Tea Antioxidants

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Abstract.. In the paper, antioxidant activity (AOA) of tea infusions as a function of infusion time and type or brand of teas as well as a concentration of caffeine were measured. The main components of green tea infusions are identified as epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and caffeine. It has been shown that the antioxidant activity depends on the time of infusion and the degree of tea grinding. while there is a linear dependence between AOA and concentration of caffeine in tea. Thus the only method to reduce content of caffeine in the infusion is a preliminary withdrawal of caffeine from the plant material

1 Introduction

Tea began to be used in China 4-5 thousand years ago as a medicine. Tea leaves were ground with rice and made into flat cakes. Until now, in some countries, tea was consumed as a food in the form of a salad or soup cooked from tea leaves. The first book about tea as a drink appeared in the VIII century. It was written by LU Yu and was called "the Canon of tea". In Russia, tea appeared for the first time during the reign of Ivan III.

Tea is currently the most popular drink in the world. In Uzbekistan, tea lovers are 99.6% of the population, in Azerbaijan-99.1%, in China-98.9% and in Egypt-98.5%. In Russia, 77.5% of residents prefer to drink tea, and 22.5%— coffee.

The range of teas grown and produced by the industry is very large, but among the variety there are main types of tea: black (fully fermented), Oolong (partially fermented) and green (unfermented). In recent years, new types of tea have appeared on the world market: white, yellow, PU-erh and others [1, 2].

Currently, tea is consumed by more than 4 billion people, as, in addition to taste, the health benefits of tea are not in doubt. Tea contains a large number of compounds that are strong antioxidants. These are primarily catechins, which are present in large quantities, especially in green tea. Green tea catechins and black tea theaflavins prevent and suppress cancer [3]. Consumption of 250-500 ml of tea per day reduces mortality from ischemic disease by 2 times [4]. the inverse relationship between tea consumption and cholesterol levels is shown. [5]. The antioxidant effect of tea on the human body has been shown [6], antibacterial [7], antimicrobial [8], antiviral [9], and antidiabetic [10].

Catechins are the most useful components of tea, making a great contribution to the antioxidant activity of the drink. The main catechins of tea are eight, but 70% are epigallocatechin gallate (EGCG) and epigallocatechin (ECG). The total content of catechins are: in green tea from 130 to 220 mg/g, in Oolong tea on average 65 mg/g, and in black tea about 5 mg / g [1].

Tea helps to increase performance and attention due to the presence of caffeine - an alkaloid with a bitter taste that excitingly affects the body. According to literature data, the content of caffeine in green tea and Oolong tea varies from 26 to 40 mg/g, and in black teas, the content of caffeine is 15- 33 mg/g, [1].

The purpose of this work is to study the influence of types and brands of teas, methods of sample preparation and conditions of infusion on the most important characteristics that determine the benefits of using tea.

2 Experimental

2.1 Sample preparation

Samples of tea or tea drinks were weighed on analytical scales at 1.0 gram and filled with 50.0 or 100 ml of boiling distilled water. Stirred and allowed to infuse for a certain time, filtered first through a paper filter, then through a syringe filter. A sample was taken from the resulting extract for HPLC analysis.

The method of high-performance liquid chromatography (HPLC) was used to determine catechins and caffeine in tea and tea drinks. The analysis was performed using an Agilent Infinity 1200 chromatograph with a diode array detector. The volume of the analyzed sample (10 μL) and the flow rate of the mobile phase (0.8 mL/min) were set. Caffeine and catechins were separated on the Repsosil-Pur C18-AQ

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column (100×4,6 mm, particle size 3 μm) and detected by absorption at a wavelength of 273nm.

A calibration graph was constructed for the quantitative determination of caffeine.

For determination of antioxidant activity was used the spectrophotometric Folin-Ciocalteu method

2.2 Preparation of reagents

A solution of sodium carbonate was prepared by dissolving (10.00±0.01) g of Na2CO3 in distilled water in a 100 cm3 flask and brought to the mark.

(0.0433±0.0001) g gallic acid monohydrate (0.002547059 mmol) was placed in a 100 cm3 volumetric flask, dissolved in distilled water, and brought to the mark.

To build a calibration line, a series of solutions were prepared: 1.0 cm3 of 10% Na2CO3 was mixed in 5 ml flasks with 0.03; 0.05; 0.07 or 0.015 ml of gallic acid solution were added. Using a pipette dispenser, 0.2 ml of Folin-Chocalteu reagent was added to each flask, mixed, and brought to the mark with water. The solutions are kept for 30 minutes at room temperature, and the optical density of the solutions relative to water was measured at a wavelength of 760 nm (Fig.1).

