PHYSIO-CHEMICAL AND BIOCHEMICAL CHARACTERISATION OF GREEN TEA (*CAMELLIA SINENSIS*) OBTAINED THROUGH TWO DIFFERENT EXTRACTION METHODS

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RESEARCH ARTICLE (REVIEW ARTICLE)

Abstract
The objective of the study was to observe the physio-chemical differences between two different extractions of green tea (Camellia sinensis).
To conduct the study, two green tea extractions were obtained: through classical infusion at 85 ℃ for two minutes and through cold extraction at 45 ℃, under void for 45 minutes. Based on the green tea extractions, total antioxidant capacity, polyphenols, flavonoid, and vitamin C concentrations were determined, as well as pH, conductivity, and dry soluble matter values, after which the results were compared to observe the differences.
The obtained results showed that even though the results were similar between the two infusions, a better extraction of the bioactive compounds was determined from the classical infusion.

Keywords: green tea, physio-chemical analysis, cold extraction, infusion
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INTRODUCTION

Green tea is obtained through minimal processing of the *Camellia sinensis* plant. The main purpose of this process is to keep the fresh flavour and to preserve the highest number of bioactive compounds found in the fresh leaves. After harvesting the leaves, the next step is to neutralise the enzymes to avoid tea degradation. The leaves are rolled, and the cells walls are broken, leading to the expression of the juice from the leaves, thus reducing the humidity as much as possible during the drying phase [1].

According to specialty literature, it is demonstrated that the temperature and the infusion time influence the bioactive composition of the tea, as well as the antioxidant capacity [2]. Traditionally, green tea is infused 2-3 minutes at 80-90°C. Researchers observed that the longer the infusion time is, the more the antioxidant capacity grows, reaching a maximum at 5 minutes [3]. Also, the temperature of the infusion, influences the antioxidant capacity and increases the solubility of the catechins [4]. Another research discovered that infusing tea at room temperature, up to two hours, presented an antioxidant activity with 65% higher than infusions at 70°C for 360 seconds [5]. A study from 2011, states that green tea brewed with hot water has a more astringent taste compared with the green tea brewed with cold water. This happens because hot water extracts epigallocatechin gallate and caffeine, compounds that elicit bitterness and a strong astringent taste. These compounds are hard to extract in cold water, enhancing only the extraction of epigallocatechin and the theanine that are easily extracted in cold water and flavour the green tea infused this way[6].

The main **objective** of this study was to observe the differences in green tea extractions obtained through two methods: classical extraction at 85°C for 2 minutes and through cold extraction at 45°C for 45 minutes under void, and to analyse if there are significant differences between the two extractions through physio-chemical and biochemical determinations.

MATERIAL AND METHOD

1.Material

To elaborate this study, green tea packaged in tea bags was bought in April 2024.

The devices used were ceramic mugs, a water heater, a thermometer, a Heidolph Laborota 4010 digital rotavapor, analytical balance, mini-Shimadzu spectrophotometer, WTW pH-meter and KRUSS digital refractometer.

2.Characterisation of the green tea

Green tea (*Camellia sinensis*) is one of the most consumed beverages around the world. It contains more antioxidants than any other form of tea. The composition in bioactive compounds varies and is directly dependant on the fermentation processes employed in production [7].

The chemical composition of green tea is complex. Some of the elements present in it are vitamins B, C, E, caffeine and theophylline, pigments: chlorophyl and carotenoids, minerals such as Ca, Mg, Mn, Fe, Zn, Mo, Se, Na, P, K, etc., and volatile compounds: esters, alcohols, aldehydes and lactones [8].

Green tea also has many health benefits as seen below:

- enhanced antioxidant activity and protection against oxidative damages through responsible consumption while following a healthy diet [9];
- hypotensive effect and lowered risk of cardiovascular diseases when minimum 10 cups of tea are consumed daily [10];
- anti-mutagenic potential against cancer demonstrated through studies upon cell cultures [11];
- enhanced oral health through lowering the number of cavities after frequent consumption of green tea [12];
- controlled body weight through thermogenesis from the synergic interaction between caffeine, polyphenols and prolonged stimulation of the sympathetic nervous system [13];
- UV protection through oral consumption or topical application of polyphenols from green tea [14];
- increased glucose tolerance [15].

3.Method

The experiment was carried out in 7 steps with 5 repetitions each and the average of the obtained results was written in the paper.

Step 1 was the extraction of the infusion with:

- the classical method at 85°C for 2 minutes,
- the cold extraction under void at 45°C for 45 minutes in the Heidolph Laborota 4010 digital rotavapor.

Step 2 was the determination of the total antioxidant capacity with the FRAP method as it was elaborated by Benzie and Strain in 1996. After mixing the green tea extracts with the FRAP reactive and the other components, the obtained solution was stirred and heated at 37°C. After cooling the samples, the absorbance was read at 593nm. The results were expressed as a correspondent of the antioxidant activity in Trolox equivalents [16].

Step 3 Total phenolic compounds were determined through the Folin-Ciocâlteu method, and the samples were read at 760nm. The results were expressed in Gallic acid equivalents/100ml analysed material [17].

Step 4 Ascorbic acid content was determined through the volumetric method – iodometry. The determination is based on the reducing property of the ascorbic acid by transformation through oxidation in dehidroascorbic acid [16].

