (g), m_3 is the weight of dried bag with sample after incubation (g), m_4 is the weight of both samples and sacks after drying and combustion (g) [46].

4.6 Sample preparation – extraction of the native and undigested form of matcha teas

Because matcha is consumed in whole leaf parts the extraction conditions to simulate human body temperature was the main focus.

In order to determine the TPC values, antioxidant activities, and phenolic profile in the native form of matcha powder, 0.1 g of the native powdered form of matcha tea was weighted into Eppendorf plastic tubes and mixed with 2 ml of redistilled water. Subsequently, the tubes were placed into a thermo-shaker under 900 rpm for extracting those compounds at 37°C for 30 minutes. Then, samples were cooled to an ambient temperature and filtered (0.22 µm) to obtain extracts for analyzing them.

Regarding the undigested powdered part of matcha samples, the process of digestibility assessment (chapter 4.5) was terminated by drying samples (in this case at 40°C for 12 hours). The solid undigested part of the matcha sample was extracted the same way as described in the previous paragraph.

4.7 Total polyphenolic content

The total polyphenolic contents were determined by Folin-Ciocalteu reaction. Briefly, 5 ml of redistilled water, 50 µl of matcha extract, 0.5 ml of Folin-Ciocaulteu reagent, and 1.5 ml of 20% Na_2CO_3 were mixed together in a 10-ml volumetric flask to react. Afterward, the volumetric flask was filled up to the mark with redistilled water and kept away from sunlight for 30 minutes. Consequently, the absorbance was measured at 765 nm with a UV/VIS spectrophotometer Lambda 25. Instead of tea infusion water was added as a blank [47].

For the calibration, the same procedure was used, while instead of tea infusion the standard solutions of gallic acid were added. Stock solution with a concentration of 4000 mg/l was diluted as follows: 50, 100, 200, 400, 600 and 800 mg/l.

4.8 Individual phenolics determination using HPLC

The most typical phenolic compounds were determined by a HPLC system (type of Dionex Ultimate 3000 RS) that consists of Thermo Scientific Dionex UltiMate 3000 Diode Array Detector type DAD-3000RS, an UltiMate 3000 rapid separation autosampler, a binary pump HPG-3x00RS, and a solve selector valve HPG-3400RS. Signals provided by the HPLC system were processed on a PC running the LC Chromeleon TM 7.2 Chromatography data

system. The profile of the polyphenols was measured according to Deng et al. (2012) with a slight modification.

Kinetex column C18 (150 x 4.6 mm; 2.6 μm) (Phenomenex, USA) was used to separate the phenolic acids. The volume of a sample of 10 μl was brought to the column and eluted under gradient conditions performed with redistilled water:acetic acid in a ratio of 99:1 (A) and redistilled water:ACN:acetic acid in a ratio of 67:32:1 (B).

The solvent gradient was programmed as follows: 10% B at 0 min, increasing from 0−10 min to 20%, 10−16 min 20−40% B, 16−20 min 40−50% B, 25−26 min 50−70% B, 26−30 min 70% B, 30−40 min 70−10% B, 40−45 min 10% B. The solvent flow rate was 1 ml per minute, the temperature of the column was set at 30°C and the chromatogram was evaluated at a wavelength of 275 nm. DAD response was linear for all phenolic acids within the calibration range of 0.05−150.0 μg/ml. The correlation coefficients were exceeding 0.9998. The appropriate phenolics were identified using their retention time [48].

4.9 Antioxidant activity assay

4.9.1 Scavenging of DPPH radicals

Stock and working solutions

The stock solution was prepared by dissolving 24 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) in 100 ml of methanol. From this stock, the working solution was prepared by mixing 10 ml of stock solution with 45 ml of methanol. This was followed by measuring the absorbance of the working solution at a wavelength of 515 nm against methanol as a blank. The measured value was listed as A_0.

Sample measurements

For measuring, 8.55 ml of working solution of DPPH radicals and 30 μl of sample extracts were pipetted into a test tube to mix together. This mixture was left in the dark for 60 minutes. After this, the decreasing of absorbance was measured at a wavelength of 515 nm to calculate the value of inactivation (%), which was calculated according to equation 8. According to the linear regression curve the antioxidant values were calculated and expressed as trolox equivalents (μg TE/g).

$$Inactivation\ (\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (8)$$

where A_0 is the absorbance of the working solution, and A_1 is the absorbance of the sample.

Calibration curve

Trolox as a reference was used. The standard stock solution was prepared by dissolving it in methanol for the final concentration of 800 mg/l. By dilution, calibration sets of 40, 80, 120, 160, and 200 mg/l were prepared. Each concentration of volume of 450 µl was added to 8.55 ml of the working solution. After one hour in the dark, the decrease in absorbance was measured at 515 nm. From the measured data, the value of inactivation (%) was counted. The calibration curve was compiled as the dependence of the inactivation on the trolox concentration.

4.9.2 Scavenging of ABTS radicals

Preparation of ABTS radicals

Into a 10 ml volumetric flask, 0.018 g of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was weighted and filled with redistilled water up to the mark. Next, 0.2 ml of 0.06M $K_2S_2O_8$ was added to this mixture to sit for 16 hours at laboratory temperature in the dark. After this time the ABTS radicals were created and were ready for further use.

ABTS reaction mixture

An acetate buffer was prepared by mixing 63.5 ml of 0.2M CH_3COONa and 136.5 ml of 0.2M CH_3COOH. The final pH was 4.3. The buffer volume of 97.5 ml was mixed with 2.5 ml of ABTS radical solution. This reaction mixture was measured at a wavelength of 734 nm against the acetate buffer as a blank. By measuring the absorbance of the reaction mixture, the value of absorbance (A_0) was obtained that was used for the calculation of inactivation (%). The value of inactivation was calculated according to equation 8 as described in chapter 4.9.1.

Calibration curve

For the scavenging of ABTS radicals, trolox as a reference standard was used. By dissolving 40 mg of trolox in 100 ml of methanol the stock solution of 0.40 mg/ml was obtained. From this solution the calibration series of 0.01, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 and 0.40 mg/ml were prepared. The volume of 75 µl of calibration solutions was added to 6 ml of the

ABTS reaction mixture. The samples were mixed and left for 30 minutes in the dark and after this time the decreasing absorbances were measured at a wavelength of 734 nm. The calibration curve was constructed from the measured values as the dependence of the inactivation on the concentration of trolox.

Sample measurements

For measuring, 6 ml of the ABTS reaction mixture together with 10 µl of the matcha sample were added into the test tube. The tubes were kept for 30 minutes out of the sunlight. After, the decrease of the absorbance was measured at a wavelength of 734 nm. The total antioxidant activity was counted from linear regression, expressed as the equivalent value of trolox in micrograms per 1 gram of the sample (µg TE/g).

4.10 Statistical evaluation

Basic chemical, total, and individual phenolic contents, antioxidant activity, and digestibility values were repeated 3–4 times (n = 3–4), and the results were reported as mean ± standard deviation (SD). The results were statistically evaluated using a parametrical test comparing mean values of two independent assortments (Student *t*-test). The level of significance was set at 5%.

5 RESULTS AND DISCUSSION

Experimental part A

In this section, this diploma thesis determines and discusses the total polyphenolic contents (TPC), the profile of individual phenolics, and the antioxidant activities (measured by scavenging of ABTS and DPPH radicals) of five kinds of matcha teas under different leaching conditions (at 60, 70 and 80°C for 3 and 5 minutes).

Experimental part B

In this section, the digestion process was applied to matcha samples to determine the digestibility values of matcha teas and to prepare undigested parts of matcha tea. After, native and undigested matcha tea samples were extracted to determine the release of polyphenols from the tea matrix after digestion. Apart from phenolics, the antioxidant activity values were measured too.

Experimental part A

5.1 Total polyphenolic content

For the determination of total polyphenolic content (TPC), the extracts were prepared according to the process described in 4.4. The analysis itself was done as per the process mentioned in 4.7. The TPC results are expressed in an equivalent amount of gallic acid (GAE) in mg per 1 g of the sample (mg GAE/g sample). The results are shown in Table 5.

Table 5 Results of TPC measurements

Sample	**T(min)**	**TPC (mg GAE/g ± SD)**		
		80°C	**70°C**	**60°C**
1	3	$1290\pm40^{a,A}$	$1460\pm50^{b,A}$	$1300\pm50^{a,A}$
	5	$1210\pm10^{a,B}$	$1430\pm40^{b,A}$	$1250\pm10^{c,B}$
2	3	$1280\pm30^{a,A}$	$1200\pm40^{b,A}$	$1310\pm40^{c,A}$
	5	$1240\pm20^{a,B}$	$1160\pm20^{b,B}$	$1360\pm20^{c,B}$
3	3	$1570\pm30^{a,A}$	$1530\pm30^{b,A}$	$1580\pm40^{a,A}$
	5	$1580\pm40^{a,A}$	$1470\pm20^{b,B}$	$1500\pm20^{c,B}$
4	3	$1900\pm40^{a,A}$	$1870\pm30^{b,A}$	$1840\pm30^{c,A}$
	5	$1730\pm30^{a,B}$	$1780\pm40^{a,B}$	$2060\pm50^{b,B}$
5	3	$1530\pm30^{a,A}$	$1670\pm40^{b,A}$	$2000\pm50^{c,A}$
	5	$1480\pm40^{a,B}$	$1620\pm10^{b,B}$	$1750\pm50^{c,B}$

All results are presented on a dry matter basis as means ± SD, n=4 (the mean of four measurements). Means within a line with at least one identical small superscript (in case of each matcha sample leaching at 80, 70, and 60°C) do not differ significantly ($p\geq0.05$), means within a column with at least one identical capitalized superscript (in case of each matcha sample leaching 3 and 5 minutes) do not differ significantly ($p\geq0.05$).

From the measured results, the leaching temperature did not always have a significant effect on the content of total polyphenols. For example, in Sample 1, the same polyphenol content was measured at both 80 and 60°C at an infusion time of 3 minutes. The situation is similar for sample 3. If we look at the infusion time of 5 minutes, there are always statistically significant differences in the content of total polyphenols if they were infused at different temperatures. Also, it is not possible to unequivocally say that a leaching time of 5 minutes would always provide a higher amount of TPC compared to a leaching time of 3 minutes.

Because the extraction time is very short, the leaching of polyphenols will certainly be influenced by the matcha tea matrix and its particle size. Also, the fact that some polyphenols are present freely, while others bind to plant cell structures, cellulose, or hemicelluloses may have an influence as well [49][50].

The highest TPC level was measured in sample 4 and the lowest in samples 1 and 2. During cultivation, matcha is shaded which reduces the photosynthesis and causes a higher content of bioactive compounds in the final product. According to Zaiter et al. [51], the grinding process also leads to a higher content of polyphenols. Therefore, the quality of these two samples might not be as good as of the other ones due to insufficient matcha shading or grinding.

Kowalska et al. [52] demonstrated the influence of brewing parameters on the content of polyphenolic compounds. In most of the samples, longer brewing time and higher temperature lead to a higher number of compounds. This does not correspond to these results since the brewing time and temperature did not have a significant impact on the content of polyphenols.

Even though it is not usual to present the same results in two forms, a graphic illustration is more suitable to see the results. Total phenolic contents measured under different leaching conditions are presented in Figure 8. We can see that a different extraction temperature is individually suitable for each sample. Sample 1 contained the highest number of polyphenols at 70°C while sample 3 at 80°C. The temperature of 60 °C led to a higher content of polyphenols in samples 2 and 5 at both leaching times.

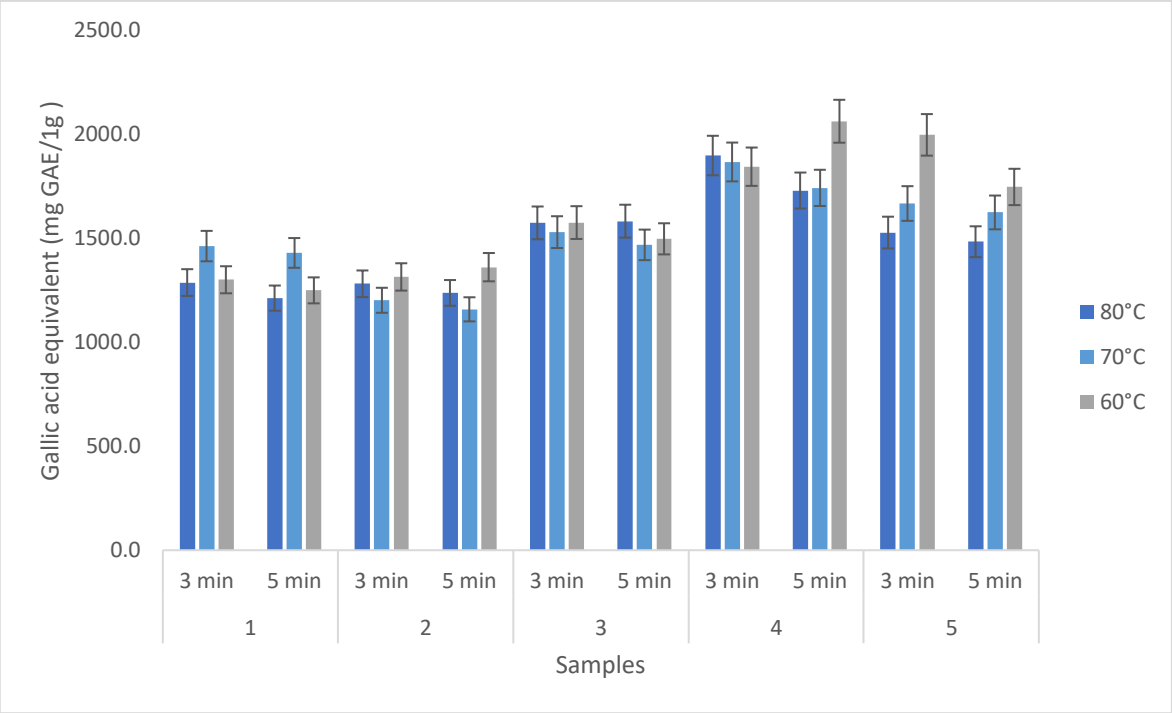

Figure 8 TPC values in graphic illustration

To construct the calibration curve for the determination of TPC values, gallic acid as a reference standard was used. The process is fully described in chapter 4.7. The equation of linear regression was y = 0,013x – 0,0222. The absorbances obtained are displayed in Table 6.

Table 6 Calibration data for TPC measurements

Gallic acid concentration (mg/l)	**Absorbance**
0	0.000
50	0.029
100	0.081
200	0.206
400	0.518
600	0.781
800	0.948

Figure 9 shows a graphical visualization of the calibration curve.

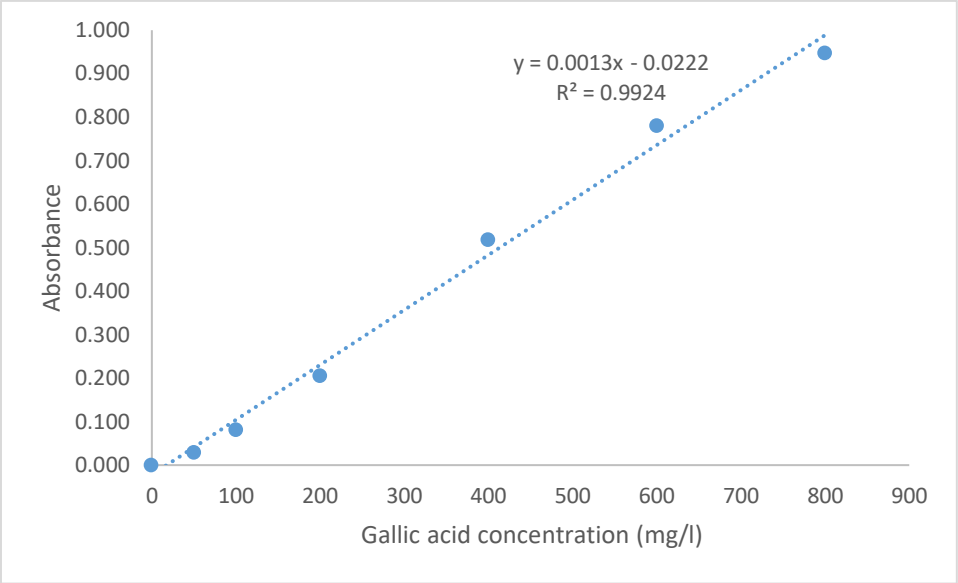

Figure 9 Calibration curve for TPC measurements

5.2 Phenolic compounds determination by using HPLC

The determination of 22 different polyphenolic compounds was performed by HPLC with DAD detection (wavelength at 275 nm) in matcha tea extracts prepared by the same procedure as described in chapter 4.4. The analysis was performed according to the process mentioned in 4.8. The equations of linear regression that were used for qualification are displayed in Table 7.

