Questions of Authentication and Standardization of White Varieties of Ceylon Tea Imported to Russia

Alexandr Simakov
Saratov State Agrarian University named after N.I. Vavilov
Saratov, Russia
simakovaiv@yandex.ru

Elena Artemova
Oryol State University named after I.S. Turgeneva
Orel, Russia

Inna Simakova
Saratov State Agrarian University named after N.I. Vavilov
Saratov, Russia
simakovaiv@yandex.ru

Natalia Durnova
Saratov State Medical University named after V.I. Razumovsky
Saratov, Russia

Elena Didusenko
Saratov State Agrarian University named after N.I. Vavilov
Saratov, Russia
cool.lenaelena2013@yandex.ru

Nina Myachikova
Belgorod State National Research University
Belgorod, Russia
myachikova@bsu.edu.ru

***Abstract*—The article raises questions concerning the reasons for the falsification of white tea varieties in Russia, the possibility of determining its authenticity by instrumental and sensory methods. The technological process and parameters of brewing leaves of white tea varieties, for example, silver tea, are considered. The criteria for the organoleptic evaluation of the silver tea infusion are developed with repeated brewing by "spillage" method.**

***Keywords*—Silver tea, falsification, identification, anatomical and morphological characteristics of the leaf tea, spectral profile, organoleptic evaluation**

I. INTRODUCTION

Tea is the most widely consumed drink worldwide, and it is a "national drink" in Russia. Every year, the average Russian consumes more than 1.2 kg of tea per year, according to the average consumption of tea, Russia ranks the 5th in the world. Black tea is the most popular in Russia, in recent years consumption of green tea and tea bags has increased noticeably. In the context of tough competition, large and small players in the tea market are trying to increase their share at the expense of expensive varieties of Chinese and Ceylon tea. The marketing strategy for the products of this segment, as well as for the segment of green tea, is mainly based on the trend of a healthy lifestyle. In accordance with this, white sorts of tea began to occupy a special place in the choice of the Russian consumer [1, 2].

Studies had shown that awareness of the benefits of tea had recently increased. Most of the beneficial effects of tea are associated with the major polyphenol components of tea and strong antioxidant potential. Tea has medicinal action in cancer, arthritis, cardiovascular disease and diabetes. Tea is rich in polyphenol compounds known as tea flavonoids. Having this group of compounds in its structures, the tea has high antioxidant activity and radical removal properties. The main catechins present in tea are epigal-locatechin, catechin, epigallocatechin gallate, epicatechin and epicatechin gallate [3-5].

White tea varieties began to take a special place in the choice of the Russian consumer. Comparing the white, green and black main tea types derived from the same tea plant (Camellia sinensis), white tea (silver needle) has been proven to exhibit the highest antioxidant activity, followed by green and less frequently black tea. The antioxidant ability (silver needle) can be attributed to various parts of the plant and the treatment it undergoes, followed by the production of green tea and black tea from different parts of the plant (leaves) and a change in the degree of treatment [6-8].

The high polyphenol content of white tea gives it antiseptic and antioxidant properties that can prevent free radicals, inhibit oxidative stress and inflammation associated with various diseases such as obesity, diabetes, and other degenerative diseases. Oral administration of white tea ethanol extract has proven to be an alternative in the treatment of diabetes mellitus, as flavonoids, especially catechin compounds, play an important role in reducing fasting blood glucose levels [6, 9-11].

According to Gonbad, R.A. et al., white tea can significantly regulate the function of tumor suppressor genes and block the pathway of colorectal cancer to metastasis [12].

Study of the inhibitory effect of Iranian white tea (Camellia sinensis L.) on the proliferation of colon cancer cell line, HCT-116 [13-15].

Russian scientists, studying teas from different countries, have found that the value of white Chinese tea in terms of the content of substances capable of reducing oxidative stress in the human body is obvious and this makes it a leader among teas represented on the wide consumer market. They concluded that the promising types of tea for the prevention of diseases resulting from oxidative stress are white Chinese tea and green Krasnodar tea. At the same time, it was noted that Russian green tea grown and produced in Krasnodar region has slightly lower values of active substances content, but much more affordable than white Chinese tea [2, 3, 8].

However, it should be noted that there is no normative documentation for this type of tea in the Russian Federation, conformity declarations are prepared on the basis of the Technical Regulations of the Customs Union TP TC 021/2011 “On food safety” or interstate standard GOST 33481-2015 Tea partially fermented.

Technical conditions.

However, there is a huge difference between white and other varieties of tea, due not only to the price, but also to the technology of production, growing, harvesting, taste characteristics, health benefits and other qualitative characteristics [3]. The absence of a standard that is at least recommendatory creates the prerequisites for numerous methods of its falsification.

