potravinárstvo®

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 13, 2019, no. 1, p. 32-37
doi: https://doi.org/10.5219/990
Received: 12 December 2018. Accepted: 9 January 2019.
Available online: 31 January 2019 at www.potravinarstvo.com

ISSN 1337-0960 (online)

THE COMPOSITION AND CONTENT OF PHENOLIC COMPOUNDS IN TEA, GROWN IN HUMID SUBTROPICS OF RUSSIA

Nataliia Platonova, Anton Astanin, Sergey Sedykh, Lidiia Samarina, Oksana Belous

ABSTRACT

The article presents the results of studies on the dynamics, qualitative and quantitative composition of phenolic compounds of tea raw materials and tea of new forms of selection of the Institute. The regularity in their synthesis by months is determined, which affects the quality indicators of the end product and necessitates blending to the repaired tea. The accumulation of tannins in tea raw materials depends on hydrothermal conditions, in particular, the amount of precipitation. The content of tannin increases from May to June, then there is some decline in their content, due to hydrothermal stress, slowing the synthesis of tannins in the tea leaf. The content of the water-soluble fraction gradually increases from May to July, and then there is a slight decline. It is shown that the accumulation of phenolic compounds in tea raw materials varies during the collection season. The contents of theaflavins increased from the beginning of the collecting sheet to its completion. The content of thearubigins showed peaks: the lowest rate in June, the highest in August. It was revealed that a sharp drop in the synthesis of thearubigins in June is associated with the onset of the summer dormancy of growth and synthetic processes. The synthesis of both indicators depends on meteorological conditions. The comparative analysis of the samples of tea raw materials collected from experimental plants is carried out. It is shown that the content of tannin and extractive substances in the raw materials of the studied varieties and mutant forms is high. In terms of the ratio of theaflavins and thearubigins, tea made from experienced raw materials meets international requirements. Determination of the qualitative composition of the catechin group of green tea, produced from raw materials of new forms, showed a high level of accumulation of the main groups of catechins.

Keywords: *Camellia sinensis*; cultivar; black tea; green tea; hydrothermal condition; tannin; extractive compounds; flavonoid; catechin

INTRODUCTION

Tea plant (*Camellia sinensis* (L.) O. Kuntze) is the most ancient crop. As a cultivated plant, tea has been cultivated in China since ancient times, since the XVIII century – in India and Sri Lanka, since the XVII century – in Russia, and since the XIX century – on vast areas in various parts of the globe. But now the only European tea producer is Russia, and the only place in Russia where tea is grown on an industrial scale is the humid subtropics of the Krasnodar region **(Ryndin et al., 2017; Belous and Platonova, 2018)**.

The mass distribution of tea is due not only to the fact that it is a pleasant, thirst-quenching drink, but to a large extent, and its effect on the human body. Active substances of tea are polyphenols, flavonoids, aromatic substances, vitamins, plant pigments, amino acids, mineral salts, etc. **(Willson, 1975; Gramza et al., 2005; Khan and Mukhtar, 2007; Belous, 2013)**. The tea contains more than 600 chemicals involved not only in the creation of specific properties of tea-aroma, taste and color of the infusion, but also the healing properties of the product **(Vorontsov, 1946; Mgaloblishvili and Tsutsunava, 1979; Salah et al., 1995; Khvedelidze and Gvinianidze, 2004)**. In the last decade, the interest in tea has increased, due to the content of polyphenolic compounds with antioxidant effect **(Salah et al., 1995; Wright, 2005; Platonova, Belous and Ostadalova, 2017; Platonova and Belous, 2018)**.

Industrial plantations of Krasnodar region are a mixture of different morphological groups, as laid seed populations of Georgian and Chinese origin. In this regard, plants differ in yield, growth ability, and most importantly, biochemical parameters that determine the quality of the finished product. Improving the varietal composition of tea plantations is one of the urgent tasks of tea growing. On the basis of the all-Russian research Institute of floriculture and subtropical crops for a long period of work on the culture of tea created varieties characterized by high yield and product quality (cv. Sochi, Karatum, Matsestinsky, Vano, etc.) **(Prokopenko and Tuov, 1994)**. But work in this direction continues. The presence at the Institute 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 catechin complex of new forms of tea, selected by scientists of the Institute. We have determined the composition of the catechin complex in green tea, made from raw materials grown on the plantation of the Institute.

