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INFLUENCE OF TEMPERATURE AND BREWING TIME ON THE TOTAL POLYPHENOL CONTENT IN TEA

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Abstract: Tea is a crucial source of antioxidant compounds – polyphenols – in a standard daily diet. On average, Poles drink 2–3 cups of tea daily. Its regular consumption may prevent cardiovascular and metabolic disorders. The objective of the study was to compare the total polyphenol content (TPC) in various types of tea infusions prepared at different temperatures (T = 70/100°C) and brewing times (t = 2/10 minutes). Organic and conventional black and green teas were tested. The TPC was measured using the Folin-Ciocalteu spectrophotometric method and expressed as gallic acid equivalents (mg GAE/g of the sample). Each tea extract was assayed three times independently. In the organic types of teas, the highest TPC was found in green tea (95.65 ±3.96 mg GAE/g) brewed at 100°C for 10 minutes, while the lowest in black tea (51.65 ±2.39 mg GAE/g) brewed at 70°C for 2 minutes. Among the conventional teas, the highest TPC was also noted in green tea (91.83 ±2.29 mg GAE/g) brewed at 100°C/10 minutes and the lowest in black tea (48.88 ±2.20 mg GAE/g) at 70°C/2 minutes. Longer brewing times increased TPC in organic teas (in both black and green) and in conventional black tea (p <0.05), whereas higher temperature increased TPC only in organic teas (black and green); (p <0.01).

Key words: antioxidant properties, black tea, brewing time, green tea, infusion, total polyphenol content

INTRODUCTION

Oxidation-reduction processes occur continuously within all living cells [1]. During some of these reactions, free radicals – reactive oxygen species – are formed. These can lead to the modification and damage of cell structures, including proteins, lipids and nucleic acids. Antioxidants are involved in reducing the production of excessive free radicals [2]. One group of compounds that possess antioxidant properties is polyphenols [3]. These natural substances, which are stored in various parts of plants, constitute an essential component of the standard daily diet [4]. The most important subgroups of polyphenols include phenolic acids and flavonoids. Among the phenolic acids, notable examples are caffeic acid, vanillic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, and gallic acid. On the other hand,

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flavonoids constitute a broad class of compounds, which can be further divided into subgroups such as flavonols (e.g., kaempferol, quercetin), flavones (e.g., apigenin), and flavanols, which include catechin and epicatechin [5].

Tea, as the most widely consumed beverage, is a significant source of polyphenols in the standard diet [6]. They demonstrate robust antioxidant properties that exert a protective effect against cardiovascular diseases and neurodegenerative disorders. They are also attributed to the ability to lower blood pressure, cholesterol and glucose levels in the blood. Regular polyphenol consumption may reduce the risk of cardiovascular disease due to their antioxidant effect on low-density lipoprotein (LDL). Additionally, some studies suggest that these compounds may protect the skin from the harmful effects of UV radiation [7, 8, 9].

Data indicate that the average Pole consumes approximately 2–3 cups of tea per day [10]. Tea consumed without the addition of sugar and milk does not provide calories and plays an important role in maintaining the body's hydration [11].

Leaves of the tea plant are characterised by their dark green colour and hard texture, while the flowers are soft and white. There are many varieties of tea. A typical example is the Chinese-origin *Camellia sinensis var. sinensis*, which demonstrates resistance to drought and cold. It is the most prevalent tea variety and the longest used in production [12, 13]. The raw materials to produce tea can be harvested up to several times a year, or even throughout the year in areas such as southern India, Ceylon and Indonesia. The traditional approach to harvesting involves laying the leaves on a surface such as a flat basket, bamboo mat or the floor. This is usually done under conditions of fresh air, sunlight (which provides additional stimulation and increases the number of catechins due to UV radiation) or in a ventilated room with a controlled temperature. The period of leaf wilting lasts between three and twelve hours, during which their moisture is reduced by 30–50%. Water loss facilitates leaf rolling [14]. At the time that the leaf is plucked from the tea branch, the oxidation in the leaf is already underway. It occurs gradually and without human intervention. The process is halted by the enzymes that initiate the oxidation, which are then deactivated [15]. The next point of production is rolling, during which the leaves are appropriately shaped and flattened. Most of these steps are now mechanised. However, high-quality tea is still twisted by hand. This stage influences the prolongation of the tea's shelf life. Next, the tea is sorted, classified, and packaged [16].