Table 7 Equations of linear regression for individual phenolics

Compounds	**Equations**
Gallic acid	y = 0.5896x + 0.0007
Protocatechuic acid	y = 0.2359x – 0.0022
Neochlorogenic acid	y = 0.1559x + 0.0003
4-hydroxybenzoic acid	y = 0.5114x + 0.0010
Epigallocatechin	y = 0.0221x – 0.0001
Catechin	y = 0.1449x + 0.0023
Vanillic acid	y = 0.4383x + 0.0015
Chlorogenic acid	y = 0.2125x + 0.0007
Caffeic acid	y = 0.7015x + 0.0011
Syringic acid	y = 0.4632x + 0.0016
Epicatechin	y = 0.1366x – 0.0003
EGCG	y = 0.0875x – 0.0020
*trans-p-C*oumaric acid	y = 0.6377x – 0.0022
Ferulic acid	y = 0.3616x + 0.0014
Sinapic acid	y = 0.1767x + 0.0001
ECG	y = 0.2608x – 0.0008
Ellagic acid	y = 0.2005x – 0.0004
Rutin	y = 0.1653x – 0.0012
trans-2-Hydroxycinnamic acid	y = 1.2589x + 0.0035
Protocatechuic acid ethyl ester	y = 0.2618x + 0.0007
Resveratrol	y = 0.5899x + 0.0041
trans-Cinnamic acid	y = 1.4799x + 0.0051
Kaempferol	y = 0.2241x + 0.0010
Quercetin	y = 0.2074x + 0.0007

EGCG – epigallocatechin-3-gallate, ECG – epicatechin-3-gallate

Concerning individual phenolics, some of the polyphenols measured were not detected and identified in matcha samples. Those are vanillic, chlorogenic, syringic acids, and resveratrol. *Trans*-Cinnamic acid, kaempferol, and quercetin were assessed when the brewing temperature was 80 and 70°C. Conversely, when brewing at 60°C, they were not detected.

As can be seen from the results (Figure 10), the lowest amount of gallic acid (4.52 µg/g) was measured in sample 2 when the brewing time was set to 3 minutes and the temperature at 80°C. The higher values of gallic acid were found in matcha sample no. 3 when its concentration reached 10.9 µg/g.

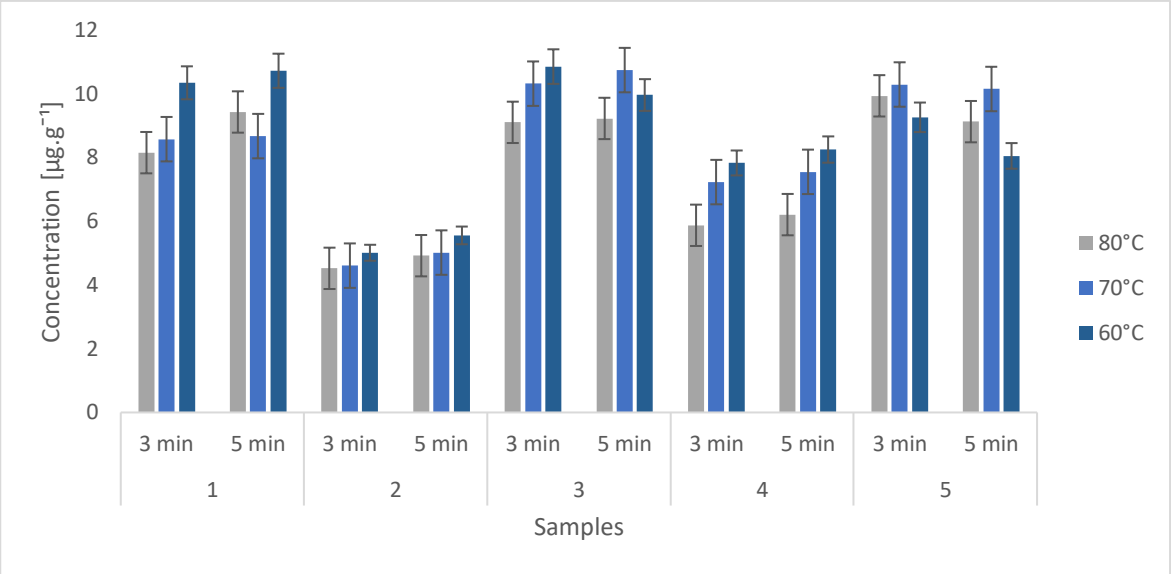

Figure 10 Gallic acid – HPLC

To magnitude, the higher concentrations were obtained in the case of protocatechuic acid determination (Figure 11). Their contents were measured in a wide range (7.22–57.2 µg/g). It is discernable that the most convenient temperature for this compound is 60°C.

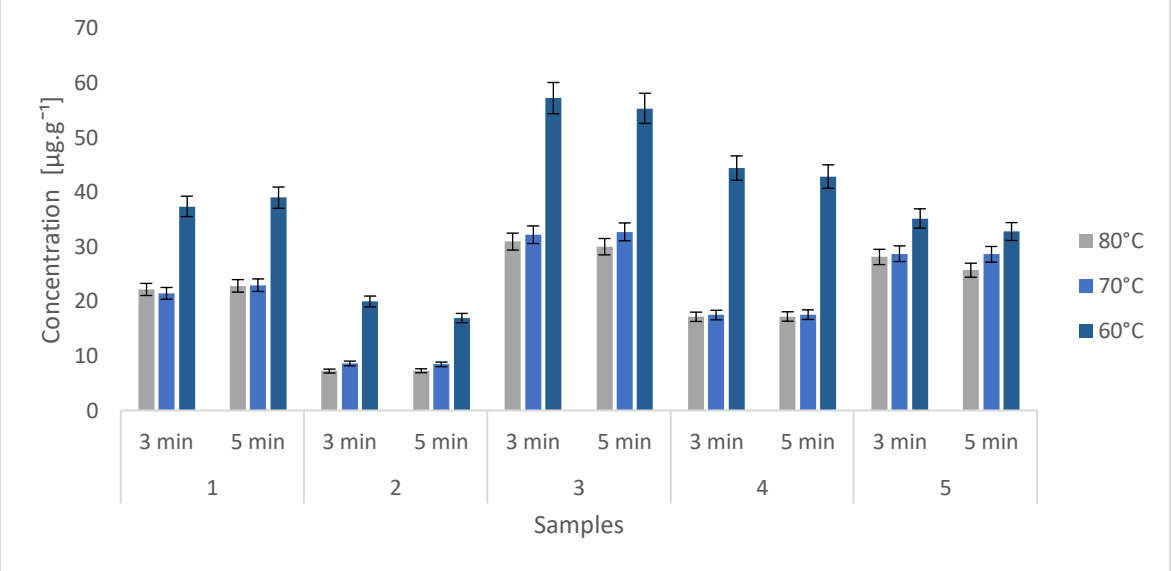

Figure 11 Protocatechuic acid – HPLC

In the case of neochlorogenic acid (Figure 12) the highest concentration (33.0 µg/g) was measured in sample 5 when a leaching temperature of 80°C for 5 minutes was applied. Although the highest concentration was measured for sample no.5, for most of the samples, the highest amount of this phenolic compound was measured at 60°C. The results significantly vary across different samples and temperatures.

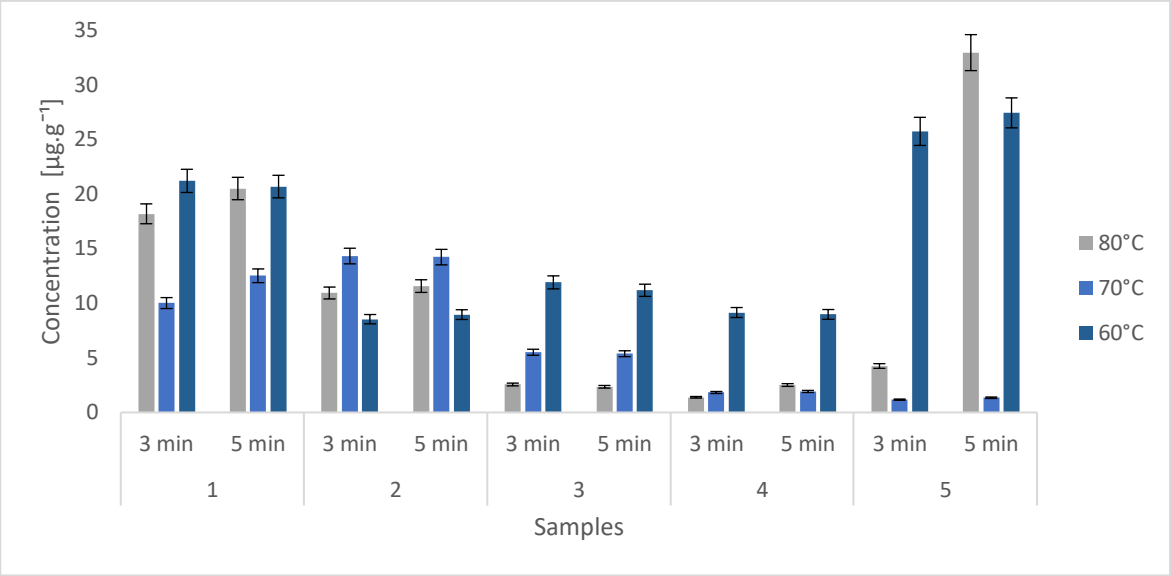

Figure 12 Neochlorogenic acid – HPLC

The polyphenolic compound of 4-hydroxybenzoic acid appears to be one of the minor polyphenols present in matcha tea (as we can see in Figure 13). The concentrations were less than 8.00 µg per 1 gram of sample for all tested samples. The highest amount was present in sample 1.

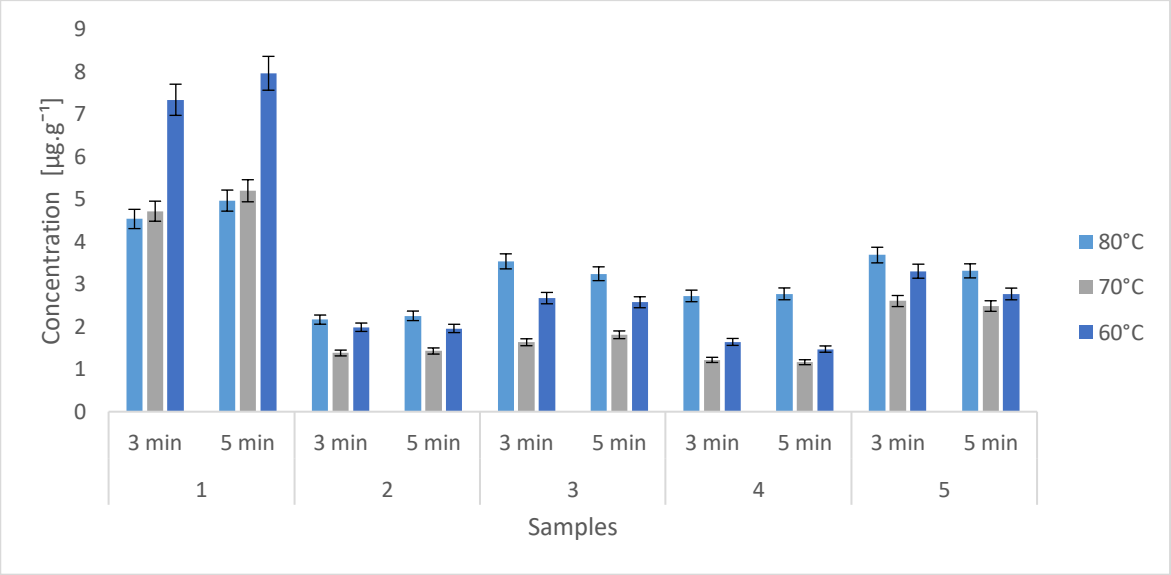

Figure 13 4-hydroxybenzoic acid – HPLC

Green teas contain five major catechins: epigallocatechin, catechin, epicatechin, epicatechin-3-gallate and epigallocatechin gallate [53]. As expected, matcha teas, as a representative of green teas, are rich in epigallocatechin. It is visible in Figure 14 that the amount of epigallocatechin in matcha tea is more than 1000 µg per 1 gram. Samples 4 and 5 contained the highest amounts of epigallocatechin while sample 2 had the lowest.

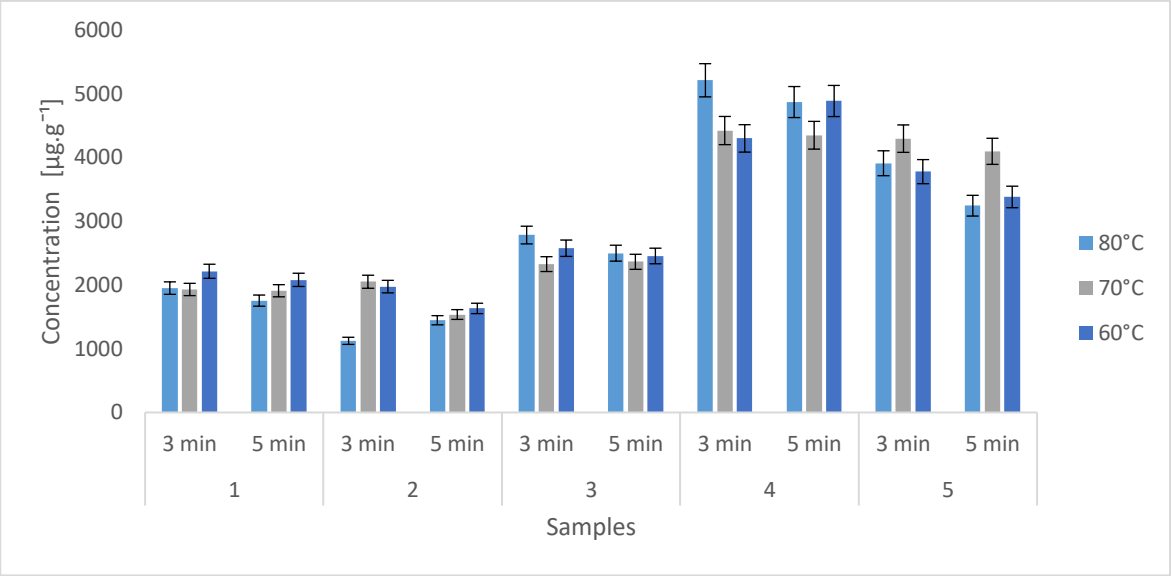

Figure 14 Epigallocatechin – HPLC

Conversely, the catechin concentrations (Figure 15), as a representative of flavanoids, were measured only in low amounts (approx. between 0.44 and 18.5 µg/g). Because green teas are rich in flavanoids, especially from several catechins, the expected concentration would be higher. It can be assumed that when the tea leaves are covered, catechin synthesis in leaves is significantly reduced [28][54].