Scientific hypothesis

All over the world works on studying of a green tea leaf and transformations which proceed in it at technological processing are conducted, biochemical researches of raw materials and a finished product are carried out. The study of the content, accumulation and transformation of substances during growth, development of green leaf, it's processing, is necessary to improve the quality of raw materials and finished tea. The main purposes in tea biochemistry are the transformation and accumulation of phenolic compounds, as they provide the most valuable properties of tea. The compositions of phenolic compounds of tea are change and depending from season and the technology of tea processing.

MATERIAL AND METHODOLOGY

Objects of study were samples of green tea made from 2 – 3 leaf sprouts following promising forms: form No. 3823, No. 582, No. 855, No. 2264, grown for an experienced collector-uterine site, founded in 1984 – 1985 in the village of Uch-Dere (Sochi, Lazarevsky region). Control – cultivar Colchida.

Colchida is a clone of large-leaved Chinese tea, high-crop productivity, in 1995 entered in the State register of selection achievements. Form No. 3823 – source population Kimini, high gustatory qualities and yield. Form No 582 – the initial population of Colchida, the variety productivity is high, has a specific flavor infusion. Form No. 855 – the original Georgian population of the No. 8, the average crop productivity, specific aroma with a light rose fragrance. Form No. 2264 – the original Georgian population of the No. 15, crop productivity and quality indicators are above average **(Gvasaliya, 2018; Prokopenko and Tuov, 1994)**.

Green tea was produced in the laboratory of biotechnology, physiology and biochemistry of plants (BPhBP) of the Russian research Institute of floriculture and subtropical crops.

The study was performed on the chromatograph MiLiChrom A-02 (Institute of Chromatography "EcoNova", Novosibirsk, Russia), 2 × 75 mm column with a sorbent ProntoSIL 120-5-C18 AQ (Bischoff Analysentechnik und Geräte, Germany). Chromatograms were processed using a computer program AlphaChrom (Institute of Chromatography "EcoNova", Novosibirsk, Russia). Aqueous 0.2 M $LiClO4$ in 0.005 M $HClO4$ was used as eluent A, and acetonitrile (NPK Cryochrome, St. Petersburg, Russia) was used as eluent B. The following catechins 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)** with some modifications. The dried tea leaf was homogenized in a mortar, sieved through a 0.5 mm sieve; a portion of 20 mg was placed in a 1.5 mL tube and extracted with 1 mL of ethanol 70% for 30 minutes at 70 °C. After extraction, the sample was filtered through a 0.45 µm filter and used for HPLC analysis.

The conditions of chromatographic analysis are: column temperature 40 °C, flow 200 $\mu L.min^{-1}$, detection 210, 220, 230, 240, 250, 260, 280, 300 nm. Elution was made in gradient mode from 0% to 40% B for 2800 µL. All measurements were performed in triplicate.

Flavonoids were determined on a PE – 5400 wi spectrophotometer at a wavelength of 665 nm (theaflavins) and 825.5 nm (thearubigins) **(AOAC International, 2009)**.

The tannin content was determined by the method of Levental with a conversion factor of 5.82 according to **Dzhemuhadze (1946)**, extractive substances – by the gravimetric method according to **Vorontsov (1946)**.

Statisic analysis

Statistical processing of the experimental data was carried out using the ANOVA package in STATGRAPHICS Centurion XV (version 15.1.02, StatPoint 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. Correlation analysis with calculation of pair correlation coefficient, for establish the dependence of parameters on abiotic factors was used.

RESULTS AND DISCUSSION

One of the main places among the substances that make up the tea leaves is a complex of tannins. All the basic properties of the finished product – its color, taste and aroma – are to varying degrees related to their transformations in the tea leaf. Tannins are the most mobile and active compounds, so they are primarily subject to certain changes under different growing conditions or processing of tea.

Studying the content of tannins in tea raw materials in the dynamics, we identified the main patterns in their synthesis by months, which significantly affects the quality indicators of the finished product and necessitates the blending of finished tea (technological method of mixing the semi-finished product to obtain a quality brand). We noted that the total content of tannins in the three-leaf flush varies during the tea leaf collection season (Figure 1).