Worldwide, tea consumers prefer to drink black tea (76–78%), and next green tea (20–22%). Black tea undergoes the most extensive oxidation and fermentation processes compared to other types of tea. During the oxidation of black tea, catechins are transformed into theaflavins and thearubigins, which retain their antioxidant properties. These compounds, often referred to as tannins, contribute to the characteristic dark colour of black tea. Tannins may exhibit therapeutic effects on gastric and intestinal disorders. Green tea is particularly rich in catechins, which are a subclass of flavanol monomers, a type of flavonoids. The catechins in green tea comprise epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). Apart from these antioxidant compounds, green tea also contains a small amount of vitamin C. The constituents of tea demonstrate antioxidant, antimutagenic, and anticarcinogenic properties. Regular consumption of green tea may prevent several types of cancer (lung, colon, oesophagus, oral cavity, stomach, small intestine, kidney, pancreas and mammary glands). Numerous epidemiological studies and clinical trials have indicated that green tea, and in some ways also black tea, may reduce the risk of various chronic diseases [17, 18, 19, 20].

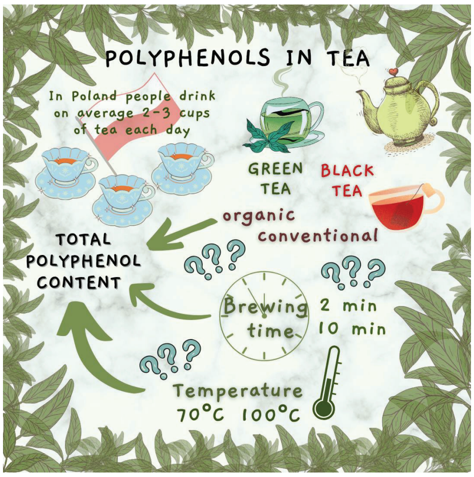

Figure 1. Do the type of tea, brewing time and temperatures influence the total polyphenol content in tea infusions?
Source: own elaboration.

It seems that the conditions under which the tea is brewed determine its antioxidant potential and the amount of antioxidative compounds in its infusion. The objective of the study was to compare the total polyphenol content (TPC) in organic and conventional types of tea infusions, prepared at different temperatures and brewing times (Fig. 1).

MATERIAL AND METHODS

This study included black and green tea in both options, organic and conventional. The organic tea, produced by *Dary Natury*, exhibited the requisite labelling indicative of organic certification. Conventional black tea (produced by *Oriental Delight*) and conventional green sencha (produced by *Sencha Classics*) were obtained from the same entity, *Basilur Tea*. The tea was provided in sachet form. The TPC of the tea was quantified according to the Folin-Ciocalteu (FC) spectrophotometric method [21] and expressed as mg of gallic acid equivalent per gram of the sample (mg GAE/g of the sample) using the equation obtained from the gallic acid calibration curve (R^2 >0.99); (Fig. 2). Determination of the TPC using this method is based on the reversible reaction of the reduction of molybdenum (VI) by polyphenols in an alkaline medium to molybdenum (V) contained in the FC reagent. The blue-coloured product formed shows an absorption maximum at λ = 745÷750 nm.

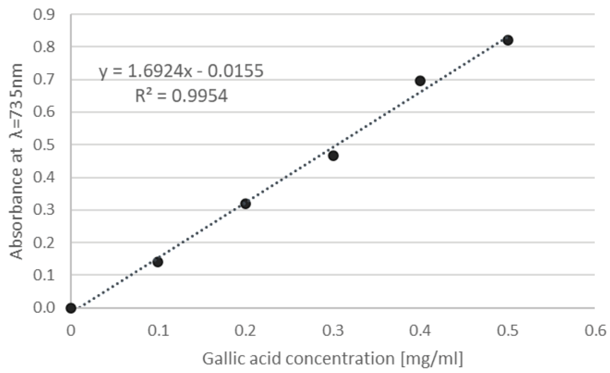

Figure 2. Calibration curve for a standard solution of gallic acid for determination of total polyphenol content

Source: own elaboration.

To prepare aqueous extracts, 1 g of tea was weighed into glass beakers (250 ml), which were then filled to a volume of 100 ml with demineralised water. The tea was infused with the water at temperatures (*T*) of 70°C and 100°C (immediately after boiling) for two different durations (*t*): 2 and 10 minutes. Each tea was brewed under four condition configurations:

- 70°C for 2 minutes,
- 70°C for 10 minutes,
- boiling water for 2 minutes,
- boiling water for 10 minutes.

After the designated brewing time, the infusions were decanted. The resulting extracts were then transferred to new beakers, taking 5 ml of the sample from each infusion.