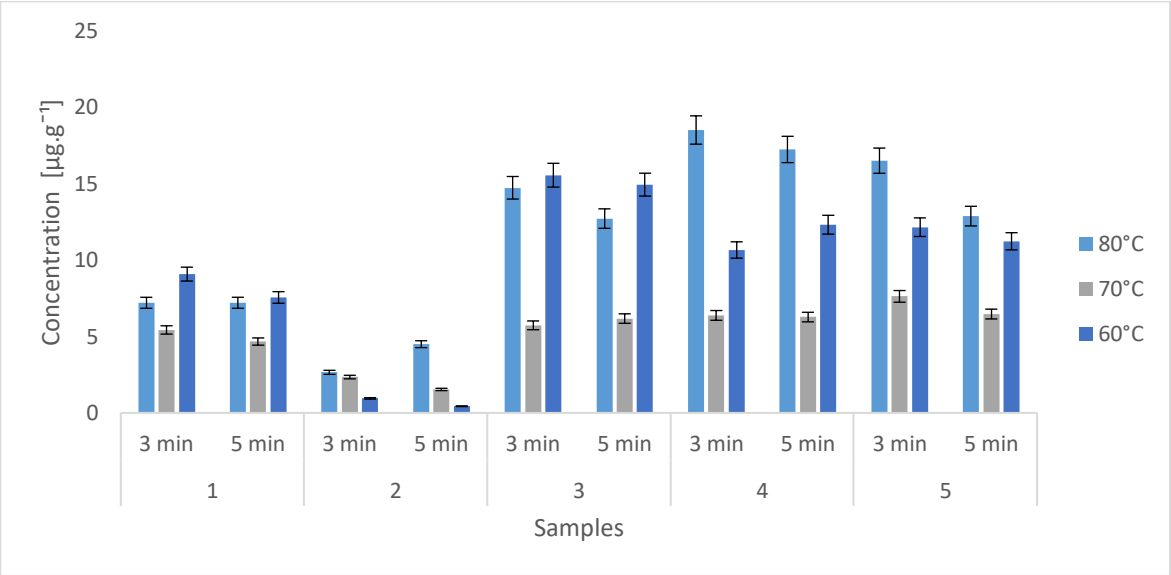

Figure 15 Catechin – HPLC

As can be seen from the data (Figure 16), the highest concentrations of caffeic acid were measured. Caffeic acid as a representative of polyphenolic acids is a major acid in this group of polyphenols. Its concentration reached up to 988 µg/g, and higher concentration values were measured mainly during the 60°C water extraction.

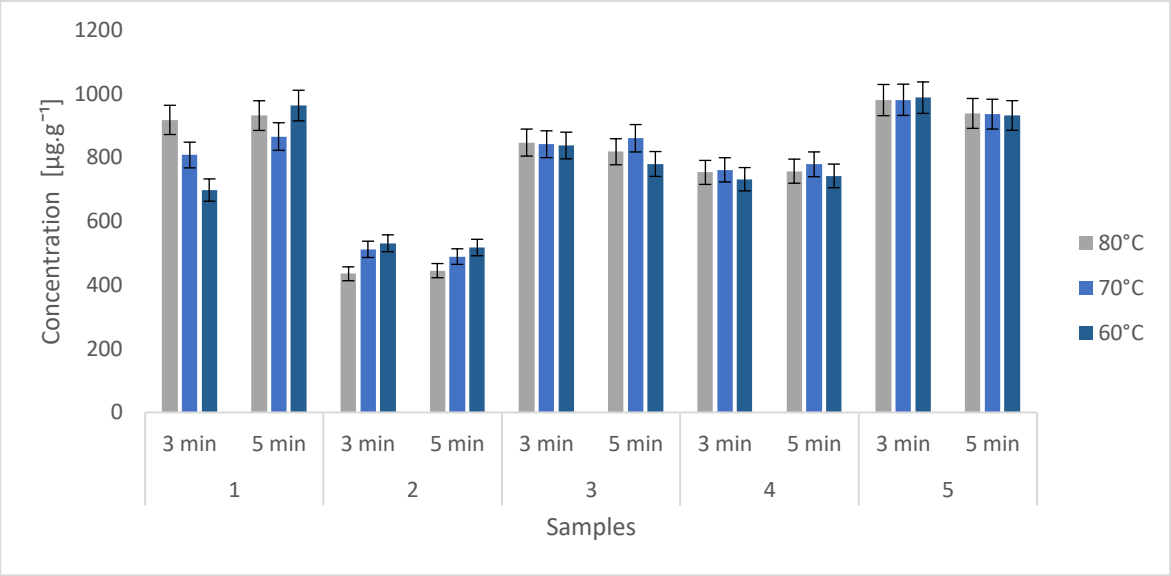

Figure 16 Caffeic acid – HPLC

The concentrations of epicatechin (Figure 17) reached up to 500 µg/g. The highest content of epicatechin was measured in samples 4 and 5, while the lowest was in sample 2. Higher concentrations were mostly measured for 60°C extractions.

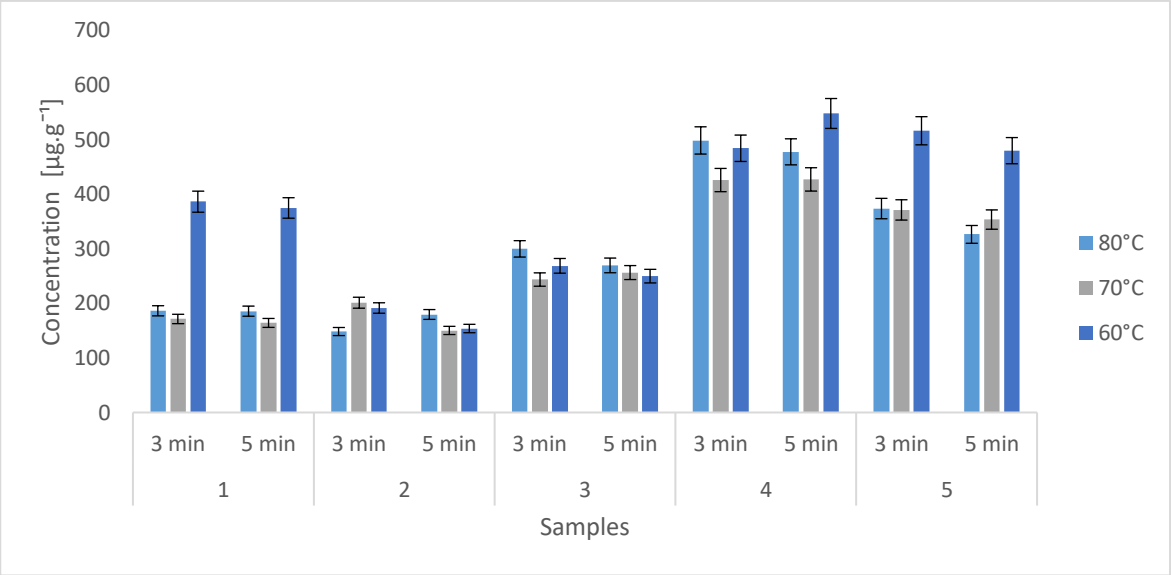

Figure 17 Epicatechin – HPLC

The concentrations of EGCG significantly vary across samples. The range of values goes from 0.00 µg/g (sample no.2 at 60°C, 5 min) to 3100 µg/g (sample no.4 at 80°C, 3 min). From the graphic illustration (Figure 18) the most convenient temperature for this phenolic compound is 80°C.

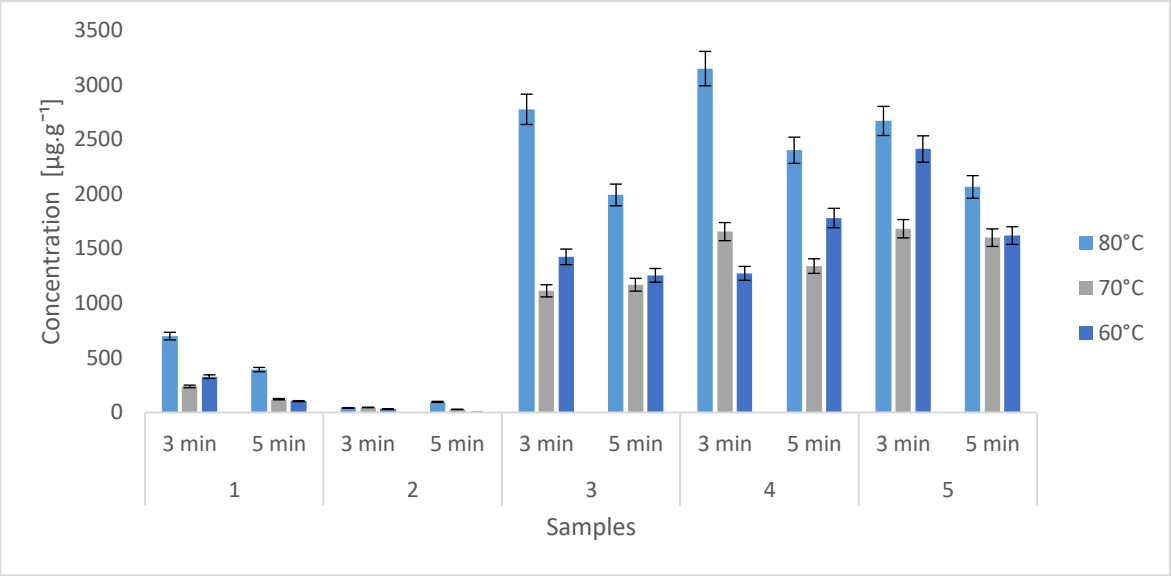

Figure 18 EGCG – HPLC

It is clear from Figure 19 that *trans-p*-Coumaric acid is a minor compound of matcha tea. The highest concentration was measured for sample no. 5 at 80°C (6.41 µg/g). The higher amounts of this substance were measured mostly during 80°C water extractions.

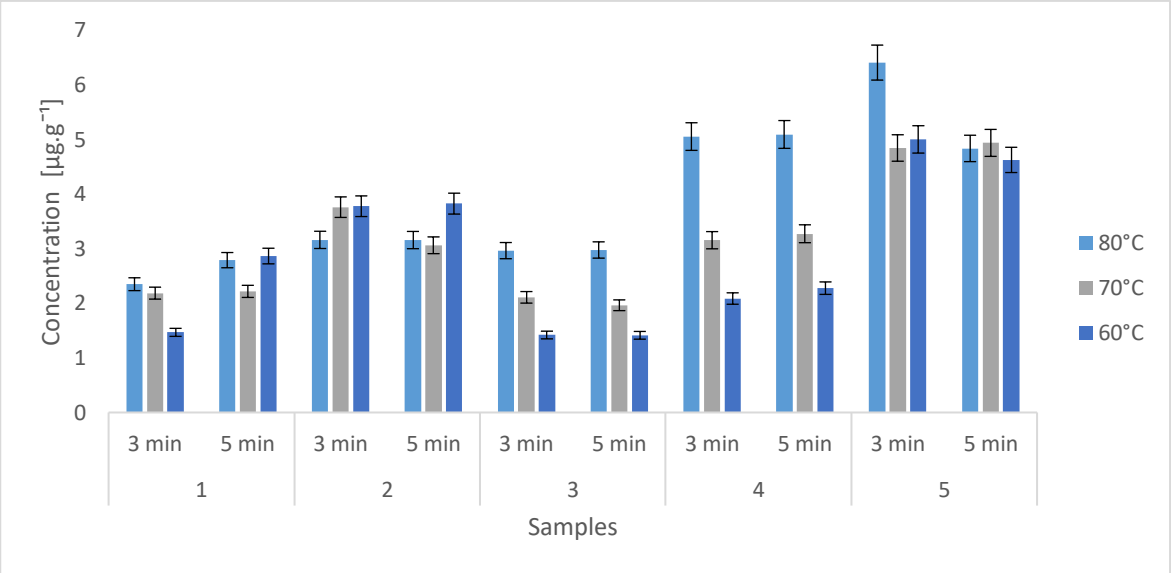

Figure 19 *trans-p*-Coumaric acid – HPLC

From Figure 20 it is visible that the lower the temperature applied, the higher the concentrations of ferulic acid were measured. The richest sample for this phenolic acid was sample no. 5 at the temperature of 60°C.

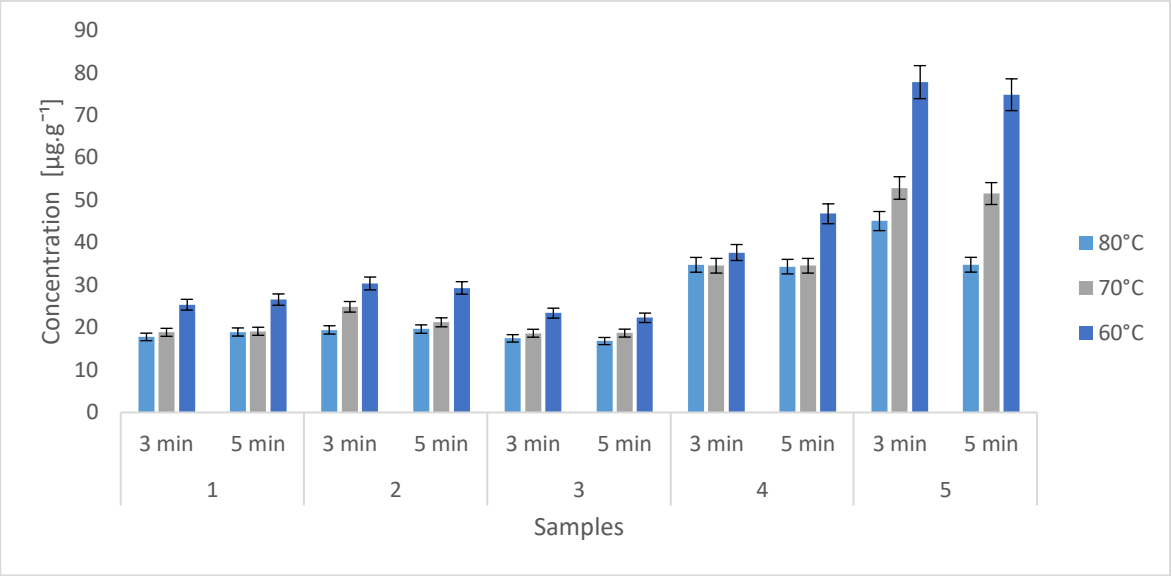

Figure 20 Ferulic acid – HPLC

The concentrations of sinapic acid (Figure 21) are the highest at 80°C for all measured samples. The highest amount appears to be in sample 4 at 80°C after 3 minutes of extraction (34.83 µg/g).

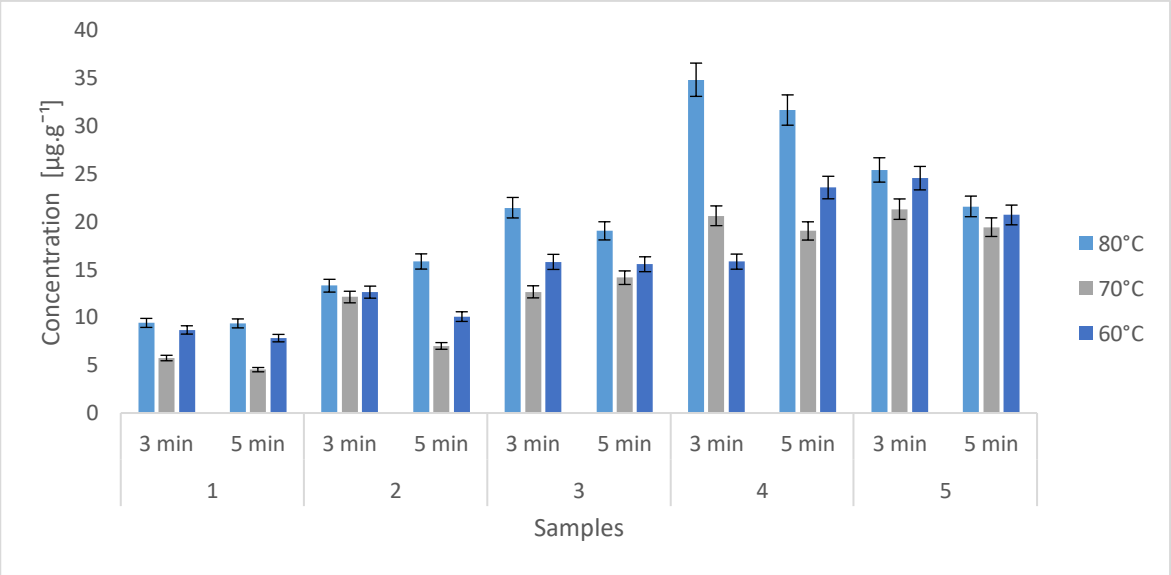

Figure 21 Sinapic acid – HPLC

For most of the samples measured, we can see that a higher concentration of ECG is connected to a lower temperature (Figure 22). The highest amounts were measured for sample no. 4 (up to 123 µg/g).