As can be seen from Figure 1, the quantitative content of tannin in Russia's humid subtropics (unlike other tea-producing countries) increases from May to June, then there is some decline in their content, which is connected, in our opinion, with temperature and arid stress slowing down the synthesis of tannins in the tea leaf.

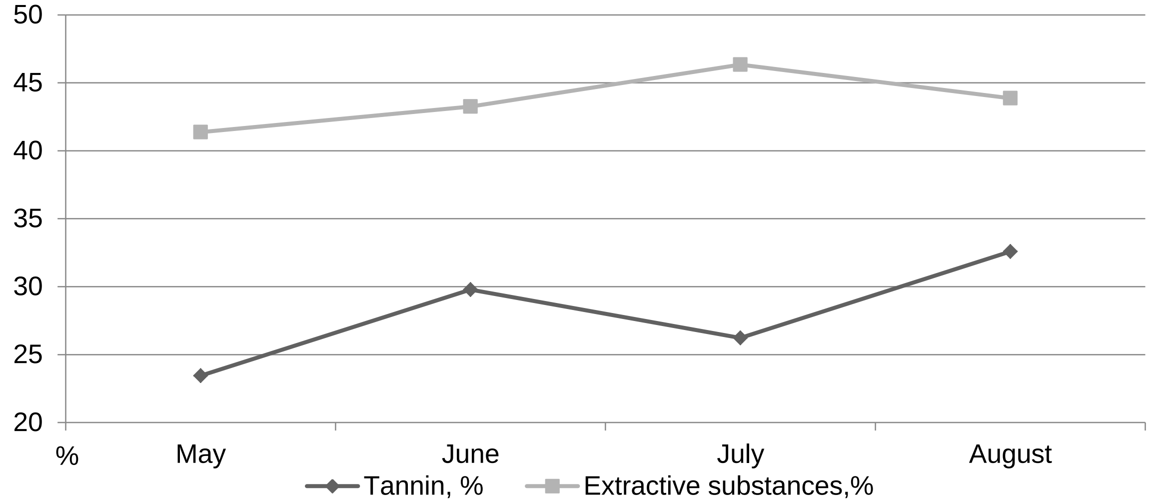

Figure 1 Seasonal changes in the content of tannins and extractive substances in tea raw materials, average over 11 years.

Table 1 Pair correlation coefficients between quality indicators of tea raw materials and hydrothermal factors, average over 11 years.

Parameter	Precipitation, mm	T, °C
Tannin, %	-0.88	-0.45
Extractive substances, %	-0.74	-0.35
Caffeine, %	-0.86	-0.54

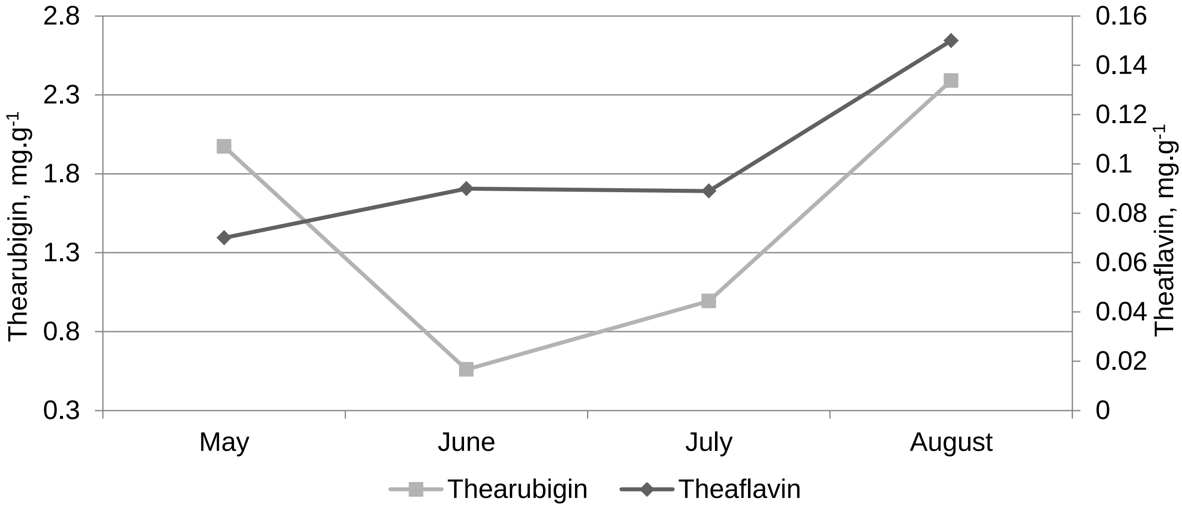

Figure 2 The content of flavonoids in the samples of finished tea made from plants of the variety Colhida, average over 2 years.