Step 5 Total flavonoid compounds were determined using the spectrophotometric method with $AlCl_3$ and the absorbance was measured at 510nm. The used standard was catechin, represented by quercetin [18].

Step 6 Determination of pH and conductivity was done with the use of the WTW pH-meter.

Step 7 Determination of dry soluble matter was done using the digital refractometer KRUSS at 20,6°C, and the correction at 20°C was made using the online programme winemaker.plus [19]

RESULTS AND DISCUSSIONS

Following the research, below are presented the obtained results and the correlations between the parameters.

Table 1 – The results of physio-chemical and biochemical determinations

Determinations	Sample 1 – classic extraction	Sample 2 – cold extraction
Total antioxidant capacity mM TE	10,117	9,287
Total phenol compounds mg GAE/100ml	92,428	89,034
Ascorbic acid content mg/100ml	50,62	46,17
Total flavonoid compounds $AlCl_3$ mg/100ml	103,645	98,437
pH	6,25	6,3
Conductivity	76,3	76,2
Dry soluble matter - ° Brix	0,45	0,45

Following the physio-chemical and biochemical determinations as seen in Table 1, it can be seen that the classical extraction method, has a higher number of bioactive compounds, a slightly higher conductivity but a lower pH compared to the cold extraction method.

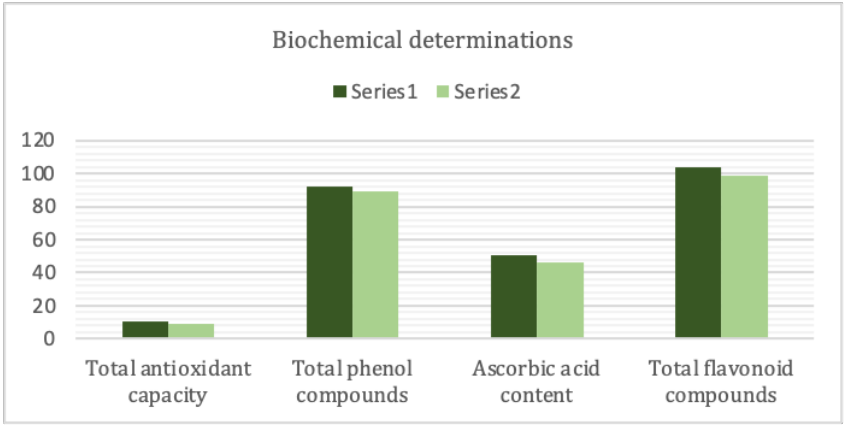

Figure 1 – Comparison between the biochemical values of the two extracts

The results that were obtained from the biochemical determination show that there are very small differences between the extraction methods, with the classical method being slightly richer in bioactive compounds.

According to newer studies, epigallocatechin has a beneficial effect on the immune system, enhancing it and theanine reduces the psychosocial stress, but these compounds are inhibited by epigallocatechin gallate and caffeine, thus in order to obtain the aforementioned effects, cold brewing should be applied to green tea[6].

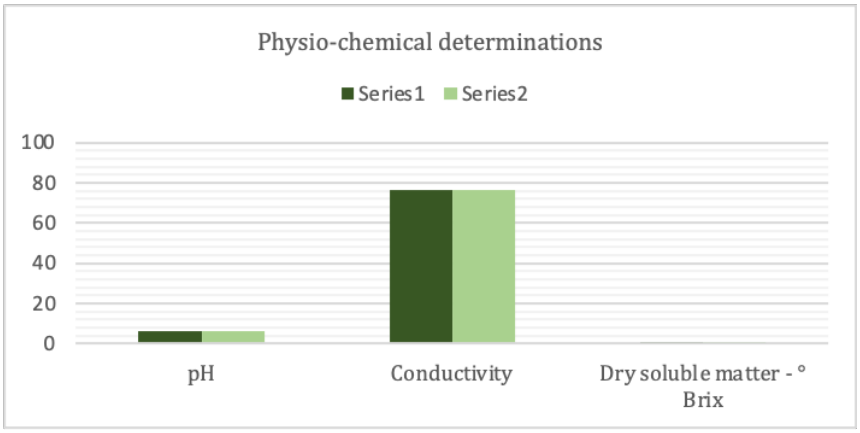

Figure 2 – Comparison between the physio-chemical values of the two extracts

Both tea infusions had very similar results when the pH, the conductivity and the dry soluble matter was determined.

Other studies showed as well that there are very small differences between the physio-chemical results, when talking about the method of infusion cold or classical, the differences appearing when the samples change concentrations of plant used for extraction[20].

CONCLUSIONS

1. Cold extraction of tea infusion showed similar results to the classical extraction.

2. The results found in this paper were consistent with previous reports regarding the biochemical and physio-chemical composition of teas brewed in cold and hot water.

3. Considering that epigallocatechin gallate and caffeine extracted in hot water inhibit the presence of epigallocatechin and theanine that are soluble in cold water, cold extraction is recommended when the latter compounds are desired.

4. Classical hot infusion is bitter, and astringent compared to the cold infusion that has a softer flavour thus making the cold extraction to be an option that is better tolerated by consumers and is also a rich source of bio-compounds.

ACKNOWLEGMENTS

This work was co-funded from the project CNFIS FDI-2024-F-0334 – "Interdisciplinary research and innovation using sustainable technologie – CIIUTS" funded by UEFISCDI.

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