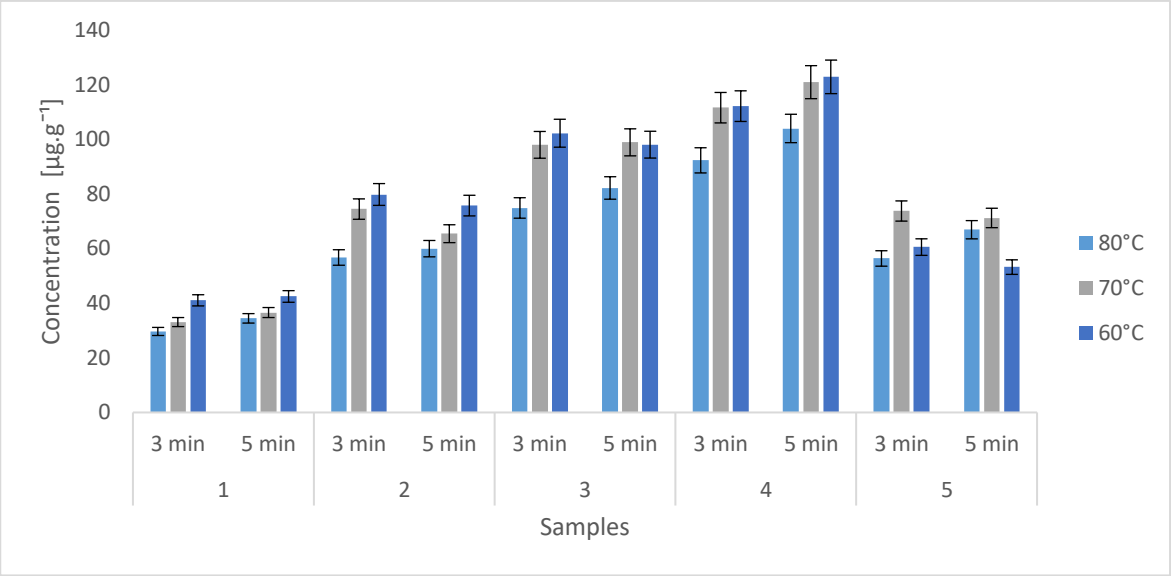

Figure 22 ECG – HPLC

The concentrations of ellagic acid (Figure 23) were measured in the range of 51.6–127 µg/g. The highest content of this phenolic acid was present in sample 4 at 80°C. There does not seem to be a pattern in the influence of the temperature used.

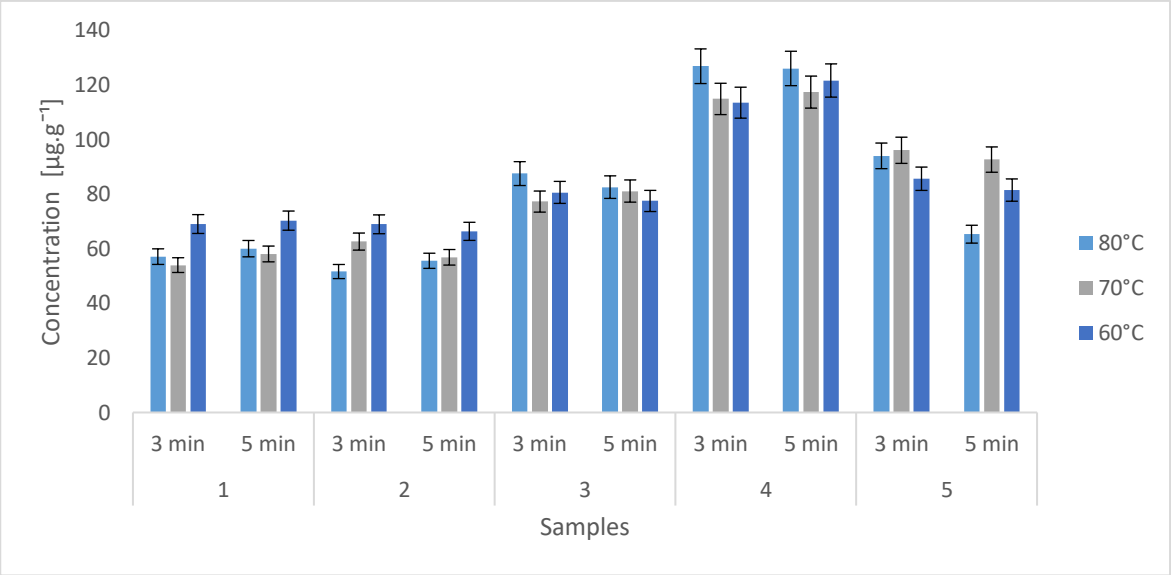

Figure 23 Ellagic acid – HPLC

Rutin, quercetin, and kaempferol as well as other flavonoids can also be found in green teas. In Figure 24, there is a display of rutin concentration. We can see that rutin is a minor compound appearing in green tea. The highest amount of rutin was present in sample 3, which was brewed for three minutes at 80°C (11.2 µg/g).

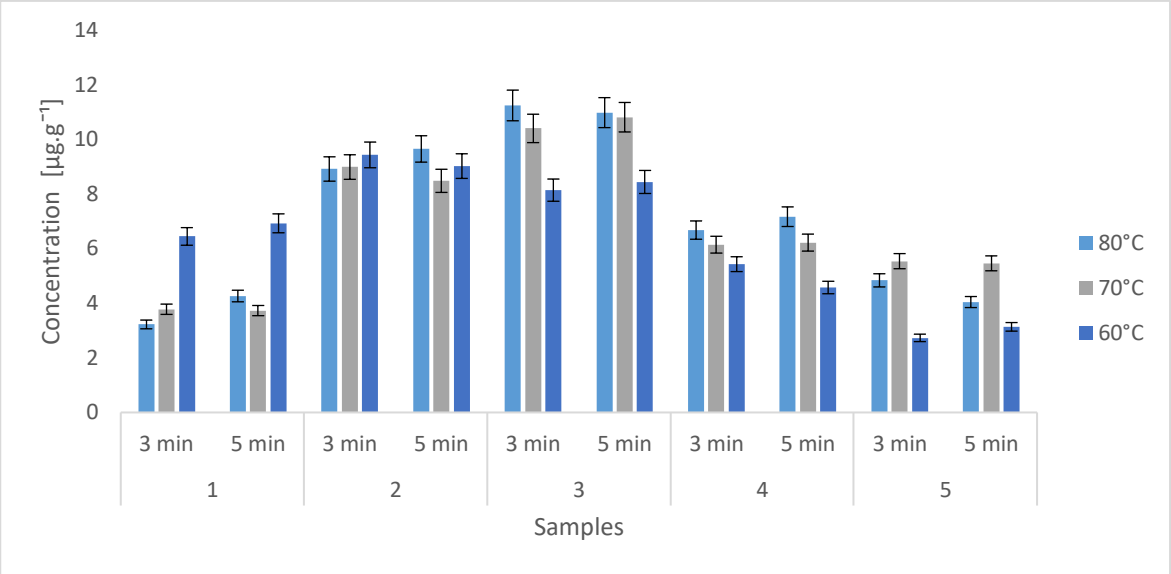

Figure 24 Rutin – HPLC

In Figure 25 there is a display of *trans*-2-Hydroxycinnamic acid. This polyphenolic compound is also a minor part of the green tea matrix. The lowest concentrations are in sample no. 5 at 60°C (0.16 µg/g), while the highest are in sample no. 2 at 70°C (2.19 µg/g).

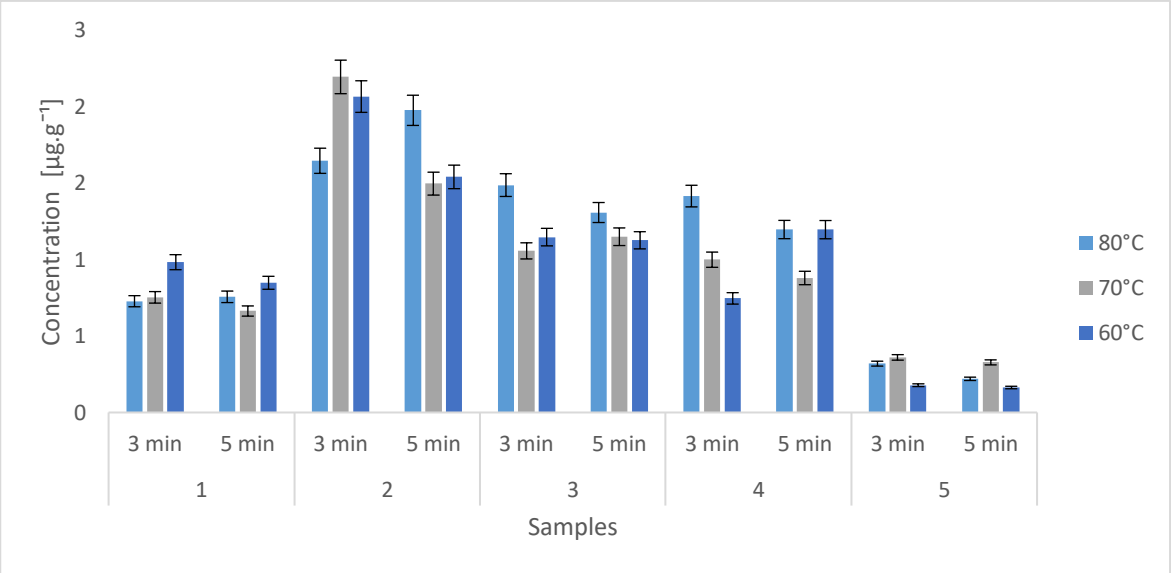

Figure 25 *trans*-2-Hydroxycinnamic acid – HPLC

The temperature influence on the release of protocatechuic ethyl ester differs for samples (Figure 26). The highest concentrations for samples 1 and 2 were measured at 80°C. On the other hand, the rest of the samples showed better extractions when lower temperatures were applied. Especially the extraction of sample no. 4 contained less than 1 µg/g at 80°C.

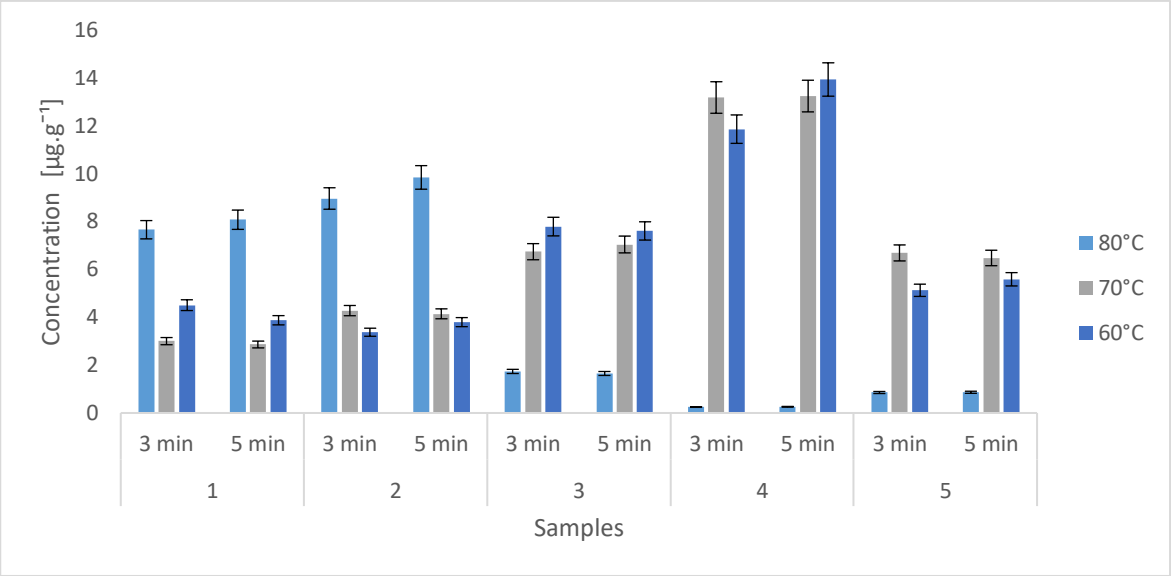

Figure 26 Protocatechuic ethyl ester – HPLC

The compound of *trans*-Cinnamic acid was not measured at 60°C at all (Figure 27). It is also a minor compound of green tea as the highest concentration measured was 2.00 µg/g, which was present in sample no. 4 brewed for 3 minutes at 80°C.

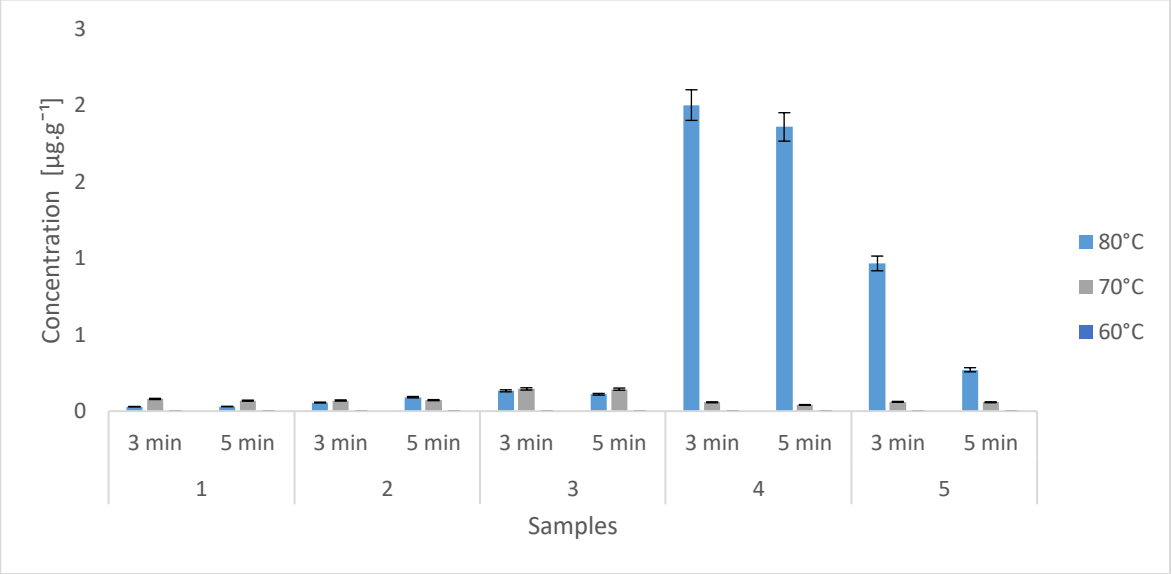

Figure 27 *trans*-Cinnamic acid – HPLC

For four out of five samples, the temperature of 70°C had a positive influence on the kaempferol release (Figure 28). While at 80°C extractions, very low concentrations were observed. At 60°C, there were no contents measured. The highest amount was present in sample no. 3 at 70°C brewed for 5 minutes (24.9 µg/g).

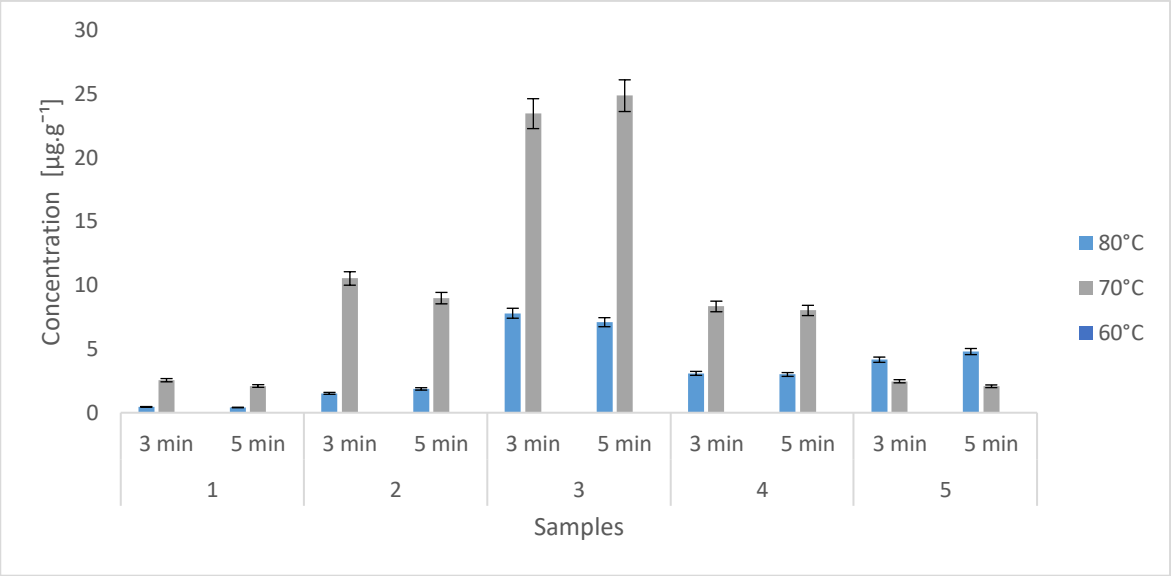

Figure 28 Kaempferol – HPLC

The temperature influence on the concentration of quercetin varies from sample to sample. For samples 2, 3, and 4, the temperature of 80°C was more convenient; for the rest of the samples (1 and 5), it was a temperature of 70°C. At the temperature of 60°C, no concentrations of quercetin were measured. The highest content was present in sample no. 4 at 80°C (13.2 µg/g).