In brief, 5 ml of the sample with 2 ml of distilled water was mixed with 50 µl of the tea extract and 250 µl of Folin-Ciocalteu solution. After 3 minutes, 750 µl of Na_2CO_3 solution was added and vortexed. The blank was prepared using the same process but with 50 µl of distilled water instead of the sample. The tubes were covered with aluminium foil and placed in a water bath at 40°C for 30 minutes. After incubation, the absorbance was measured at a wavelength equal to 735 nm using a UV-Vis spectrophotometer (Pharmacia LKB Ultrospec III). Each measurement was repeated three times independently [22].

Statistical analysis was performed using the Statistica 13.3 software. Analysis of variance (ANOVA) was conducted to evaluate the influence of the different temperatures and tea brewing times on the TPC. To assess the significance of differences between the individual samples of tea, post-hoc tests were used. The significance level was set at p <0.05.

RESULTS AND DISCUSSION

The results of the TPC determinations are presented in Figures 3 and 4.

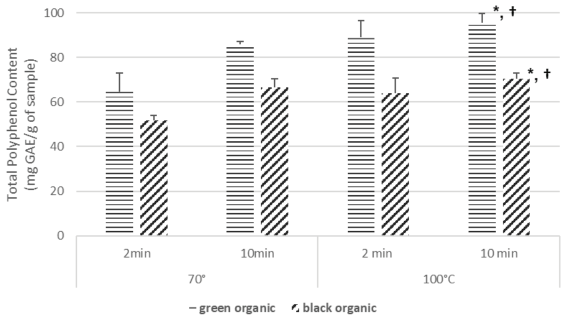

Figure 3. Comparison of total polyphenol content in organic tea brewed for 2 and 10 minutes at temperatures of 70°C and 100°C. Results are presented as mean ± standard deviation, * – p <0.05, † – p <0.01

Source: own elaboration.

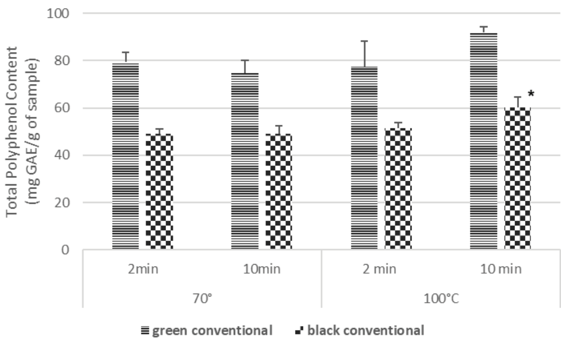

Figure 4. Comparison of total polyphenol content in conventional tea brewed for 2 and 10 minutes at temperatures of 70°C and 100°C. Results are presented as mean ± standard deviation, * – p <0.05

Source: own elaboration.

Of the organic tea varieties, the highest TPC was found in the green tea, amounting to 95.65 ±3.96 mg GAE/g of the sample, when brewed at 100°C for 10 minutes. In contrast, the lowest TPC was recorded in the black tea, with a value of 51.65 ±2.39 mg GAE/g of the sample, under brewing conditions of 70°C for 2 minutes (Fig. 3). Tea brewed with boiling water (for both times: 2 and 10 min) showed a higher TPC than those brewed at T = 70°C. Tea steeped for 10 minutes (in both T: 70°C and boiling water) had a higher TPC compared to those brewed for a shorter time across all organic tea types: black and green.

In the conventional types of tea, the highest TPC was also identified in green tea, reaching 91.83 ±2.29 mg GAE/g of the sample, when brewed at 100°C for 10 minutes. Conversely, the lowest TPC was found in black tea, with a value of 48.88 ±2.20 mg GAE/g of the sample, brewed at 70°C for 2 minutes (Fig. 4).

When analysing the differences in polyphenol content in conventional tea (depending on brewing temperature and time), it was found that conventional green tea had the highest polyphenol content (under conditions of T = 100°C, t = 10 minutes), while conventional black tea had the lowest (at T = 70°C, t = 2 minutes). Both conventional black and green tea brewed with boiling water had higher polyphenol contents compared to those brewed at 70°C.

Green tea (both organic and conventional) exhibited a higher TPC compared to black tea. The longer brewing time (t = 10 minutes) increased the TPC in organic tea (in both black and green) and in conventional black tea (p <0.05), whereas the higher temperature increased the TPC only in the organic teas (black and green) (p <0.01).