Table 2 Pair correlation coefficients between hydrothermal factors and flavonoid content ($p \leq 0.05$).

Parameters	**Theaflavins, mg.g^{-1}**	**Thearubigins, mg.g^{-1}**
Thearubigins, mg.g^{-1}	0.928	-
Temperature, °C	0.860	0.184
Precipitation, mm	-0.776	-0.562

As the hydrothermal conditions reach their optimum, the tannin content is rapidly increasing, reaching an average of 30.29 – 32.58% in August.

Other dynamics were noted in the accumulation of extractive substances in the tea leaf (Figure 1). From May to July there is a gradual increase in the content of the water-soluble fraction, followed by a slight gradual decline.

Statistical processing showed that the accumulation of tannins in tea raw materials directly depends on hydrothermal conditions, and to a greater extent, not on temperature conditions of vegetation, but on the amount of precipitation (Table 1). Moreover, the relationship between quality indicators and climatic factors is opposite, as the strength of the factor increases, the content of tannins decreases significantly ($p \leq 0.05$).

Also, we were able to trace the dynamics of the formation of thearubigins and theaflavins in finished tea by months and their dependence on hydrothermal factors. It was established that the content of theaflavins increased from the beginning of the collection of the leaf (in May 0.07 $mg.g^{-1}$) to its completion (in August 0.15 $mg.g^{-1}$). Peaks were observed in the content of thearubigins: the lowest value in June was 0.56 $mg.g^{-1}$, the highest – in August, 2.39 $mg.g^{-1}$ (Figure 2).

The sharp decline in the synthesis of thearubigins in June may be due to the onset of a period of summer dormancy of growth and synthetic processes, especially since during the same period the accumulation of theaflavins reached a plateau - from June to July there were no changes in their content (Figure 2).

To establish the influence of hydrothermal factors on the accumulation of flavonoids, the relationship between these indicators was calculated (Table 2).

As can be seen from the given data presented in Table 2, the synthesis of both indicators is quite closely related, which is a well-known fact: it is precisely during oxidation that theaflavins quickly turn into thearubigins. At the same time, the accumulation of these flavonoids by the tea plant directly depends on meteorological conditions: as the air temperature rises and the amount of precipitation decreases, the content of both flavonoid groups increases. This may be due to a high concentration of cell sap and, as a consequence, activation of the antioxidant system of plants. Dispersion analysis indicates that it is theaflavins that closely correlate with hydrothermal factors, while thearubigins, being secondary metabolites relative to theaflavins, react poorly to the temperature factor. Rainfall are affecting the functional state of the plant itself **(Belous, 2008)**, it was provided an optimal water balance of cells and the synthesis of flavonoids. Flavonoids in the manufacture of tea, during oxidative processes, can indirectly affect the synthesis of thearubigins.

In addition to identifying dependencies and establishing the dynamics in the accumulation of tannins, we conducted a comparative analysis of samples of tea raw materials collected from experimental plants (Table 3). As can be seen from Table 3, the content of tannin and extractive substances in the raw materials of the studied varieties and mutant forms are high.

Tea produced from plants of mutant forms No. 582 and No. 2264 in terms of the content of theaflavins showed the highest values, while the highest content of thearubigins was observed in tea obtained from raw materials of the Colkhida variety and form No. 2264 (Table 3). Since theaflavins are unstable compounds and easily oxidize to thearubigins during oxidation, at present there is no single standard for their content in the finished product. But, according to international rules, 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 this indicator, all the tea produced in the laboratory of the institute`s laboratory from raw materials harvested from experimental plants complies with international requirements.

Determination of the qualitative composition of the catechin group in green tea, using the example of the most promising form No. 582 (Figure 3), showed that the largest amount of epigallocatechin gallate, 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 selection of the institute is characterized by a high level of accumulation of various groups of catechins (Table 4).