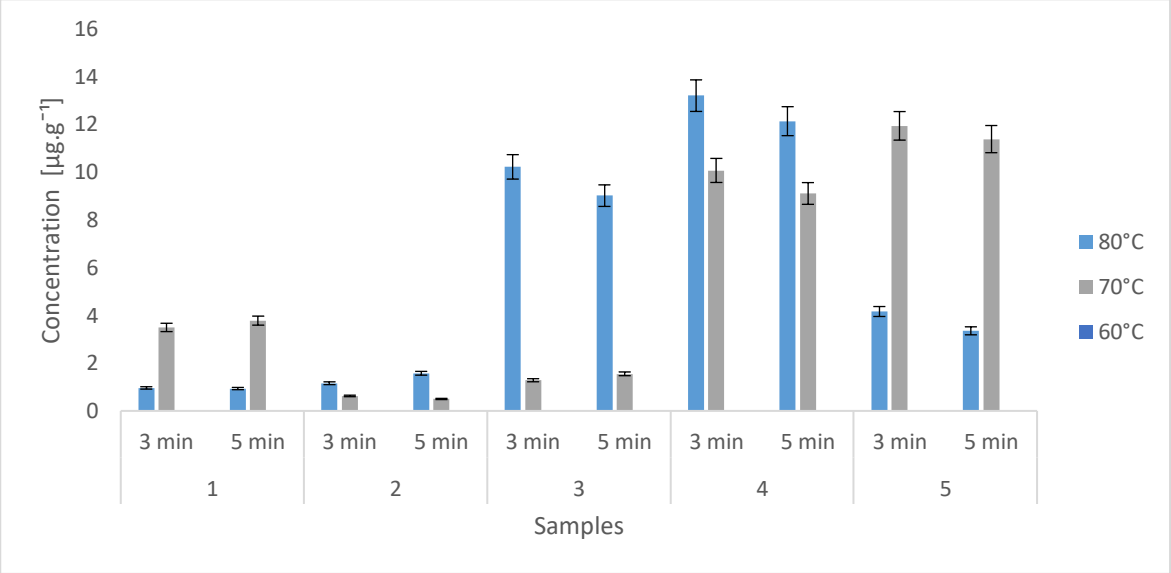

Figure 29 Quercetin – HPLC

In Table 8 there are total sums of individual phenolics. From the analysis results, it is obvious that there were always significant differences in the leaching at different temperatures within each sample. The only exception is for sample no. 2 between temperatures 80 and 70°C. The total sum of individual phenolics was often surprisingly lower when the sample was

leached for five minutes rather than three minutes. This might be due to the bad stability of catechins that significantly degrade in the sunlight and with time. This hypothesis needs additional support from future research.

Table 8 Total sum of individual phenolics

Sample	T (min)	µg/g ± SD		
		80°C	**70°C**	**60°C**
1	3	$3940\pm50^{a,A}$	$3330\pm40^{b,A}$	$3870\pm50^{c,A}$
	5	$3470\pm40^{a,B}$	$3250\pm40^{b,B}$	$3770\pm50^{c,B}$
2	3	$1940\pm20^{a,A}$	$3050\pm30^{b,A}$	$2970\pm30^{c,A}$
	5	$2380\pm30^{a,B}$	$2410\pm30^{a,B}$	$2540\pm20^{b,B}$
3	3	$7000\pm60^{a,A}$	$4830\pm60^{b,A}$	$5450\pm60^{c,A}$
	5	$5870\pm50^{a,B}$	$4970\pm60^{b,B}$	$5070\pm50^{c,B}$
4	3	$9980\pm60^{a,A}$	$7630\pm50^{b,A}$	$7160\pm60^{c,A}$
	5	$8880\pm50^{a,B}$	$7260\pm60^{b,B}$	$8370\pm50^{c,B}$
5	3	$8240\pm70^{a,A}$	$7660\pm70^{b,A}$	$8040\pm50^{c,A}$
	5	$6870\pm60^{a,B}$	$7300\pm40^{b,B}$	$6740\pm50^{c,B}$

All results are presented on a dry matter basis as means ± SD, n=4 (the mean of four measurements). Means within a line with at least one identical small superscript (in case of each matcha sample leaching at 80, 70, and 60°C) do not differ significantly ($p\geq0.05$), means within a column with at least one identical capitalized superscript (in case of each matcha sample leaching 3 and 5 minutes) do not differ significantly ($p\geq0.05$).

The effect of temperature and leaching time was different for each sample and compound. Therefore, it is hard to determine the most suitable brewing conditions. The major polyphenols determined in the samples are EGC, EGCG, epicatechin, and caffeic acid. Finding studies performed under the same extraction conditions is challenging.

Regarding the presence of flavanols, Sakakibara et al. [55] determined polyphenols in teas. Of all the catechins measured, the highest concentrations were measured for EGC (17900 µmol/100 g leaf) and EGCG (14900 µmol/100 g leaf) in sencha green tea, while the least present was catechin (278 µmol/100 g leaf). This corresponds with our results that the highest catechins were EGC (up to 5213 µg/g), EGCG (up to 3150 µg/g) and the lowest one was catechin (up to 18.5 µg/g).

On the other hand, in [56] and [57] it was determined that EGCG is the primary flavanol present while EGC was the secondary. However, among all flavanols, the concentration of catechin was always the lowest.

Another study showed [58] that the best extraction conditions for green tea catechins are a temperature of 70°C and a leaching time of 5 minutes.

According to Bindes et al. [59] the most suitable temperature for polyphenols extraction is 80°C. Balci et al. performed a study on Turkish green tea [60]. Their study concluded that

the content of phenolics and flavonoids increased with higher temperatures and longer extraction times. They also determined a decrease in catechins when the leaching temperature was increased from 75 to 85°C.

Regarding the sensory evaluation of green tea, Lee et al. determined [61] that the most appropriate conditions for green tea preparation are infusions brewed at 60°C for 3 minutes and at 80°C brewed for 1 minute.

5.3 Results of antioxidant activity measurements

The determination of radical scavenging activity was performed on the extracts prepared according to the procedure described in 4.4. The antioxidant activity was measured by two different methods: using synthetic DPPH and ABTS radicals. Both methods mentioned previously are fully described in chapters 4.9.1 and 4.9.2, respectively.

5.3.1 Results of antioxidant activity values measured using DPPH

The decrease in absorbance value was measured at 515 nm against methanol as a blank. The results of antioxidant activities are expressed as a µg of trolox equivalent per 1 gram (µg TE/g) of dry matter sample.

Table 9 shows the antioxidant activities obtained. The highest antioxidant activities were showing samples 4 and 5 at 80°C, sample 1 at 70°C, and sample 3 at 60°C. On the other hand, the lowest antioxidant activity was measured for sample 2 at all temperatures.

Table 9 Results of radical scavenging activity in matcha teas measured using DPPH

Sample	**T (min)**	**µg TE/g ± SD**		
		80°C	**70°C**	**60°C**
1	3	$307\pm6^{a,A}$	$423\pm12^{b,A}$	$327\pm10^{c,A}$
	5	$305\pm7^{a,A}$	$405\pm12^{b,B}$	$367\pm8^{c,B}$
2	3	$306\pm6^{a,A}$	$270\pm5^{b,A}$	$303\pm2^{a,A}$
	5	$292\pm8^{a,B}$	$257\pm9^{b,B}$	$256\pm8^{c,B}$
3	3	$366\pm8^{a,A}$	$339\pm3^{b,A}$	$380\pm7^{c,A}$
	5	$339\pm5^{a,B}$	$323\pm6^{b,B}$	$344\pm7^{a,B}$
4	3	$530\pm8^{a,A}$	$363\pm3^{b,A}$	$299\pm3^{c,A}$
	5	$521\pm10^{a,B}$	$351\pm2^{b,B}$	$370\pm3^{c,B}$
5	3	$498\pm10^{a,A}$	$325\pm2^{b,A}$	$331\pm3^{c,A}$
	5	$435\pm10^{a,B}$	$350\pm5^{b,B}$	$336\pm4^{c,A}$

All results are presented on a dry matter basis as means ± SD, n=4 (the mean of four measurements). Means within a line with at least one identical small superscript (in case of each matcha sample leaching at 80, 70, and 60°C) do not differ significantly ($p \geq 0.05$), means within a column with at least one identical capitalized superscript (in case of each matcha sample leaching 3 and 5 minutes) do not differ significantly ($p \geq 0.05$).

From the obtained data, it is obvious that the measured values differ depending on leaching temperatures and times. Samples 4 and 5 showed the highest antioxidant activities. These samples were also rich in polyphenols; both in TPC and individual phenolics by HPLC assays. From these observations, it is possible to conclude that the antioxidant activity of green tea is dependent on the content of polyphenols.

Calibration data for antioxidant activity assay to construct the calibration curve are displayed in Table 10.

Table 10 Calibration data for scavenging of DPPH radicals

Trolox concentration (mg/l)	**Inactivation (%)**
0	0
40	22
80	42
120	55
160	72
200	92

In Figure 30, there is a graph of the calibration curve obtained. The equation of linear regression was y = 0,4443x + 2,6548.

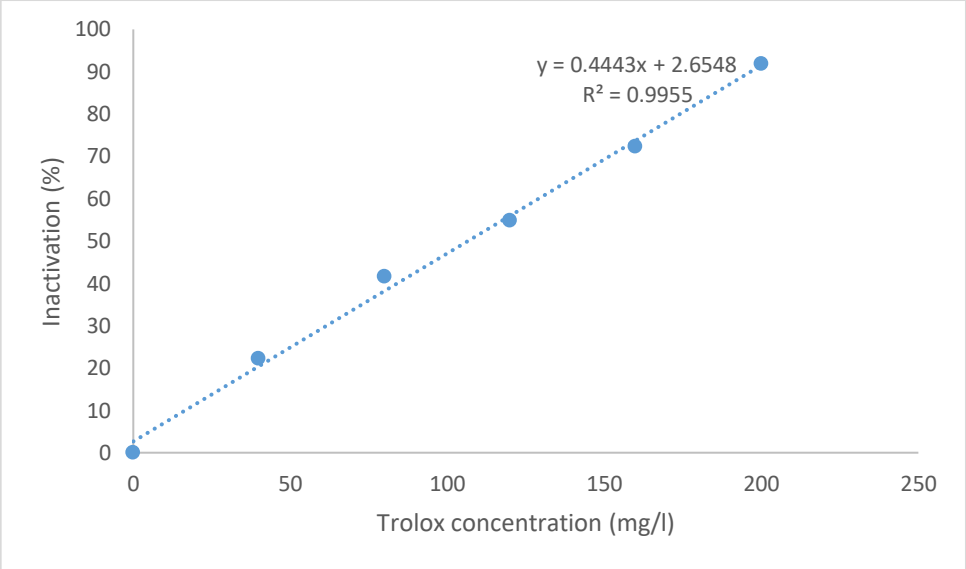

Figure 30 Calibration curve for scavenging of DPPH radicals

5.3.2 Results of antioxidant activity values measured using ABTS

The decrease in absorbance was assessed at 734 nm against acetate buffer as a blank. The method is fully described in chapter 4.9.2. The results of antioxidant activities are expressed as µg of trolox equivalent antioxidant capacity (µg TE/g) per 1 gram of dry matter sample.

In Table 11 there are the values obtained by measuring the antioxidant activities. The strongest antioxidants were samples no. 4 at 80 and 70°C, and sample no. 3 at 60°C. The lowest values were measured from sample 1 (80°C), sample 2 (70°C), and sample 4 (60°C).

Table 11 Results of radical scavenging activity in matcha teas measured using ABTS

Sample	**T (min)**	**µg TE/g ± SD**		
		80°C	**70°C**	**60°C**
1	3	$743\pm30^{a,A}$	$870\pm21^{b,A}$	$907\pm25^{c,A}$
	5	$846\pm25^{a,B}$	$906\pm20^{b,B}$	$996\pm27^{c,B}$
2	3	$778\pm10^{a,A}$	$593\pm10^{b,A}$	$829\pm25^{c,A}$
	5	$833\pm12^{a,B}$	$793\pm3^{b,B}$	$655\pm20^{c,B}$
3	3	$815\pm30^{a,A}$	$909\pm30^{b,A}$	$1100\pm20^{c,A}$
	5	$891\pm20^{a,B}$	$1060\pm30^{b,B}$	$924\pm10^{c,B}$
4	3	$1020\pm40^{a,A}$	$1140\pm40^{a,A}$	$1040\pm30^{a,A}$
	5	$937\pm30^{a,B}$	$1010\pm30^{b,B}$	$650\pm10^{c,B}$
5	3	$1000\pm40^{a,A}$	$849\pm22^{b,A}$	$1000\pm40^{a,A}$
	5	$916\pm30^{a,B}$	$1000\pm10^{b,B}$	$868\pm25^{c,B}$

All results are presented on a dry matter basis as means ± SD, n=4 (the mean of four measurements). Means within a line with at least one identical small superscript (in case of each matcha sample leaching at 80, 70, and 60°C) do not differ significantly ($p\geq0.05$), means within a column with at least one identical capitalized superscript (in case of each matcha sample leaching 3 and 5 minutes) do not differ significantly ($p\geq0.05$).

From the results, it is obvious that different temperatures are appropriate for each sample. For example, sample no. 1 showed the best antioxidant activity at 60°C, and sample no. 4 at 70°C.

When comparing both methods used for the determination of antioxidant activity, there are some similarities. Even though the values in µg TE/g differ, samples no. 3 (at 60°C) and no. 4 (at 80°C) showed the highest activity in both methods used. On the contrary, sample no. 2 at 70°C was the poorest antioxidant.

In Table 12, there is a display of percentage inactivation depending on trolox concentration in mg/ml.

Table 12 Calibration data for scavenging of ABTS radicals

Trolox concentration (mg/ml)	**Inactivation (%)**
0	0
0.01	1.5
0.05	9.5
0.10	15.6
0.15	28.8
0.20	39.9
0.25	52.3
0.30	62.5
0.40	79.3

In Figure 31 we can see the calibration curve of the inactivation which depends on the trolox concentration in mg/ml. The equation of linear regression was y = 205,25x – 1,1453.

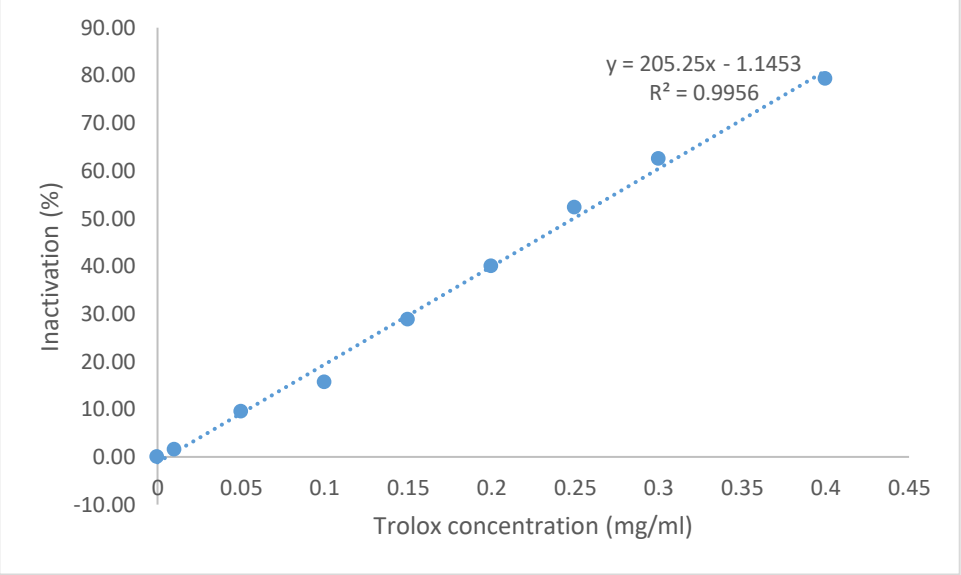

Figure 31 Calibration curve for scavenging of ABTS radicals

Experimental part B

5.4 Results of *in vitro* digestibility assessment

In Table 13, there is a display of measured digestibility values, dry matter, and ash contents. The samples were prepared according to chapter 4.5.

