Phenolic Compounds of Krasnodar Tea

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The quality of the finished tea depends on the composition of the substances that make up the raw material (2 or 3 leaves). Extractive substances are the main components that determine the taste and aroma of tea. An equally important role is played by alkaloid-containing substances – caffeine, theobromine and theophylline (Leong, et al., 2001; Valiulina, Makarova, 2018; Wright, 2002; Chowdhury, et al., 2018; Zeyi Ai, et al., 2017). From alkaloid-containing compounds during the growing season, mainly caffeine is formed and accumulated (2-3%). Tea leaves, both fresh and processed, contain pigments (chlorophyll, carotenoids, anthocyanins, flavonoids), on which the color of tea depends. The quantitative content of flavonoids is one of the indicators of the quality of tea (Wan et al., 2015; Potapovich A. I., Kostyuk, 2003; Ivanova et al., 2017; Zaprometov, 1993). Therefore, for the primary assessment of the quality of plant raw materials, quantitative determination of the content of flavonoids is often used. The metabolism of tea plants is based on the processes of accumulation and transformation of tannins (catechin complex, mainly (-)-epigallocatechingallate), the content of which reaches 30% or more. Tannins, namely teotanin, are a complex mixture of the Gallic esters of catechins and the catechins themselves. Oxidized teotanin gives the tea infusion a yellow-brown or reddish color, astringency, and taste.

On the basis of the Subtropical Scientific Centre for a long period of work on the culture of tea was created varieties characterized by high yield and product quality (cv. Sochi, Karatum, Matsestinsky, Vano, etc.), and in the studies, only three biochemical components of tea raw materials were considered: tannins, the sum of water-soluble extractives, and caffeine. A complete study of the formation and change of the antioxidant complex of both raw materials and finished (black and green) tea has not been conducted. But work in this direction continues. The presence at the Centre of highly productive varieties, as well as promising clones and hybrids, are a reliable basis for the creation of modern tea plantations. This article is devoted to the study of the features of the composition and changes of the phenolic compounds of new forms of tea, breeding by scientists of Centre.

Materials and Methods

The objects of research are cultivars and breeding forms of tea growing in the Krasnodar region: cv. Colchida (control), cv. Sochi; forms – No. 582, 3823, 855, 2264.

Quantity analysis of flavonoids:

Each sample was measured three times for both analyses of TFs and TRs. The 95% ethyl alcohol was used as extract to extract flavonoids from raw materials (Quality assurance checklist for small laboratories, 2009). In order to determine the TFs and the TRs, the method of spectrophotometry was used applying the analyzer of PE-5400VI (Russia) in the wavelength range of 665 nm for TFs and 825 nm for TRs in contrast to water used as blind sample.

Flavonoid compounds with P-vitamin activity was determined by titration in accordance with the method of vitamin analysis (Voskresenskaya et al., 2006). The quantitative determination of Ruthin is based on its ability to be oxidized by permanganate. As an indicator, indigocarmine is used, which reacts with permanganate after all Ruthin is oxidized.

The determination of caffeine was performed by reverse-phase high-performance liquid chromatography (HPLC). The followingcatechins were used as standards: catechin, epicatechin,gallocatechin, gallocatechin gallate, epigallocatechin,epicatechin gallate, epigallocatechin gallate, gallic acid,caffeine. All reagents were chemically pure or analytical grade and contained more than 98% of the main substance. Extraction method was based on well-known method (ISO 14502-2:2005).The conditions of chromatographic analysis are: columntemperature 40 °C, flow 200 µL.min-1, detection 210, 220, 230, 240, 250, 260, 280, 300 nm. Elution was made ingradient mode from 0% to 40% B for 2800 µL. Allmeasurements were performed in triplicate at the basic Institute of Chemical Biology andFundamental Medicine, Siberian Branch of the RussianAcademy of Sciences, Novosibirsk, Russia.

Statistical processing of the experimental data was carried out using the ANOVA package in STATGRAPHICS Centurion XV (version 15.1.02, Stat Point Technologies) and MS Excel 2007. Statistical analysis included univariate analysis of variance (method of comparing

averages using variance analysis, t-test) and variance analysis (ANOVA). The significance of difference between the means of the least significant difference (LSD) results with $p < 0.05$ was considered statistically significant. All experiments were performed in triplicate and the values were expressed as mean ±SD. The differences between the samples were assessed using unpaired t-test.