The variety Colchida, being a large leaf tea, is characterized by a higher content of EGKG, which is also confirmed by literature data. **(Haslam, 1989; Gzhidzhiechvili et al., 1984)**. At the same time, all selected forms contain a lower caffeine value. 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 variety Colchida.

Table 3 Phenolic compounds of black tea and tea leaves (raw materials), average over 5 years.

Varieties, variety forms	Tannin, %	Extractive substances, %	Theaflavins $mg.g^{-1}$*	Thearubigins, $mg.g^{-1}$*
Camellia sinensis cv. Colchida	28.7 ±1.7	42.40 ±1.0	0.15 ±0.02	2.39 ±0.44
Camellia sinensis mf. № 3823	28.3 ±1.6	43.41 ±1.3	0.11 ±0.01	1.13 ±0.16
Camellia sinensis mf. № 582	30.1 ±2.1	43.92 ±1.9	0.16 ±0.01	2.04 ±0.49
Camellia sinensis mf. № 855	29.8 ±1.7	43.90 ±1.3	0.12 ±0.01	1.24 ±0.15
Camellia sinensis mf. № 2264	28.2 ±1.3	42.45 ±1.4	0.16 ±0.01	2.26 ±0.26
LSD ($p \leq 0.05$)	1.01	0.89	0.03	0.09

Note: * data over two years.

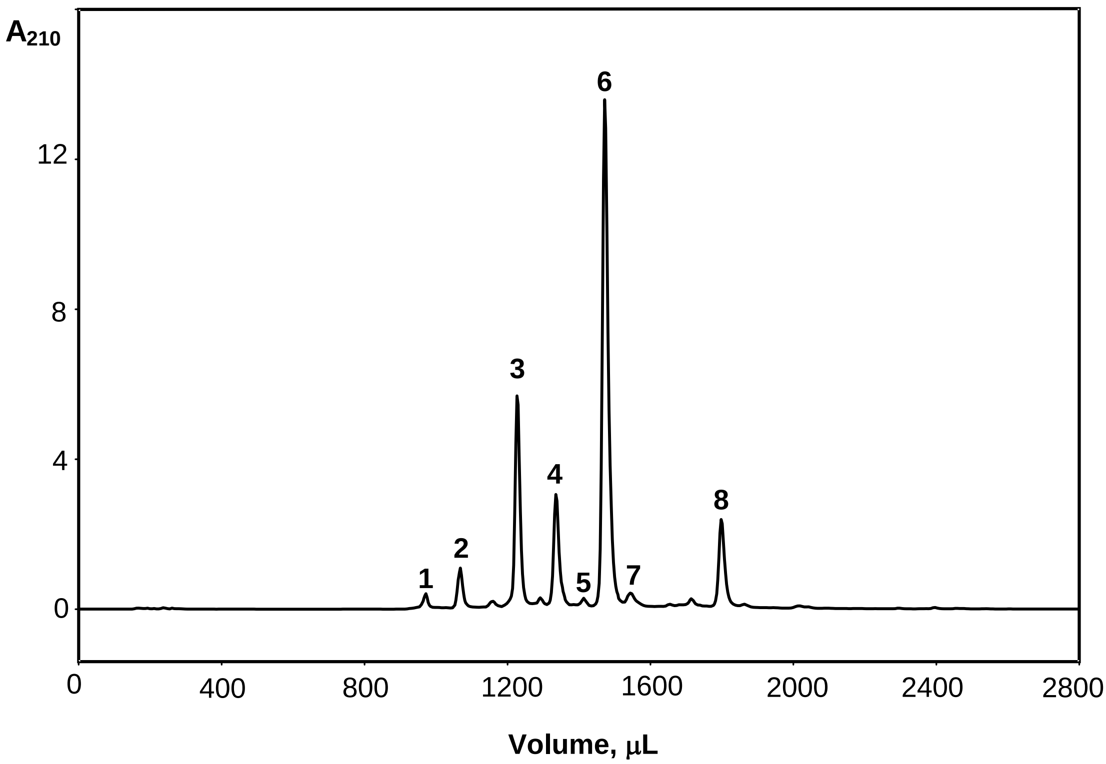

Figure 3 Chromatogram of green tea extract from sample No. 582. Note: the peak numbers correspond to: (1) gallic acid, (2) hallocatechin, (3) epigallocatechin, (4) caffeine, (5) epicatechin, (6) epigallocatechin gallate, (7) gallocatechin gallate and (8) epicatechin gallate.