Table 13 Results of dry matter, ash contents, and digestibility values

Sample	DMD (%) ±SD	OMD (%) ±SD	Dry matter (%) ±SD	Ash (%) ±SD
1	66.7 ± 2.0^{a}	72.5 ± 2.0^{a}	$96.1\pm1.0^{a,c}$	5.87 ± 0.05^{a}
2	70.0 ± 2.2^{b}	74.9 ± 2.0^{b}	$97.1\pm0.8^{b,c}$	5.85 ± 0.05^{a}
3	75.6 ± 1.5^{c}	79.3 ± 1.5^{c}	97.3 ± 0.8^{b}	5.14 ± 0.02^{b}
4	66.1 ± 2.0^{a}	71.0 ± 1.7^{d}	$97.1\pm0.9^{b,c}$	5.03 ± 0.08^{b}
5	64.5 ± 1.7^{d}	69.6 ± 2.0^{e}	96.7 ± 1.0^{c}	5.23 ± 0.04^{c}

All results are presented as means ± SD, n=3 (the mean of three measurements). Means within a column with at least one identical small superscript do not differ significantly ($p\geq0.05$).
DMD – dry matter digestibility, OMD – organic matter digestibility

As can be seen in Table 13, the dry matter contents of matcha samples varied from 96.1 to 97.3%. According to Czech regulation No. 330 [62], the dry matter value for green tea should not be less than 90%. ISO 11287 (2011) [63] stated 4% as a minimum and 8% as the maximal ash level for green teas. Our samples comply with this regulation. Dry matter digestibility (DMD) and organic matter digestibility (OMD) values ranged from 64.5 to 75.6% and from 69.6 to 79.3%, respectively. Research data evaluating the digestibility of matcha teas is limited. Nevertheless, the results can be compared at least with the study by Koláčková et al. (2020) where the digestibility values of ground tea leaves reached 69.7% [36].

5.5 The effect of *in vitro* digestion on TPC values

The samples were prepared as described in chapter 4.6. The results of TPC values expressed in mg GAE/g are displayed in Table 14.

Table 14 Total polyphenolic contents determined in native and undigested parts of matcha

Samples	Native matcha	Undigested matcha	Remaining parts (RP)
	TPC (mg GAE/g) ±SD		(%)
1	$13.1\pm0.4^{a,A}$	$2.63\pm0.10^{a,B}$	7
2	$11.5\pm1.0^{b,A}$	$2.96\pm0.20^{b,B}$	8
3	$14.1\pm0.4^{c,A}$	$5.78\pm0.20^{c,B}$	10
4	$16.5\pm0.5^{d,A}$	$2.88\pm0.20^{b,B}$	6
5	$15.0\pm0.5^{e,A}$	$3.52\pm0.10^{d,B}$	8

All results are presented on a dry matter basis as means ± SD, n=4 (the mean of four measurements). Means within a column with at least one identical small superscript (in case of each matcha sample) do not differ significantly ($p\geq0.05$), means within a line with at least one identical capitalized superscript (in case of native and undigested matcha samples) do not differ significantly ($p\geq0.05$).

Matcha is consumed directly in the form of powder as a useful source of phenolics. The release of individual substances from food matrices determines their absorption behavior in the gastrointestinal tract. Therefore, the consumption of foods high in polyphenols does not always mean that more phenolic substances are absorbed after digestion. Hence, quantifying the proportion of digestible or indigestible antioxidants available for digestion is crucial. The quantity of digested substances released during digestion and their availability for absorption by the intestinal brush border of the cells is expressed as their bioaccessibility. It has a significant impact on the bioavailability and biological activity of ingested substances. It refers to how many antioxidants have crossed through the cell membrane and are now available for usage within the cell. It also monitors the amounts of antioxidants released into the targeted cell to show their biological activity and assesses the stability of antioxidants during the process [64].

The bioaccessibility index is the prerequisite for bioactive substances to express their impacts on human health. Since animal or human trials are labor-intensive and unethical, *in vitro* digestion models have been widely established and used for the investigation of changes in the phytochemical profile during digestion processes and the prediction of their bioaccessibility [65][66].

Even though matcha tea may be consumed directly (and entirely) in a powdered form of all leaf parts, there is still little information on its digestibility values and ability to release biologically active substances during digestion. Therefore, a two-step *in vitro* digestion process with pepsin and pancreatin under 37°C was applied.

In our laboratory experiment, the remaining parts (RP) of all analytes measured in the undigested parts were calculated using equation 9:

$$RP\ (\%) = \frac{Concentration\ of\ analytes\ in\ undigested\ part\ of\ matcha\ x\ (100 - digestibility\ value)}{Concentration\ of\ analytes\ in\ native\ part\ of\ matcha} \quad (9)$$

Except for sample no. 3, it is clear that most polyphenols were digested as for the remaining parts, there were less than 10% of polyphenols present (Table 14). Concretely, the results of the remaining parts show that there are still polyphenolic compounds present in the undigested part (6–10%). Therefore, it might be possible that these polyphenols reach the large intestine bound to fiber and are released during fiber digestion by bacteria in the colon. This might lead to a further positive impact on the protection of the intestinal tissue,

and other antioxidant and anti-inflammatory effects. Anyway, this is only a hypothesis. These suggestions need to be confirmed by further research.

These results were also supported by measuring the remaining parts for individual phenolics using HPLC. This is described in chapter 5.6.

5.6 The effect of *in vitro* digestion on releasing of individual phenolics

The highest concentration values in the native form of matcha samples were measured for epigallocatechin, caffeic acid, EGCG, and epicatechin. The results from HPLC measurements confirmed that polyphenolic substances were still present in the undigested portion of tea leaves.

As can be seen from our results (Table 15), the remaining parts were the highest for rutin, gallic, ellagic, and *trans*-2-Hydroxycinnamic acids. Across all the samples, the lowest remaining parts (RP) were measured for epigallocatechin, EGCG, epicatechin, and epicatechin-3-gallate. The lowest RP value was calculated concerning phenolic acids in the case of neochlorogenic acid. This suggests that the group of catechins could be released very well during the *in vitro* digestion process unless they degrade. This assumption has been verified only by an *ex vivo* study from Dai et al. (2020), who claimed there is low stability of EGCG in the small intestine and suggested it is delivered through nanoparticles [67]. Due to the lack of studies related to the bioaccessibility of individual phenolic compounds analyzed in matcha tea (either in the native or undigested part), it is very difficult to compare the results obtained in this study with other findings. For instance, focusing on cereals, Seczyk et al. (2021) found the highest and lowest bioaccessibility values of *p*-coumaric (up to 93%) and gallic acid (23.7%), respectively [68]. Drawbridge et al. (2022) suggested that vanillic acid might be less susceptible to degradation during digestion [69].

Table 15 Remaining parts ranges for individual phenolics

PPA	**Remaining parts range (%)**
Gallic acid	6 – 28
Protocatechuic acid	1 – 2
Neochlorogenic acid	0 – 2
4-hydroxybenzoic acid	1 – 5
Epigallocatechin	<1
Catechin	2 – 9
Caffeic acid	1 – 4
Epicatechin	0 – 1
EGCG	0 – 1
*trans-p-C*oumaric acid	N.D.
Ferrulic acid	2 – 8
Sinapic acid	0 – 5
ECG	0 – 1
Ellagic acid	8 – 19
Rutin	9 – 19
trans-2-Hydroxycinnamic acid	0 – 4
Protocatechuic acid	1 – 13
trans-Cinnamic acid	<1
Kaempferol	0 – 18
Quercetin	2 – 5

EGCG – epigallocatechin-3-gallate, ECG – epicatechin-3-gallate

5.7 The effect of *in vitro* digestion on antioxidant activity values

The effect of *in vitro* digestibility on the antioxidant activity values was provided using two independent assays. Table 16 summarizes the results showing that native matcha tea leaves have significant antioxidant potential. Moreover, the highest values were observed for both the native and undigested parts of the tea leaves determined by the quenching of ABTS and DPPH radicals. In the case of antioxidant activity, the range of remaining parts was 42–68% for DPPH and 11–61% when the ABTS method was applied. Comparing both methods (DPPH and ABTS) there are similar results for samples 1 and 5. The values vary for samples 2, 3, and 4. Sample no. 3 showed 68% of the remaining parts when using the DPPH method; while for the ABTS method, the value was only 11%. Antioxidant properties are influenced by many factors, such as leaching time and temperature, and water yield during the extraction of tea compounds; and they cannot be described precisely by one method. Due to the lack

of data providing information about the effects of *in vitro* gastrointestinal digestion on phenolics, xanthine alkaloids, and antioxidant activity of matcha tea samples, it is difficult to compare the obtained values with already published results.

Table 16 Antioxidant activity values determined in native and undigested part of matcha

Method	**Samples**	**Native matcha**	**Undigested matcha**	**Remaining parts**
		µg TE/g ± SD		**(%)**
DPPH	1	23.4±0.1[a]	36.3±1.5[a]	52
	2	22.2±0.3[b]	31.1±1.5[b]	42
	3	27.4±0.6[c]	75.9±1.4[c]	68
	4	25.5±1.1[d]	41.7±1.6[d]	55
	5	27.1±1.0[e]	35.5±1.9[a]	46
ABTS	1	36.3±0.6[a]	66.0±1.5[a]	61
	2	31.1±1.0[b]	30.4±1.4[b]	29
	3	75.9±0.7[c]	33.2±1.0[c]	11
	4	41.7±0.2[d]	37.9±1.0[d]	31
	5	35.5±0.1[e]	42.9±0.8[e]	43

All results are presented in a dry matter basis as means ± SD, n=4 (the mean of four measurements). Means within a column with at least one identical small superscript do not differ significantly ($p \geq 0.05$).

The results indicate that even the undigested part of matcha tea that reaches all the way to the colon may still show an antioxidant activity as there are still present phenolic compounds in undigested leaves. Besides these particles, antioxidant activity is evinced by other compounds such as chlorophyll, xanthine derivates (alkaloids), xanthophyll and carotenoid pigments, and some of the residues of oligosaccharide chains from the fiber [70][71][72].

Another question is if these chains can act as a prebiotic, etc. which should be the subject of further research. Comparing the results of both methods used is difficult as each of the methods has its own mechanism and reaction conditions. Therefore, it is not possible to expect the same results.

CONCLUSION

This diploma thesis focuses on the determination of polyphenolic substances in matcha teas, antioxidant activity, and their *in vitro* digestibility.

In the first part, there is a literature review where different ways of cultivation, manufacturing, and preparation of green teas are described. There are also mentioned phenolic compounds that appear in green teas and their beneficial effects on the human organism.

The experimental part is divided into two parts. In part A, five different samples were leached under three different temperatures (60, 70, and 80°C) for 3 and 5 minutes. The results show that the leaching time did not always significantly impact the polyphenolic content in the final extract nor the antioxidant activity. This might be caused by the instability of some of the polyphenols especially catechins that are not stable in the sunlight and degrade with time. Anyway, this should be supported by further research. The influence of temperature was obvious for certain polyphenols measured. However, no temperature could be used as universal for all the polyphenols. Major polyphenols determined in the samples were EGC, EGCG, epicatechin, and caffeic acid.

In experimental part B, native and undigested parts of matcha tea samples were compared after *in vitro* digestion process. The results indicate that most polyphenols from the catechin family could be digested by the human body. The range of total polyphenols present in the remaining part was 6–10%. On the other hand, the remaining parts of the antioxidant activity were quite high, which suggests that the antioxidant potential of matcha tea is not fully used during the digestion process and might still be used in the large intestine.

When comparing the samples investigated, samples 4 and 5 were showing the highest contents of polyphenols and significant antioxidant activity values. Therefore, it can be determined that the quality of these two matcha teas was very high. Sample no. 2 was determined, most of the time, as the poorest for polyphenolic content and the antioxidant activity. This might be due to its bigger particles; it was not ground properly. This probably leads to the conclusion that the quality of this tea is not that high. Samples 3 and 1 showed average values.

BIBLIOGRAPHY

[1] MAIDEN, Zoe. The History & Processing Methods. *Hackberrytea.com* [online]. 2021 [cit. 2022-04-17]. Available from: https://www.hackberrytea.com/blogs/tea-education/the-history-of-green-tea

[2] Types of tea. *Teasource.com* [online]. Roseville [cit. 2022-04-17]. Available from: https://www.teasource.com/pages/types-of-tea

[3] JOHNSON, Jon. Matcha vs. green tea: Which is healthier?. *Medicalnewstoaday.com* [online]. 2021 [cit. 2022-04-17]. Available from: https://www.medicalnewstoday.com/articles/matcha-vs-green-tea#green-tea-benefits

[4] HO, Chi-Tang and Fereidoon SHAHIDI. *Tea and tea products.* United States of America: Taylor, 2009. ISBN 978-0-8493-8082-2.

[5] Green Tea tree leaves, Camellia sinensis. In: *Alamy.com* [online]. MaximImages.com, 2019 [cit. 2022-04-17]. Available from: https://www.alamy.com/green-tea-tree-leaves-camellia-sinensis-closeup-leaves-of-a-tea-plant-are-used-for-producing-multiple-varieties-of-tea-image256489156.html

[6] AHMED, Selena a John Richard STEPP. *Green Tea*. Tea in Health and Disease Prevention [online]. Elsevier, 2013, 2013, 19-31 [cit. 2022-04-17]. ISBN 9780123849373. Available from: doi:10.1016/B978-0-12-384937-3.00002-1

[7] SINGH, Vishal a Deepak Kumar VERMA. Processing Technology and Health Benefits of Green Tea. *Popular Kheti*[online]. 2014, 2(1), 23-30 [cit. 2022-04-17]. ISSN 2321-0001. Available from: https://www.researchgate.net/publication/261547299_Processing_Technology_and_Health_Benefits_of_Green_Tea

[8] HAN, Wen-Yan, Ji-Gang HUANG, Xin LI, Zhi-Xin LI, Golam Jalal AHAMMED, Peng YAN a John Richard STEPP. Altitudinal effects on the quality of green tea in east China: a climate change perspective. *European Food Research and Technology* [online]. 2017, 243(2), 323-330 [cit. 2022-04-17]. ISSN 1438-2377. Available from: doi:10.1007/s00217-016-2746-5

[9] KIM, Erin. Japanese Matcha Vs Chinese Matcha. *Chalait.com* [online]. 2019 [cit. 2022-04-17]. Available from: https://www.chalait.com/blogs/matcha-guide/japanese-matcha-vs-chinese-matcha

[10] NISHIDA, Kei. FROM PLANT TO YOUR TEA CUP HOW IS MATCHA TEA MADE?. *Japanesegreentea.in* [online]. [cit. 2022-04-17]. Available from: https://www.japanesegreentea.in/blogs/japanese-green-tea-lovers-in-india/from-plant-to-your-tea-cup-how-is-matcha-tea-made

[11] How Matcha is Produced. *Breakawaymatcha.com* [online]. [cit. 2022-04-17]. Available from: https://breakawaymatcha.com/blogs/masterclass-in-matcha/how-matcha-is-produced

[12] Matcha: How Powdered Green Tea is Produced. *Livejapan.com* [online]. 2017 [cit. 2022-04-17]. Available from: https://livejapan.com/en/in-hokkaido/in-pref-hokkaido/in-sapporo_chitose/article-a0001058/

[13] Matcha production process – how matcha is made. *Matchawellness.com.au* [online]. [cit. 2022-04-17]. Available from: https://www.matchawellness.com.au/blogs/matcha-education/matcha-production-process-how-matcha-is-made

[14] How matcha is made in Japan. *Naokimatcha.com* [online]. 2020 [cit. 2022-04-17]. Available from: https://naokimatcha.com/articles/how-matcha-is-made-in-japan/

[15] How to prepare matcha: A ritual for the senses. *Ikedamatcha.com* [online]. [cit. 2022-04-17]. Available from: https://ikedamatcha.com/blogs/tea-news/how-to-prepare-matcha

[16] Cold brew matcha. *Nomnompaleo.com* [online]. [cit. 2022-04-17]. Available from: https://nomnompaleo.com/cold-brew-matcha

[17] What Are The Different Types of Japanese Green Tea?. *Afternoonteareads.com* [online]. 2022 [cit. 2022-04-17]. Available from: https://afternoonteareads.com/types-of-japanese-green-tea/