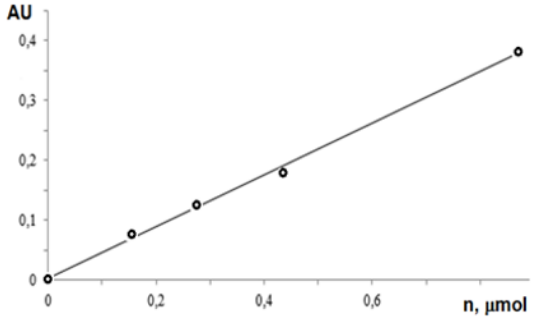

Fig.1 The calibration plot for gallic acid as a reference solute

The relationship between the amount of Gallic acid introduced (n, mmol) and the intensity (A) of the color is linear: A = 0,4303 · n + 0, 0035. (R2 =0,9968)

3 Results and discussion

Tea has a complex chemical composition; it contains more than 2000 compounds. But it is very important to establish the dependence of the quality of tea on its composition and to establish the influence of individual tea compounds on human health in order to investigate the possibility of using tea for antioxidant therapy.

First of all, it is necessary to distinguish catechins, the main catechins of tea: epigallocatechin gallate (EGCG) and epigallocatechin (EGCG). Green teas are most rich in catechins. The total content of catechins decreases significantly with the degree of fermentation. When tea is fermented to 85%, the total content of catechins can be reduced by 10 times or more, since catechins are converted to theaflavins, Accordingly, black fermented tea contains a large amount of theaflavins and products of condensation and polymerization of catechins (Fig.2.). At the same time, the caffeine is still preserved

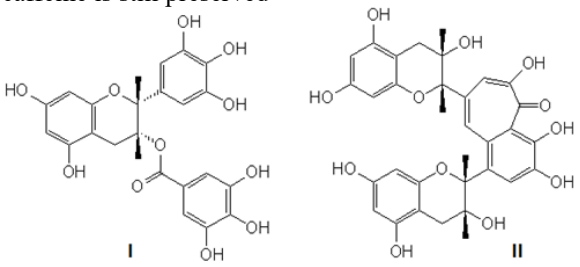

Fig.2 Structures of epigallocatechingallate (EGCG, I) and theaflavin-1 (II).

These compounds are antioxidants that largely determine the antioxidant activity of tea, and therefore being usefulness. Therefore, it is very important to choose the optimal tea varieties and methods of infusion to achieve maximum antioxidant activity.

The main catechins (epigallocatechin gallate and epicatechingallate) and caffeine were determined by reverse-phase high-performance liquid chromatography (RP-HPLC). The chromatogram of green tea extract is shown in Fig. 3., where the presence of three main components is obvious.

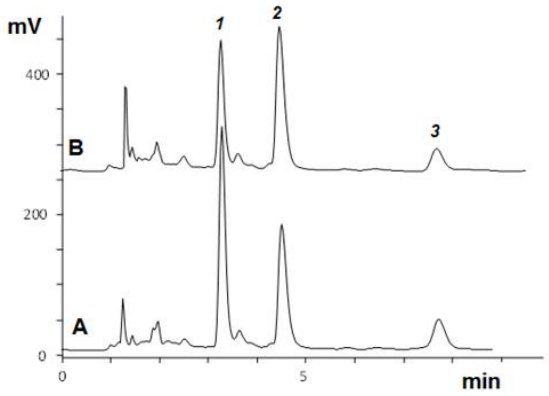

Fig 3. Green tea extract chromatogram: A-extraction by eluent, B - extraction by infusing in hot water.1-epigallocatechingallate (EGCG), 2-caffeine, 3-epicatechingallate (ECG)

Extraction was performed with two extractants: an eluent for HPLC determination and an infusion in hot water. The eluent extraction was performed to control the completeness of extraction of tea components when infused in hot water.

Catechins are substances that are unstable to oxidation, but the use of an extraction technique that involves infusing a sample of tea in hot water is necessary to assess the actual extractability of catechins when drinking tea using the traditional method of infusion.

Table 1 shows data from a 2015 beverage study on the content of catechins and caffeine in various teas.

Experimental data show that hot water can be used to extract catechins and caffeine. The highest content of catechins when brewed for 5 minutes was found for green tea "Akbar Gold "and White tea "Ice dragon". At

the same time, the highest concentration of caffeine was found in the drink made of tea "Akbar Gold".

In a series of studies performed in 2020, we compared the dependence of the extraction of caffeine in the drink depending on the time of tea brewing, Fig.4.