Table 4 Pair correlation coefficients between quality indicators of tea raw materials and hydrothermal factors, average over 11 years.

Verity	Caffeine, %	GAK, %	EGKG, %	EKG, %	GKG, %	GK, %	EK, %	EGK, %
Camellia sinensis cv. Colchida	3.10 ±0.12	0.13 ±0.007	7.03 ±0.42	1.23 ±0.04	0.40 ±0.04	0.37 ±0.07	0.033 ±0.004	1.95 ±0.10
Camellia sinensis mf. № 3823	1.96 ±0.10	0.13 ±0.010	5.64 ±0.40	0.76 ±0.04	0.23 ±0.04	0.40 ±0.03	0.024 ±0.006	2.80 ±0.14
Camellia sinensis mf. № 582	2.62 ±0.10	0.17 ±0.009	7.90 ±0.50	1.24 ±0.07	0.28 ±0.02	0.50 ±0.07	0.026 ±0.006	3.33 ±0.15
Camellia sinensis mf. № 855	2.60 ±0.08	0.15 ±0.008	5.95 ±0.33	0.99 ±0.05	0.12 ±0.01	0.38 ±0.05	0.040 ±0.005	2.91 ±0.18
Camellia sinensis mf. № 2264	1.57 ±0.08	0.08 ±0.010	5.98 ±0.37	0.93 ±0.05	0.39 ±0.05	0.45 ±0.01	0.031 ±0.005	2.97 ±0.19

The closest to the control variety is green tea, produced from raw materials of mold No. 582. The low content of catechin group substances is characteristic of forms No. 3823, 855 and 2264.

CONCLUSION

Thus, we have studied the dynamics, composition and content of phenolic compounds in tea raw materials and tea of new forms of plant selection. It is shown that the total content of tannins in the three-leaf flush varies during the harvest season and the accumulation of phenolic compounds in tea raw materials directly depends on the hydrothermal conditions, in particular, on the amount of precipitation during the growing season. The dynamics of the formation of thearubigins and theaflavins in finished tea by months is traced; it has been established that the accumulation of flavonoids directly depends on meteorological conditions: as the air temperature rises and the amount of precipitation decreases, their content increases. A comparative analysis of black tea samples was carried out, which showed that, based on the ratio of theaflavins and thearubigins, tea collected from experimental plants complies with international requirements. Determination of the qualitative composition of the catechin group in green tea showed that the greatest amount falls on epigallocatechin gallate and tea of new forms of selection of the institute characterized by a high level of accumulation of identified groups of catechins.

REFERENCES

AOAC International, 2009. *Quality assuarance check list for small laboratories*, vol. 16, no. 11, 13 p.

Belous, O. G. 2008. Diagnostics of tea plant resistance to stress factors. *Vestnik of the Russian agricultural science*, vol. 6, no. 6, p.41-43. https://doi.org/10.30850/vrsn/2018/2/27-30

Belous, O. G. 2013. Influence of microelements on biochemical parameters of tea. *Potravinarstvo*, vol. 7, no. 1, p. 149-152.

Belous, O., Platonova, N. 2018. Physiological foundations of sustainability Camellia sinensis (L.) O. Kuntze and Corylus pontica C. Koch. in the conditions of humid subtropics of Russia. *American Journal of Plant Sciences. Special Issue on Plants in Extreme Environment*, vol. 9, no. 9, p. 1771-1780. https://doi.org/10.4236/ajps.2018.99129

Dzhemuhadze, M. K. 1946. Biochemical control of withering of tea leaves. *Biochemistry of tea production*, vol. 5, no. 1, p. 125-142.

Gramza, A., Pawlak-Lemanska, K., Korczak, J., Wasowicz, E., Rudzinska, M. 2005. Tea extracts as free radical scavengers. *Polish Journal Environment Studies*, vol. 14, p. 861-867.

Gvasaliya, M. V. 2018. Economic estimation of new varieties and mutant forms of tea (*Camellia sinensis* (L.) Kuntze) in Russian subtropics. *Pomiculture and small fruits culture in Russia*, vol. 53, no. 1, p. 104-111.