[18] Types of Japanese Green Tea. *Bento.com* [online]. [cit. 2022-04-17]. Available from: https://bento.com/fexp-greentea.html

[19] Best Types of Japanese Teas. *Simplelooseleaf.com* [online]. 2020 [cit. 2022-04-17]. Available from: https://simplelooseleaf.com/blog/tea-culture/best-types-of-japanese-teas/

[20] CHACKO, Sabu M, Priya T THAMBI, Ramadasan KUTTAN a Ikuo NISHIGAKI. Beneficial effects of green tea: A literature review. *Chinese Medicine* [online]. 2010, 5(1) [cit. 2022-04-17]. ISSN 1749-8546. Available from: doi:10.1186/1749-8546-5-13

[21] Beneficial Properties of Green Tea Catechins. *International Journal of Molecular Sciences*[online]. 2020, 21(5) [cit. 2022-04-17]. ISSN 1422-0067. Available from: doi:10.3390/ijms21051744

[22] SHAHIDI, Fereidoon a Priyatharini AMBIGAIPALAN. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods* [online]. 2015, 18, 820-897 [cit. 2022-04-17]. ISSN 17564646. Available from: doi:10.1016/j.jff.2015.06.018

[23] JAKUBCZYK, Karolina, Joanna KOCHMAN, Aleksandra KWIATKOWSKA, Justyna KAŁDUŃSKA, Karolina DEC, Dorota KAWCZUGA a Katarzyna JANDA. Antioxidant Properties and Nutritional Composition of Matcha Green Tea. *Foods*[online]. 2020, 9(4) [cit. 2022-04-17]. ISSN 2304-8158. Available from: doi:10.3390/foods9040483

[24] BAE, Joonseo, Nayoung KIM, Yunyoung SHIN, Soo-Yeon KIM a You-Jeong YOU-JEONG KIM. Activity of catechins and their applications. *Biomedical Dermatology* [online]. 2020, 4(8) [cit. 2022-04-17]. ISSN 2398-8460. Available from: doi:https://doi.org/10.1186/s41702-020-0057-8

[25] *Life Sciences* [online]. 78. 2006 [cit. 2022-04-17]. ISSN 00243205. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0024320505012415

[26] BERNATONIENE, Jurga a Dalia KOPUSTINSKIENE. The Role of Catechins in Cellular Responses to Oxidative Stress: The main chemical structures of catechins. *Molecules* [online]. 2018, 23(4) [cit. 2022-04-18]. ISSN 1420-3049. Available from: doi:10.3390/molecules23040965

[27] HIGDON, Jane V. a Balz FREI. Tea Catechins and Polyphenols: Health Effects, Metabolism, and Antioxidant Functions. *Critical Reviews in Food Science and*

Nutrition [online]. 2003, 43(1), 89-143 [cit. 2022-04-18]. ISSN 1040-8398. Available from: doi:10.1080/10408690390826464

[28] WEISS, David J. a Christopher R. ANDERTON. Determination of catechins in matcha green tea by micellar electrokinetic chromatography. *Journal of Chromatography A* [online]. 2003, 1011(1-2), 173-180 [cit. 2022-04-18]. ISSN 00219673. Available from: doi:10.1016/S0021-9673(03)01133-6

[29] WANG, Huafu a Keith HELLIWELL. Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography. *Food Research International* [online]. 2001, 34(2-3), 223-227 [cit. 2022-04-18]. ISSN 09639969. Available from: doi:10.1016/S0963-9969(00)00156-3

[30] GARGI, Sen, Sarkar NILANJAN, Nath MOUTUSI a Maity SUBHASIS. Bioactive components of tea. *Archive of Food and Nutritional Science* [online]. 2020, 4(1), 001-009 [cit. 2022-04-18]. ISSN 25750194. Available from: doi:10.29328/journal.afns.1001020

[31] SCHRÖDER, Lennard, Philip MARAHRENS, Julian KOCH, et al. Effects of green tea, matcha tea and their components epigallocatechin gallate and quercetin on MCF-7 and MDA-MB-231 breast carcinoma cells. *Oncology Reports* [online]. 2018 [cit. 2022-04-18]. ISSN 1021-335X. Available from: doi:10.3892/or.2018.6789

[32] KUMAR, Naresh a Nidhi GOEL. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports* [online]. 2019, 24 [cit. 2022-04-18]. ISSN 2215017X. Available from: doi:10.1016/j.btre.2019.e00370

[33] XIA, Zhenzhen, Yongnian NI a Serge KOKOT. Simultaneous determination of caffeine, theophylline and theobromine in food samples by a kinetic spectrophotometric method. *Food Chemistry* [online]. 2013, **141**(4), 4087-4093 [cit. 2022-04-18]. ISSN 03088146. Available from: doi:10.1016/j.foodchem.2013.06.121

[34] KOCHMAN, Joanna, Karolina JAKUBCZYK, Justyna ANTONIEWICZ, Honorata MRUK a Katarzyna JANDA. Health Benefits and Chemical Composition of Matcha Green Tea: A Review. *Molecules* [online]. 2021, **26**(1) [cit. 2022-04-18]. ISSN 1420-3049. Available from: doi:10.3390/molecules26010085

[35] KOLÁČKOVÁ, Tereza, Daniela SUMCZYNSKI, Ludmila ZÁLEŠÁKOVÁ, Lenka ŠENKÁROVÁ, Jana ORSAVOVÁ a Nikoleta LANCZOVÁ. Free and bound amino

acids, minerals and trace elements in matcha (Camellia sinensis L.): A nutritional evaluation. *Journal of Food Composition and Analysis* [online]. 2020, **92** [cit. 2022-04-18]. ISSN 08891575. Available from: doi:10.1016/j.jfca.2020.103581

[36] KOLÁČKOVÁ, Tereza, Kateřina KOLOFIKOVÁ, Irena SYTAŘOVÁ, Lukáš SNOPEK, Daniela SUMCZYNSKI a Jana ORSAVOVÁ. Matcha Tea: Analysis of Nutritional Composition, Phenolics and Antioxidant Activity. *Plant Foods for Human Nutrition* [online]. 2020, **75**(1), 48-53 [cit. 2022-04-18]. ISSN 0921-9668. Available from: doi:10.1007/s11130-019-00777-z

[37] *Journal of Tea Science Research* [online]. 2015 [cit. 2022-04-18]. ISSN 1927-6494. Available from: http://biopublisher.ca/html-2152-44-jtsr

[38] KOLÁČKOVÁ, Tereza, Daniela SUMCZYNSKI, Vratislav BEDNAŘÍK, Štěpán VINTER, Jana ORSAVOVÁ a Kateřina KOLOFIKOVÁ. Mineral and trace element composition after digestion and leaching into matcha ice tea infusions (Camellia sinensis L.). *Journal of Food Composition and Analysis* [online]. 2021, **97** [cit. 2022-04-19]. ISSN 08891575. Available from: doi:10.1016/j.jfca.2020.103792

[39] YU, Xiao-Lan a Yong HE. Tea saponins: effective natural surfactants beneficial for soil remediation, from preparation to application. *RSC Advances* [online]. 2018, **8**(43), 24312-24321 [cit. 2022-04-19]. ISSN 2046-2069. Available from: doi:10.1039/C8RA02859A

[40] SHARANGI BARAN, A.B. Bioactive Compounds and Antioxidant Properties of Tea: Status, Global Research and Potentialities. *Journal of Tea Science Research* [online]. 2015 [cit. 2022-04-19]. ISSN 1927-6494. Available from: doi:10.5376/2015.05.0011

[41] ZAVERI, Nurulain T. Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications. *Life Sciences* [online]. 2006, **78**(18), 2073-2080 [cit. 2022-04-19]. ISSN 00243205. Available from: doi:10.1016/j.lfs.2005.12.006

[42] KHAN, Naghma a Hasan MUKHTAR. Tea polyphenols for health promotion. *Life Sciences* [online]. 2007, **81**(7), 519-533 [cit. 2022-04-19]. ISSN 00243205. Available from: doi:10.1016/j.lfs.2007.06.011

[43] AFZAL, M., A. M. SAFER a M. MENON. Green tea polyphenols and their potential role in health and disease. *Inflammopharmacology* [online]. 2015, **23**(4), 151-161

[cit. 2022-04-19]. ISSN 0925-4692. Available from: doi:10.1007/s10787-015-0236-1

[44] International Organization for Standardization (1980). Tea- Determination of loss of mass at 103 ^{X}C (Moisture), ISO 1573, Geneva, Switzerland, 1-4.

[45] International Organization for Standardization (1980). Tea- Determination of total ash, ISO 1575, Geneva, Switzerland, 1-3.

[46] ALTANGEREL, Bayanmunkh, Zultsetseg SENGEE, Daniela KRAMAROVA, Otakar ROP a Ignac HOZA. *The determination of water-soluble vitamins and in vitro digestibility of selected Czech cheeses* [online]. 2011, **46**(6), 1225-1230 [cit. 2022-04-19]. ISSN 09505423. Available from: doi:10.1111/j.1365-2621.2011.02601.x

[47] SINGLETON, Vernon L., Rudolf ORTHOFER, Rosa M. LAMUELA-RAVENTÓS, Otakar ROP a Ignac HOZA. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Oxidants and Antioxidants Part A* [online]. Elsevier, 1999, 1999, **46**(6), 152-178 [cit. 2022-04-19]. Methods in Enzymology. ISBN 9780121822002. ISSN 09505423. Available from: doi:10.1016/S0076-6879(99)99017-1

[48] DENG, Gui-Fang, Xiang-Rong XU, Ya-Jun GUO, et al. Determination of antioxidant property and their lipophilic and hydrophilic phenolic contents in cereal grains. *Journal of Functional Foods*[online]. Elsevier, 2012, 1999, **4**(4), 906-914 [cit. 2022-04-19]. Methods in Enzymology. ISBN 9780121822002. ISSN 17564646. Available from: doi:10.1016/j.jff.2012.06.008

[49] SIEMIŃSKA-KUCZER, Anna, Monika SZYMAŃSKA-CHARGOT a Artur ZDUNEK. Recent advances in interactions between polyphenols and plant cell wall polysaccharides as studied using an adsorption technique. *Food Chemistry* [online]. 2022, **373** [cit. 2022-05-04]. ISSN 03088146. Available from: doi:10.1016/j.foodchem.2021.131487

[50] DAS, Protiva Rani a Jong-Bang EUN. *Influence of different extraction techniques on bio-accessibility of green tea extract* [online]. Gwangju, S. Korea, 2017 [cit. 2022-05-04]. Available from: https://www.researchgate.net/publication/323257654_Influence_of_different_extra

ction_techniques_on_bio-accessibility_of_green_tea_extract. Conference Paper. Graduate School of Chonnam National University.

[51] ZAITER, Ali, Loïc BECKER, Marie-Céleste KARAM a Amadou DICKO. Effect of particle size on antioxidant activity and catechin content of green tea powders. *Journal of Food Science and Technology* [online]. 2016, **53**(4), 2025-2032 [cit. 2022-04-22]. ISSN 0022-1155. Available from: doi:10.1007/s13197-016-2201-4

[52] KOWALSKA, Jolanta, Agata MARZEC, Ewa DOMIAN, Sabina GALUS, Agnieszka CIURZYŃSKA, Rita BRZEZIŃSKA a Hanna KOWALSKA. Influence of Tea Brewing Parameters on the Antioxidant Potential of Infusions and Extracts Depending on the Degree of Processing of the Leaves of Camellia sinensis. *Molecules* [online]. 2021, **26**(16) [cit. 2022-04-22]. ISSN 1420-3049. Available from: doi:10.3390/molecules26164773

[53] NISHITANI, Eisei a Yuko M SAGESAKA. Simultaneous determination of catechins, caffeine and other phenolic compounds in tea using new HPLC method. *Journal of Food Composition and Analysis*[online]. 2004, **17**(5), 675-685 [cit. 2022-04-27]. ISSN 08891575. Available from: doi:10.1016/j.jfca.2003.09.009

[54] LIU, Linlin, Yingying LI, Guangbiao SHE, Xianchen ZHANG, Brian JORDAN, Qi CHEN, Jian ZHAO a Xiaochun WAN. Metabolite profiling and transcriptomic analyses reveal an essential role of UVR8-mediated signal transduction pathway in regulating flavonoid biosynthesis in tea plants (Camellia sinensis) in response to shading. *BMC Plant Biology* [online]. 2018, **18**(1) [cit. 2022-04-28]. ISSN 1471-2229. Available from: doi:10.1186/s12870-018-1440-0

[55] SAKAKIBARA, Hiroyuki, Yoshinori HONDA, Satoshi NAKAGAWA, Hitoshi ASHIDA a Kazuki KANAZAWA. Simultaneous Determination of All Polyphenols in Vegetables, Fruits, and Teas. *Journal of Agricultural and Food Chemistry* [online]. 2003, **51**(3), 571-581 [cit. 2022-04-28]. ISSN 0021-8561. Available from: doi:10.1021/jf020926l

[56] Sensitive Determination of Catechins in Tea by HPLC. *Thermofisher.com* [online]. Dionex Corporation, 2016 [cit. 2022-04-28]. Available from: https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/AN-275-LC-Catechins-Tea-LPN2799-EN.pdf

[57] WANG, Huafu, Gordon J PROVAN a Keith HELLIWELL. HPLC determination of catechins in tea leaves and tea extracts using relative response factors. *Food Chemistry* [online]. 2003, **81**(2), 307-312 [cit. 2022-04-28]. ISSN 03088146. Available from: doi:10.1016/S0308-8146(02)00510-1

[58] LEE, Young-Sang, Seul-A JUNG, Jung-Hwan KIM, et al. A Study on Change in Chemical Composition of Green Tea, White Tea, Yellow Tea,Oolong Tea and Black Tea with Different Extraction Conditions. *The Korean Journal of Food And Nutrition* [online]. 2015, **28**(5), 766-773 [cit. 2022-04-28]. ISSN 1225-4339. Available from: doi:10.9799/ksfan.2015.28.5.766

[59] BINDES, Marlon Menezes Maciel, Vicelma Luiz CARDOSO, Miria Hespanhol Miranda REIS a Daria Camilla BOFFITO. Maximisation of the polyphenols extraction yield from green tea leaves and sequential clarification. *Journal of Food Engineering* [online]. 2019, **241**, 97-104 [cit. 2022-04-28]. ISSN 02608774. Available from: doi:10.1016/j.jfoodeng.2018.08.006

[60] BALCI, Ferhan a Feramuz ÖZDEMIR. Influence of shooting period and extraction conditions on bioactive compounds in Turkish green tea. *Food Science and Technology* [online]. 2016, **36**(4), 737-743 [cit. 2022-04-28]. ISSN 1678-457X. Available from: doi:10.1590/1678-457x.17016

[61] LEE, SOH MIN, SEO-JIN CHUNG, OK-HEE LEE, HYE-SEONG LEE, YOUNG-KYUNG KIM a KWANG-OK KIM. Development of sample preparation, presentation procedure and sensory descriptive analysis of green tea. *Journal of Sensory Studies* [online]. 2008, **23**(4), 450-467 [cit. 2022-04-28]. ISSN 08878250. Available from: doi:10.1111/j.1745-459X.2008.00165.x

[62] Ministry of Agriculture, Reg. No. 330 (1997) Regulation for tea, coffee and coffee substitutes. The Czech Republic, Prague

[63] International Organization for Standardization (2011). Green tea. Definition and basic requirements, ISO 11287 (2011) Geneva, Switzerland

[64] KOLÁČKOVÁ, Tereza, Daniela SUMCZYNSKI a Antonín MINAŘÍK. *The Effect of In Vitro Digestion on Matcha Tea (Camellia sinensis) Active Components and Antioxidant Activity* [online]. 30 April 2022, 1-16 [cit. 2022-05-11]. Availavle from: doi:doi.org/10.3390/antiox11050889

[65] SWETHA, M. P., C. RADHA a S. P. MUTHUKUMAR. Bioaccessibility and bioavailability of Moringa oleifera seed flour polyphenols. *Journal of Food Measurement and Characterization* [online]. 2018, **12**(3), 1917-1926 [cit. 2022-05-06]. ISSN 2193-4126. Available from: doi:10.1007/s11694-018-9806-4