Table 1. Concentration of catechins and caffeine in the drink when brewed for 5 minutes, mg/g (n= 3)

№	Name and type of tea	Catechins	Caffeine
1	Green tea «Curtis»	180 ± 6	26,1 ± 0.1
2	Green tea «Real Ceylon»	178 ± 6	22,9 ± 0.1
3	Green tea «Akbar Gold»	223 ± 7	28,9 ± 0.1
4	White tea « Ice dragon »	205 ± 6	24,9 ± 0.1
5	Green tea « Java»	191 ± 6	28,1 ± 0.1
6	Green tea «Imah Moroccan mint»	95 ± 5	18,0 ± 0.1

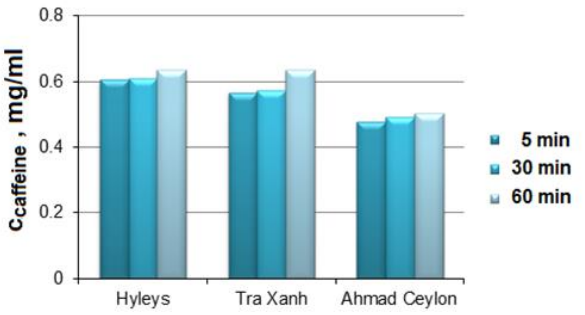

Fig. 4. The content of caffeine in different tea samples depending on the time of tea brewing.

Increasing the brewing time from 5 to 60 min. practically does not affect the degree of extraction of caffeine in tea.

In a special brand of decaffeinated tea "Milford", caffeine was not found, which cofirms the effectiveness of the supercritical CO_2 extraction used in the manufacture of the product.

Fig. 5 shows data on the content of caffeine in drinks made from different varieties and types of tea.

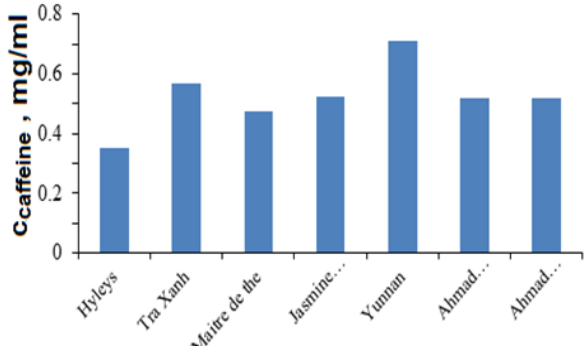

Fig.5. The caffeine content of different brands of tea when brewed for 5 minutes, (n= 3).

To determine the effect of tea brewing time on the amount of antioxidant activity of tea and the content of caffeine in it, black ground tea Grienfield Glassic Breakfast and green ground tea Richard Royal Green Tea were selected. The brewing time was selected in minutes: 1, 2, 4, 5, 17, 19.

Fig. 6-7 shows the results obtained for Greenfield Classic Breakfast black ground tea.

From experimental data, it can be seen that both the content of caffeine and even more antioxidant activity increase with an increase in the brewing time of black tea to 19 minutes. But we have found that further increase of extraction time leads to an increase of the caffeine concentration as well as to increased antioxidant activity though after 2 hours of infussion a decrease in antioxidant activity of the beverage by 12% was found. Thus we can conclude that to achieve the maximum value of the antioxidant activity of tea, it is necessary to maintain the drink during brewing for up to 20 minutes.

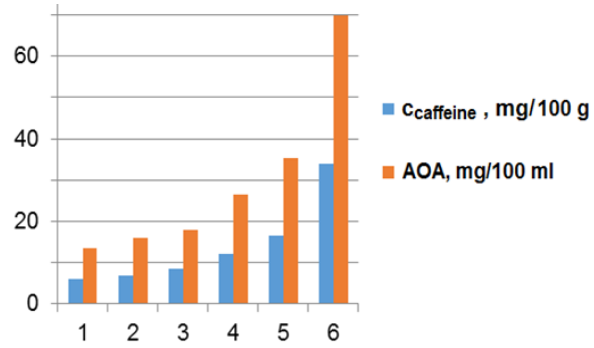

Fig.6. Influence of brewing time of Greenfield Classic Breakfast black ground tea on caffeine content and antioxidant activity brewing Time (min.): 1 - 1; 2 - 2; 3 - 4.; 4 - 5; 5 - 17; 6 - 19.