Results and Discussion

Theaflavins (TFs) and thearubigins (TRs) are formed in the fermentation process of tea. We were found that there is a direct relationship between the quality of raw materials and the content of flavonoids in tea: green tea has fewer theaflavins than raw materials and black tea. Our researches showed, that tea of perspective forms 582 and 2264 in terms of the content of theaflavins have the highest values, while the highest content of thearubigins was observed in tea obtained from raw materials of the cv. Colchida and form 2264 (0.12 – 0.16 mg.g-1 of TFs and 1.13 – 2.39 mg.g-1 of TRs). Any blend of tea should have a ratio of theaflavins and thearubigins not lower than 1:16, and in super tea 1:10, according to international rules (Kang et al., 2017; Li et al., 2018; Palanivel et al., 2020). According to this indicator, all Krasnodar tea and tea from experimental plants complies with international requirements.

Ruthin(vitamin P) is an important component of the antioxidant system of tea plants, it not only takes part in the main redox reactions, but also increases the absorption of ascorbic acid(Gulati, Ravindranath, 1996; Vinson, 2000; Sinija, Mishra, 2008; Sharangi, 2009; Skotnicka, 2011; Ferreira et al., 2020).Studies have shown that depending on the season of tea leaf collection, the content of Ruthin in green tea ranges from 36 to 41 mg.100g-1, and in black – withinthe limits 17 – 20 mg. 100g-1, which is 2 times lower than in green non-fermented tea. Genotypic features have influence on the content of vitamin P. Thus, a lower content of Ruthin is characterized by the cv. Sochi (on average 10 mg. 100g-1 in black tea and 34 mg. 100g-1 in green), a higher content has form 582 (about 23 mg. 100g-1 in black tea and 46 mg. 100g-1 in green).

A quantitative analysis of polyphenols and caffeine (epicatechin (EC), epigallocatechin (EGC), gallocatechin (GC), catechin (C), epicatechin gallate (EGCG), epigallocatechin gallate (EGCG), gallic acid (GA), caffeine (Caf)) in the tea samples under study was performed at the maximum absorption wavelength of analytes (280 nm) and their content per 1 g of dry tea was calculated (Kartsova et al., 2019).

Determination of the qualitative composition of the catechin group in green tea, showed that the largest amount of epigallocatechin gallate (5.64 – 7.90%), which is characterized by the highest antioxidant activity of the main catechins (epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate), the prevalence of this group in selected forms of teas reflects their high value. A comparison of tea in terms of the quantitative content of the catechol complex showed that tea of new forms of Centre`s breeding is characterized by a high level of accumulation of various groups of catechins (Platonova et al., 2019). The cv. Colchida, being a large leaf tea, is characterized by a higher content of EGKG (7.03 ±0.42 %), which is also confirmed by literature data. The low content of catechin group substances is characteristic of forms 3823, 855 and 2264.

Tea contains one of the varieties of caffeine – Thein. The peculiarity of Thein is a milder effect on the human body. Since caffeine is present in combination with tea tannin, when brewed, it is not extracted completely and the drink acts on the body more gently. At the same time, unlike caffeine, Thein does not accumulate in the body. However, all selected forms contain a lower caffeine value (1.57 – 2.62 %). Despite the fact that the chemical connection between caffeine tea and tannins neutralizes its effect on the human body (compared to pure caffeine coffee), the lower content of theine in new forms of tea makes them more attractive against the background of a rich caffeine cv. Colchida (3.10%). The closest to the control variety is green tea, produced from raw materials of form 582 (2.62%). The highest synthesis of caffeine in all experimental plants was observed in July (from 24.633 mg/g to 28.614 mg/g), and the excess of its amount in comparison with the rest of the growing season is 1.6 – 2.3 times, which is significant. When processing tea raw materials (3-leaf sprouts into ready-made tea, the destruction of caffeine occurs and its amount decreases by 1.2-1.4 times.

There is a difference in the content of catechin groups in tea collected in August and in July, which may indicate a possible correlation between the accumulation of catechins and the time of tea collection (Kartsova et al., 2019).

Thus, we conducted a comparative biochemical analysis of the content of antioxidant substances in tea raw materials (3-leaf sprouts) and finished (black and green) tea. There was a significant change in the content of flavonoids, caffeine, Ruthin and whole phenolic compounds in the processing of tea raw materials. The differences are due to the lack of fermentation process in the production of unfermented (green) tea, which is the most important technological method in the production of black tea. This allows green tea to retain almost all water-soluble vitamins that inhibit lipid peroxidation in cell membranes. In addition, there is a significant variability in the content of phenolic compounds, due to the genotypic features of tea plants.

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