[66] YU, Jiawen, Wu LI, Bangyan YOU, Shiying YANG, Wenyan XIAN, Yu DENG, Wei HUANG a Ruili YANG. Phenolic profiles, bioaccessibility and antioxidant activity of plum (Prunus Salicina Lindl). *Food Research International* [online]. 2021, **143** [cit. 2022-05-06]. ISSN 09639969. Available from: doi:10.1016/j.foodres.2021.110300

[67] DAI, Wenzhong, Chengcheng RUAN, Yumeng ZHANG, Jiejie WANG, Jing HAN, Zihan SHAO, Yue SUN a Jin LIANG. Bioavailability enhancement of EGCG by structural modification and nano-delivery: A review. *Journal of Functional Foods* [online]. 2020, **65** [cit. 2022-05-06]. ISSN 17564646. Available from: doi:10.1016/j.jff.2019.103732

[68] SĘCZYK, Łukasz, Danuta SUGIER, Michał ŚWIECA a Urszula GAWLIK-DZIKI. The effect of in vitro digestion, food matrix, and hydrothermal treatment on the potential bioaccessibility of selected phenolic compounds. *Food Chemistry* [online]. 2021, **344** [cit. 2022-05-06]. ISSN 03088146. Available from: doi:10.1016/j.foodchem.2020.128581

[69] DRAWBRIDGE, Pamela C., Franklin APEA-BAH, Polyanna SILVEIRA HORNUNG a Trust BETA. Bioaccessibility of phenolic acids in Canadian hulless barley varieties. *Food Chemistry* [online]. 2021, **358** [cit. 2022-05-06]. ISSN 03088146. Available from: doi:10.1016/j.foodchem.2021.129905

[70] PÉREZ-GÁLVEZ, Antonio, Isabel VIERA a María ROCA. Carotenoids and Chlorophylls as Antioxidants. *Antioxidants* [online]. 2020, **9**(6) [cit. 2022-05-04]. ISSN 2076-3921. Available from: doi:10.3390/antiox9060505

[71] MACÁKOVÁ, Kateřina, Rita AFONSO, Luciano SASO a Přemysl MLADĚNKA. The influence of alkaloids on oxidative stress and related signaling pathways. *Free Radical Biology and Medicine* [online]. 2019, **134**, 429-444 [cit. 2022-05-04]. ISSN 08915849. Available from: doi:10.1016/j.freeradbiomed.2019.01.026

[72] VIEIRA, Tatiane F., Rúbia C. G. CORRÊA, Rosely A. PERALTA, Regina F. PERALTA-MUNIZ-MOREIRA, Adelar BRACHT a Rosane M. PERALTA. An

Overview of Structural Aspects and Health Beneficial Effects of Antioxidant Oligosaccharides. *Current Pharmaceutical Design* [online]. 2020, **26**(16), 1759-1777 [cit. 2022-05-04]. ISSN 13816128. Available from: doi:10.2174/1381612824666180517120642

LIST OF ABBREVIATIONS

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

AOA – Antioxidant activity

DAD – Diode array detector

DMD – Dry matter digestibility

DPPH – 2,2-difenyl-1-pikrylhydrazyl

EC – Epicatechin

ECG – Epicatechin-3-gallate

EGC – Epigallocatechin

EGCG – Epigallocatechin-3-gallate

GAE – Gallic acid equivalent

HPLC – High-performance liquid chromatography

OMD – Organic matter digestibility

RP – Remaining part

SD – Standard deviation

TE – Trolox equivalent

TPC – Total phenolic content

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Appendix P I: Additional graphs

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APPENDIX P I: ADDITIONAL GRAPHS

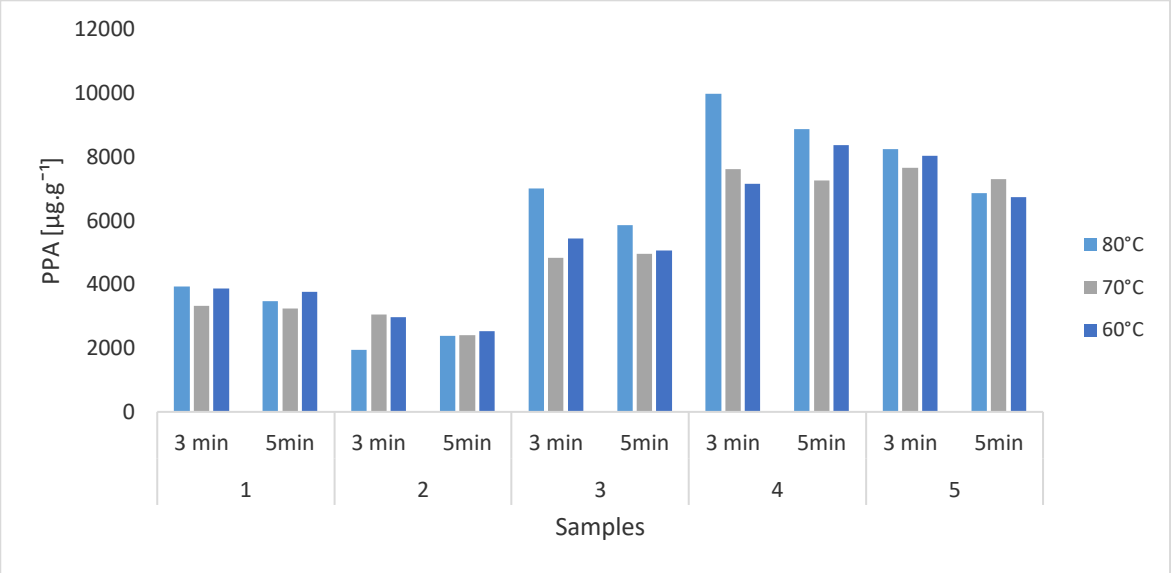

Figure 32 Total sum of individual polyphenolics by HPLC

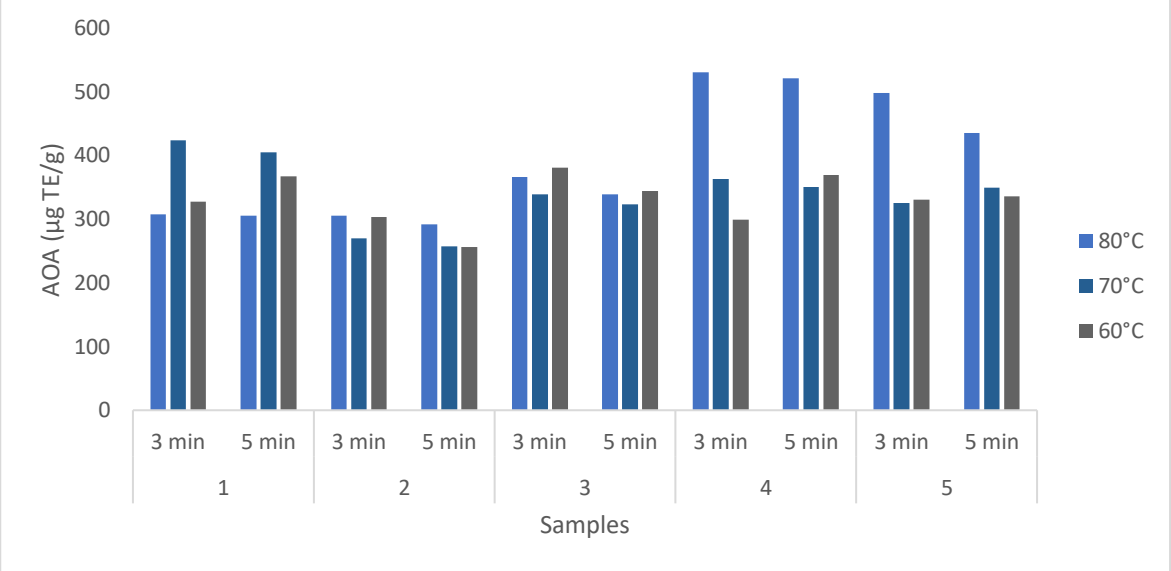

Figure 33 Results of radical scavenging activity using DPPH

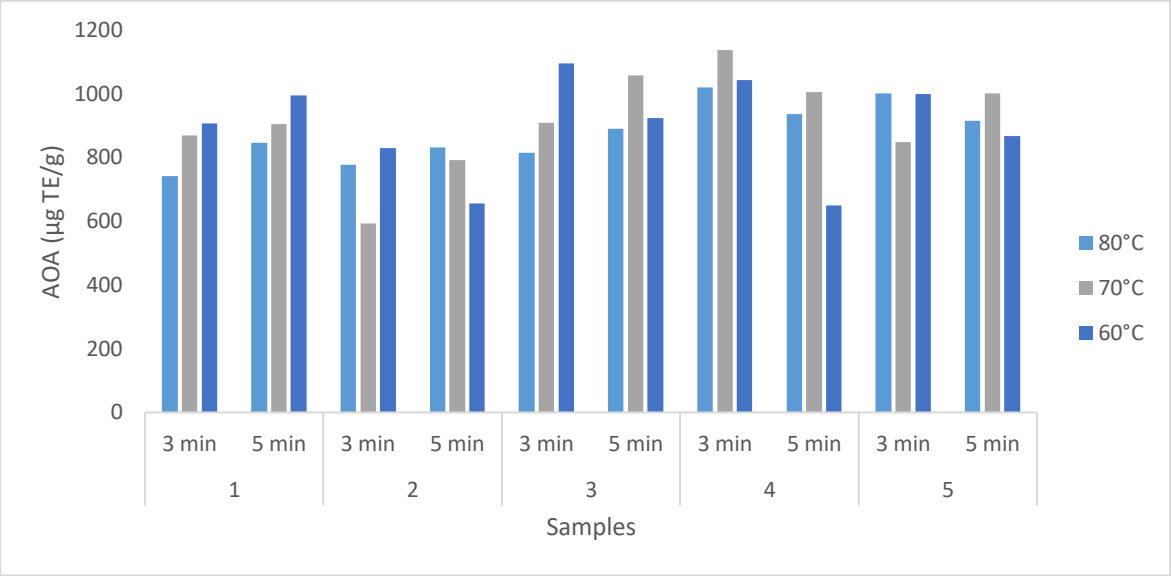

Figure 34 Results of radical scavenging activity using ABTS

APPENDIX P II: ADDITIONAL TABLE

Table 17 Individual phenolic concentrations in native and undigested parts of matcha measured using HPLC

PPA	Samples	Native matcha	Undigested matcha	Remaining parts (RP)
		µg/g ± SD		(%)
Gallic acid	1	604±20[a]	217±10[a]	12
	2	362±10[b]	153±8[b]	12
	3	1280±30[c]	339±10[c]	6
	4	379±10[d]	281±10[d]	25
	5	365±6[b]	293±8[e]	29
Protocatechuic acid	1	496±10[a]	11.9±1.0[a]	1
	2	190±10[b]	12.0±1.0[a]	2
	3	685±12[c]	49.5±2.0[b]	2
	4	413±13[d]	18.8±0.5[c]	2
	5	631±12[e]	28.9±1.2[d]	2
Neochlorogenic acid	1	318±10[a]	3.81±0.20[a]	<1
	2	473±10[b]	3.42±0.30[b]	<1
	3	137±8[c]	5.91±0.30[c]	1
	4	58.9±2.8[d]	2.30±0.10[d]	1
	5	34.2±1.4[e]	2.21±0.10[e]	2
4-hydroxybenzoic acid	1	73.5±4.0[a]	2.00±0.20[a]	1
	2	30.0±1.3[b]	1.53±0.04[b]	2
	3	43.4±0.4[c]	4.80±0.05[c]	3
	4	14.1±0.2[d]	2.21±0.10[d]	5
	5	48.0±2.0[e]	2.92±0.10[e]	2
EGC	1	56500±200[a]	310±8[a]	<1
	2	61800±200[b]	54.8±1.2[b]	<1
	3	73400±100[c]	69.0±1.5[c]	<1
	4	12600±200[d]	73.9±1.5[d]	<1
	5	109000±300[e]	134±5[e]	<1
Catechin	1	300±30[a]	21.6±0.2[a]	2
	2	141±10[b]	17.6±0.3[b]	4
	3	191±10[c]	30.8±0.2[c]	4
	4	140±10[b]	25.6±0.3[d]	6
	5	126±3[d]	32.1±0.4[e]	9
Caffeic acid	1	16130±40[a]	699±15[a]	1
	2	10260±50[b]	664±10[b]	2
	3	15090±50[c]	1650±30[c]	3
	4	14980±30[d]	1000±50[d]	2
	5	17240±30[e]	1780±40[e]	4
EC	1	3790±30[a]	144±10[a]	1
	2	4610±30[b]	119±6[b]	1
	3	7410±50[c]	199±2[c]	1
	4	1270±100[d]	155±3[d]	<1
	5	12300±20[e]	246±2[e]	1

EGCG	1	19400±200[a]	281±2[a]	1
	2	10000±200[b]	311±8[b]	1
	3	58400±300[c]	857±3[c]	<1
	4	56900±300[d]	672±7[d]	<1
	5	62000±300[e]	730±15[e]	<1
Ferulic acid	1	101±6[a]	12.7±0.5[a]	4
	2	136±5[b]	18.8±0.5[b]	4
	3	117±4[c]	39.7±0.4[c]	8
	4	392±7[d]	25.2±0.3[d]	2
	5	235±6[e]	38.7±0.5[e]	6
Sinapic acid	1	16.5±1.0[a]	2.51±0.06[a]	5
	2	47.5±3.0[b]	5.80±0.04[b]	4
	3	368±10[c]	6.11±0.05[c]	<1
	4	43.4±1.0[d]	4.53±0.07[d]	4
	5	55.9±2.2[e]	8.00±0.03[e]	5
ECG	1	1950±30[a]	28.5±0.4[a]	1
	2	2550±50[b]	112±3[b]	1
	3	6160±20[c]	126 ±3[c]	1
	4	6990±30[d]	114±3[b]	1
	5	5720±30[e]	135±8[d]	1
Ellagic acid	1	36.6±0.6[a]	10.6±0.4[a]	10
	2	55.0±1.9[b]	14.0±0.3[b]	8
	3	68.9±1.4[c]	23.1±0.6[c]	8
	4	45.4±1.5[d]	13.6±0.3[d]	10
	5	50.4±0.6[e]	27.7±1.0[e]	20
Rutin	1	9.90±0.20[a]	2.62±0.05[a]	9
	2	11.5±0.1[b]	3.81±0.05[b]	10
	3	10.7±0.5[c]	7.80±0.04[c]	18
	4	14.9±0.5[d]	7.61±0.05[d]	17
	5	16.5±0.1[e]	8.95±0.08[e]	19
trans-2-Hydroxycinnamic acid	1	11.3±0.2[a]	6.92±0.04[a]	20
	2	39.9±1.3[b]	13.6±0.5[b]	10
	3	23.1±0.6[c]	10.0±0.10[c]	11
	4	14.5±0.4[d]	5.30±0.08[d]	13
	5	5.90±0.04[e]	3.42±0.10[e]	21
Protocatechuic acid	1	226±8[a]	19.0±0.26[a]	3
	2	304±10[b]	11.3±0.3[b]	1
	3	19.2±0.4[c]	10.6±0.4[c]	13
	4	26.6±0.8[d]	3.22±0.05[d]	4
	5	21.9±0.4[e]	4.21±0.05[e]	7
trans-Cinnamic acid	1	0.50±0.01[a]	0.42±0.02[a]	27
	2	1.60±0.02[b]	0.20±0.01[b]	5
	3	1.30±0.10[c]	0.43±0.02[a]	8
	4	1.11±0.10[d]	0.10±0.01[c]	3
	5	1.11±0.10[d]	0.22±0.01[d]	7
Kaempferol	1	68.3±2.6[a]	15.4±0.4[a]	8
	2	346±10[b]	49.3±1.0[b]	4
	3	725±6[c]	122±7[c]	4

	4	214±8[d]	35.2±0.8[d]	6
	5	23.1±0.7[e]	2.20±0.03[e]	3
Quercetin	1	13.6±0.1[a]	1.60±0.02[a]	4
	2	47.7±1.7[b]	3.62±0.06[b]	2
	3	77.2±0.6[c]	16.6±0.3[c]	5
	4	343±12[d]	24.0±0.4[d]	2
	5	292±8[e]	35.5±0.5[e]	4

All results are presented on a dry matter basis as means ± SD, n=4 (the mean of four measurements). Means within a column with at least one identical small superscript (in case of each phenolic) do not differ significantly ($p \geq 0.05$)