Gzhidzhiechvili, G. A., Guguchvili, T. A., Hurdidze, G. A., Tsanava, V. P. 1984. Phenolic compounds of tea materials of the Kolkhida variety. *Subtropical cultures*, vol. 1, no. 1, p. 39-43.

Haslam, E. 1989. Plant polyphenols: vegetable tannins revisited. In Phillipson, J. D. et al. *Chemistry and Pharmacology of Natural Products*. Cambridge : Cambridge University Press, 230 p. ISBN: 0-521-32189-1.

ISO 14502-2: 2005. *Determination of substances characteristic of green and black tea - Requirements.*

Khan, N., Mukhtar, H. 2007. Tea polyphenols for health promotion. *Life Science*, vol. 81, no. 7, p. 519-533. https://doi.org/10.1016/j.lfs.2007.06.011

Khvedelidze, V. G., Gvinianidze, T. N. 2004. New aspects of biochemistry and pharmacology of tea. *Beer and beverages*, vol. 6, no. 5, p. 56-57.

Mgaloblishvili, E. K., Tsutsunava, A. Y.1979. *Tea and medicine*. Batumi : Sabchota Adjara, 35 p.

Platonova N., Belous, O. 2018. The content of flavonoids, depending on the growing conditions and varieties of tea plants. In Material symposium: Phenolic compounds: fundamental and applied aspects. Moscow, Russia : IFR RAS, p. 319-326. ISBN 978-5-6040654-5-7.

Platonova, N., Belous, O., Ostadalova, M. 2017. Comparative analysis of biochemical components of tea. *Subtropical and Ornamental Gardening*, vol. 61, no. 1, p. 180-189.

Prokopenko, I. A., Tuov, M. T. 1994. New perspective clones of tea in the subtropics of Russia. *Subtropical and Ornamental Gardening*, vol. 38, no. 1, p. 153-160.

Ryndin, A., Belous, O., Abilfazova, Y., Prytula, Z. 2017. The regulation of the functional state of subtropical crops with micronutrients. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 175-182. https://doi.org/10.5219/669

Salah, N., Miller, N., Paganga, G., Tijburg, L. 1995. Polyphenolic Flavanols as Scavengers of Aqueous Phase Radicals and as Chain-Breaking Antioxidants. *Archives of Biochemistry and Biophysics*, no 322, p. 339-346. https://doi.org/10.1006/abbi.1995.1473

Vorontsov, V. E. 1946. *Tea biochemistry*. Moscow, Russia : Pishepromizdat, 279 p.

Willson, K. C. 1975. Studies on the mineral nutrition on tea. *Plant and Soil*, vol. 42, no 3, p. 501-516. https://doi.org/10.1007/BF00009939

Wright, L. P. 2005. Biochemical analyses for identification of quality in black tea (*Camellia sinensis*) Pretoria, South Africa : dissertation theses, University of Pretoria. 216 p.

Contact address:

Nataliia Platonova, Russian Institute of Floriculture and Subtropical Crops, Plants Biotechnology, Biochemistry and Physiology Laboratory, Yana Fabritsiusa st., 2/28, Sochi, Russia, 354002, Tel.: +7(918)3057387, E-mail: natali1875@bk.ru

Anton Astanin, Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia, Tel.: +7(383)3635116, E-mail: astanin.anton@gmail.com

Sergey Sedykh, Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia, Tel.: +7(383)3635127, E-mail: sedyh@niboch.nsc.ru

Lidiia Samarina, Cand. Biol. Sci., Russian Institute of Floriculture and Subtropical Crops, Plants Biotechnology, Biochemistry and Physiology Laboratory, Yana Fabritsiusa st., 2/28, Sochi, Russia, 354002, Tel.: +7(966)7709038, E-mail: q11111w2006@ya.ru

*Oksana Belous, Dr. Biol. Sci., professor, Russian Institute of Floriculture and Subtropical Crops, Plants Biotechnology, Biochemistry and Physiology Laboratory, Yana Fabritsiusa st., 2/28, Sochi, Russia, 354002, Tel.: +7(918)1099115, E-mail: oksana191962@mail.ru

Corresponding author: *