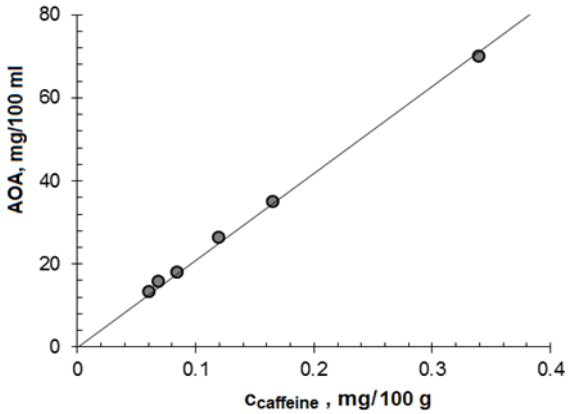

Fig.7. Correlation between caffeine content in Greenfield Classic Breakfast black ground tea on the antioxidant activity of tea

Fig. 8-9 shows the results on the effect of brewing time of Richard Royal Green Tea on the caffeine content and antioxidant activity.

The caffeine content and antioxidant activity of green tea increase with increasing brewing time only up to a certain time (5 min), and then decrease. Therefore, in order to achieve maximum antioxidant activity when brewing green tea, it is necessary to maintain the drink during brewing for up to 5 minutes

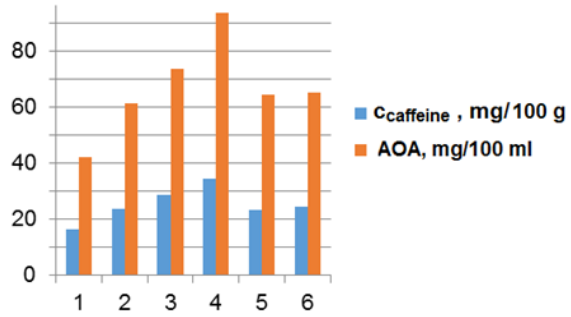

Fig.8. Influence of brewing time of Richard Royal Green Tea on the caffeine content and antioxidant activity. Brewing time (min.): 1 - 1; 2 - 2; 3 - 4.; 4 - 5; 5 - 17; 6 - 19.

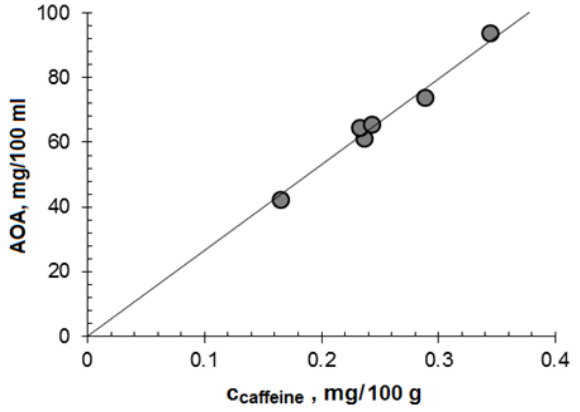

Fig.9. Influence of the growth of caffeine content in Richard Royal green tea on the antioxidant activity of tea.

Fig. 7 and 9 show that both black tea and green tea show a directly proportional relationship between an increase in antioxidant activity and an increase in their caffeine content.

To find out the influence of the degree of grinding of dried tea leaves on the amount of antioxidant activity, identical samples of leaves of different types of tea were brewed in the state of the leaf and after grinding. The antioxidant activity of 6 samples of green tea and two samples of black tea and tea drink were determined depending on the degree of grinding of vegetable raw materials, Table 2.

Finely crushed tea allows to get when brewing at the same conditions an infusion being 1.5-3.0 times higher by antioxidant activity than non-ground tea. And only for the roiboos drink, the antioxidant activity does not depend on the degree of grinding of the dried raw material. It is no accident that powdered raw materials are used for brewing tea ceremonies.

4 Conclusion

Hot water can be used to completely extract caffeine and catechins from tea.

The content of caffeine and antioxidant activity increases with increasing brewing of black tea up to 20 minutes, green tea - up to 5 minutes, then the antioxidant activity decreases. The antioxidant activity of crushed tea is 1.5-3 times greater than non-crushed tea.

Table 2. Antioxidant Activity Of Some Teas And Tea Beverages (As Gallic Acid Equvalent) (n=3).

№	Name of tea	Manufacturer	crushed	non-crushed
1	Tan Cuong green tea	Vietnam	207 ± 7	135 ± 4
2	green tea Yunnan	China	295 ± 8	125 ± 4
3	Green Jasmine tea	China	255 ± 7	99 ± 3
4	Maitre de The Napoleon	France	201 ± 7	65 ± 3
5	Ahmad Tea Bluebberry	Russia	210 ± 7	96 ± 5
6	Hyleys Green Tea	Sri Lanka	258 ± 9	127 ± 5
7	Grienfield Classic Breakfast black tea	London	155 ± 6	89 ± 4
8	Welsrhous, black tea	Nepal	136 ± 6	75 ± 3
9	Grienfield Herbal Tea Creamy Roiboos	London	67 ± 4	62 ± 4

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