As stated above, according to the statistics on tea consumption among people in Poland, on average, 2–3 cups of tea are consumed daily. Based on our results, we can conclude that drinking three cups (we assumed that 1 cup is defined as 1.5 g of tea brewed with about 150 ml of water) of organic green tea daily (brewed for 2 minutes at 70°C) will provide the organism with 290.7 mg of polyphenols daily. If the same tea is brewed with water at 100°C for 10 minutes, the supplied amount of polyphenols increases to 430.4 mg. If the individual usually drinks organic black tea (brewed for 10 minutes at 100°C), they may consume 316.4 mg of polyphenols.

The polyphenol contents of black and green teas reported in the literature are within very wide limits. Comparison of the results obtained is hampered by the use of different solvents (water, methanol), the use of various methods for measuring polyphenols, but also various methods for presenting the results in terms of gallic acid or catechin equivalents per 1 g of tea dry weight or volume of 100 or 200 cm^3. It is also necessary to take into account the variability resulting from the specific characteristics of different tea cultivars, production, and storage conditions.

The Folin-Ciocalteu method used in this study is the most widely applied and convenient international standard method (ISO 14502-1:2005) to determine the total phenolic content in foods, herbs, and other plant extracts [23]. The disadvantage of this method lies in the fact that it is based on the oxidation of -OH groups in tea polyphenols, which can easily oxidise some non-tea polyphenol compounds, making the theoretical result higher than the actual value. More accurate quantitative analysis of functional constituents in tea is possible with near-infrared (NIR) spectroscopy with non-destructive detection and electrochemical sensor technology capable of rapid on-site detection. Moreover, enhanced liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) are used for the simultaneous determination of multiple polyphenols and the identification of new polyphenols [24]. Although the latter methods are characterised by high sensitivity, precision and resolution, they are more costly and require specialised, expensive equipment.

In the study conducted by Cody [24], the polyphenol content of ten different tea infusions was determined using Matrix-Assisted Ionisation in Vacuum (MAIV) mass spectrometry (MS). The analysis encompassed nine infusions derived from *Camellia sinensis*, including three green teas, two black teas, two oolong teas, jasmine tea, and white tea, as well as one rooibos infusion. The polyphenol content in tea infusions ranged from 19.2 to 108.6 mg/100 mL across the different tea types, with green teas generally exhibiting higher polyphenol concentrations compared to black teas. These findings align with our results, where both the organic and conventional green teas showed the highest TPC.

In the study by McAlpine et al., eight types of tea were characterised in terms of TPC and antioxidant capacity with respect to brewing time [25]. As in the present study, the TPC in the tea infusions was determined using the F-C method. Tea samples were brewed for durations ranging from 1 to 10 minutes, in 1-minute intervals and the TPC was subsequently measured, which increased with longer brewing times; however, most of the polyphenols observed after 10 minutes were already extracted within the first 5 minutes, regardless of the type of tea. Green teas demonstrated higher TPC values than black ones, similar to the findings of the current study. For instance, in the referenced study, the TPC for sencha (a type of green tea) brewed for 2 minutes was 52.42 ±6.90 mg GAE/g of tea, while for 10 minutes it was 112.37 ±3.03 mg GAE/g. Similarly, in the current study, the TPC for organic green tea increased with the longer brewing time, reaching, for example, 64.59±8.49 mg GAE/g of the sample at T= 70°C for t = 2 minutes, and 85.79 ±1.44 mg GAE/g after 10 minutes of brewing. A comparable trend was observed for black teas.

The study by Vinci et al. investigated the influence of various brewing parameters (type of water, brewing time, temperature, and pH) on the TPC and antioxidant activity of green and black tea infusions [26]. An increase in the TPC was observed with longer brewing times for each type of water. As in the present study, higher TPC values were consistently recorded for green tea. Black tea demonstrated a comparable trend to green tea, with a higher TPC observed in infusions brewed at T = 100°C compared to T = 80°C (in our study, T = 70°C). The parameters that had the most significant impact on the TPC were the brewing time and the type of water used for infusion preparation. Similarly to our findings, the brewing time had a significant effect on the TPC levels in the tea samples.

In the study by Mehrabi et al., the impact of various tea preparation techniques on the content of bioactive compounds, antioxidant capacity, antibacterial properties, and polyphenol bioavailability in green, black, and oolong tea infusions was compared [27]. All tea types demonstrated an increase of TPC over longer brewing times. Consistent with our findings in this study, higher TPC values were reported for green tea, regardless of the preparation method, compared to black tea. The study highlighted the significant influence of the oxidation process on the tea's properties. Microwave-assisted brewing was identified as the most effective technique for maximising the bioactive compound content and bioavailability, which was attributed to rapid and uniform heating, which facilitates efficient extraction while minimising degradation. While all preparation methods showed a positive correlation between the TPC and antioxidant and antibacterial activities, the traditional steeping method resulted in the highest release of phenolic and flavonoid compounds after 10 minutes of brewing. This suggests that the brewing parameters, such as time and temperature, significantly influence the extraction efficiency of phenolic compounds, emphasising the importance of optimising these factors for maximising health-related benefits from tea infusions.

A Polish study by Dmowski et al., also using the Folin-Ciocalteu method, assessed the impact of the steeping time and the degree of fragmentation of black tea on its antioxidant properties [22]. The tea was brewed at approximately 90°C. The results presented in the study indicated that steeping time affected the level of polyphenolic compounds. Infusions brewed for 15 minutes exhibited higher values (average content in the analysed samples was 239.57 mg GAE/100 ml) compared to those brewed for 3 minutes (67.70 mg GAE/100 ml). Furthermore, it was noted that the degree of fragmentation and the brand of tea were not statistically significant factors. In the present study, the steeping time also influenced the polyphenol content. In this study, the teas steeped for 10 minutes contained more polyphenols than those steeped for 2 minutes (for instance, in organic black tea, the polyphenol content after 10 minutes of brewing was higher at 70.30 ±2.74 mg GAE/g of the sample, compared to 63.92 ±6.72 mg GAE/g of the sample for the 2-minute infusion, both at a constant brewing temperature of $T = 100$°C).

The antioxidant properties of four green teas, depending on the extraction temperature, were compared in the study by Ramirez-Aristizabal et al. [28]. Various methods were employed for the measurements, including the Folin-Ciocalteu method. The brewing time for the tea was set at 5 minutes. Higher temperatures (80°C) for tea brewing, compared to lower (in this case, it was at room temperature, 25°C), resulted in a greater polyphenol content (for example, in one of the green teas, the TPC at $T = 80$°C was 55.06 ±1.03 mg GAE/g of sample, whereas at $T = 25$°C it was 14.63 ±0.53 mg GAE/g of the sample). In our study, higher brewing temperatures also resulted in an increase in the TPC. The TPC of organic green tea brewed for 2 minutes at 70°C was 64.59 mg GAE/g of the sample, whereas, at 100°C, the TPC increased to 88.94 mg GAE/g of the sample.

In contrast, a study by Pintać et al. compared the phenolic profiles and antioxidant activities expressed as real serving concentrations of the most popular plant-based drinks: coffee, tea, and wine [29]. Similar to our study, the TPC of green tea reached higher values than black tea. For example, looking at teas from the same manufacturer: green tea contained 340 ±26.2 mg GAE/serving, while black tea contained 164 ±0.42 mg GAE/serving. All three categories of beverages demonstrated a substantial concentration of phenolic compounds, exhibiting significant antioxidant potential. Nevertheless, the green tea infusions displayed markedly superior antioxidant activity. Based on the findings, moderate consumption of coffee and red wine may also be recommended as a valuable source of polyphenols. In our study, conventional green tea brewed for 2 minutes at 70°C achieved a TPC of 79.56 mg GAE/g of the sample, while conventional black tea, under the same brewing conditions, exhibited a TPC of 48.88 mg GAE/g of the sample.

Our study provides interesting information regarding the amount of polyphenol in tea and influencing factors, but it is not free from some limitations. Further studies should involve a larger number of samples in order to confirm the present findings. Other, more accurate, modern methods could be used to carefully assess the contributions of individual polyphenols to the TPC. Chromatographic techniques, particularly High-Performance Liquid Chromatography (HPLC), remain the standard for the qualitative and quantitative analysis of individual polyphenolic compounds in tea, while spectrophotometric techniques continue to be widely employed. These methods, when used in combination, would provide a more comprehensive evaluation of the polyphenolic profile in tea, supporting researchers in understanding the relationship between tea's composition and its potential health benefits.

Despite the increasing adoption of advanced methods, such as near-infrared spectroscopy and electrochemical sensors, the Folin-Ciocalteu method remains the most commonly used approach for determining TPC. As noted by Sun et al. [24], this method is favoured for its stability, reliability, and simplicity. Other comparative studies between various organic and conventional teas from different producers would also be desired.

CONCLUSIONS AND FUTURE PERSPECTIVES

A higher brewing temperature resulted in an increased polyphenol content in infusions from both organic (green and black) teas, as well as from conventional teas. However, a longer tea brewing time led to an increase in polyphenol levels, but only in the case of organic tea (both black and green). It is worth paying attention not only to the brand of tea but also to its brewing parameters, which certainly translates into the amount of polyphenols in the daily diet.

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