The purpose of this work was to determine the possibility of silver tea identification according to morphological characteristics of leaves, preliminary the study of the qualitative composition of the silver tea extracts by HPLC-UV and development of criteria for sensory assessment of the quality of drinks from it.

The object of the study was:

- silver tea labeled Silver Tea (Nandana Tea Factory, Akuressa, Sri Lanka). Packed in metallized foil, the weight of the package is 10 grams;
- processes and parameters of preparation of drinks from it.

The research was carried out on the basis of the Center for collective use of scientific equipment in the field of physical and chemical biology and nanobiotechnology "Symbiosis" FGBE IBPPM RAS (Saratov).

II. Experimental

Microscopy of the tea leaf was carried out on a microscope with a light stereo Leica M 165 C (Leica-microsystems, Germany). The qualitative composition of extracts from tea samples was carried out by HPLC method with ultraviolet detection.

Sampling. Two-step extraction was carried out by low-polar (chloroform) and polar (96% ethanol) solvents. Approximately 1 g (accurate sample) of dry tea leaves was weighed for extraction.

Extraction. To carry out the extraction a sample of carefully dried tea leaves, thoroughly ground in a porcelain mortar, was put in a round-bottomed flask, 30 ml of chloroform was added, the mixture was boiled for 1 hour with a reflux condenser, then the leaves were filtered with a Schott filter, which were after that placed in round-bottomed flasks for ethanol extraction. 30 ml of ethanol were added, the mixture was boiled for 1 hour with a reflux condenser, after which the extract was filtered off on a Schott filter. The extracts were evaporated to dryness on a rotary evaporator under reduced pressure and their weights were determined. For the analysis of IR Fourier spectrometry, the sample was lyophilized.

Preparation of samples

The dried extracts were dissolved in a mixture of acetonitrile-ethanol (1: 1), 500 μl were taken for HPLC analysis. Identification of the components was performed by comparing the retention times of standard flavonoid samples (rutin in the form of hydrate (≥ 94%, “Sigma-Aldrich”, USA), quercetin in the form of dihydrate (97%, “Alfa Aesar”, UK), naringin (≥ 95% “Sigma-Aldrich”, USA), apigenin (≥ 97%, “Sigma-Aldrich”, USA), naringenin (≥ 95%, “Sigma-Aldrich”, USA), as well as prunin obtained as a result of partial acid hydrolysis of naringin), gallic acid with retention times of components of hydrolysates.

To record the IR Fourier spectrum, a lyophilized sample of the tea extract was placed on a gilded mirror.

The process and parameters of brewing different types of tea play a decisive role in the formation of the sensory characteristics of the drink. Based on the analysis of published data, it is better to brew white tea varieties by the “spillage” method that is, by repeatedly brewing one portion of the tea leaves, gradually increasing the time and temperature of the subsequent brewing. The tea leaf brewing process was carried out in a heated porcelain teapot, with boiled water, and cooled to a temperature of 75-60°C, by the “spillage” method. Every time the received drink was completely poured into a special vessel equal in volume to the teapot, then poured into small cups (volume not exceeding 40 ml) and sensory evaluation was carried out.

There are no standards for organoleptic analysis of silver tea, therefore, the standards of GOST R ISO 3972-2005 “Organoleptic analysis”.

Methodology.

Method for the study of taste sensitivity GOST R ISO 11035-2005 “Organoleptic analysis: Identification and selection of descriptors for establishing organoleptic properties in a multilateral approach” [16].

The moisture content and water-soluble extractive substances were determined according to GOST 1936-85 “Tea. Rules of acceptance and methods of analysis” [17] and GOST R ISO 9768-2011 “Tea. Method for determination of water-soluble extractive substances” [18].

III. Results and discussion

The general identifying attributes of tea include characteristic features (shape, size and anatomical structure of the leaf) and specific features (taste and aroma of the infusion, the content of water-soluble extractive substances).

The formation of specific identifying attributes occurs during the processing of tea leaves.

Confirmation of the authenticity of the brand name is the most difficult task of assortment identification of tea.

Assortment identification of tea includes the solution of the following tasks:

- determination of the nature of the tea raw materials;
- determination of geographic location of the place of growth;
- confirmation of the main classification features determining the tea assortment and indicated in the labeling;
- confirmation of the authenticity of the brand name (if it is indicated).

To determine the nature of tea raw materials, in particular, to detect falsification by replacing the tea leaf with leaves of other plants, the determination of organoleptic indices and the study of the morphological features of tea are used.

Morphological characteristics are the identification criteria not only for determination of the nature of raw materials, but also for determination of the place where tea grows. For the study of individual morphological features, visual inspection and the microscopy method are used.

Visual inspection of silver tea samples has shown the absence of impurities, broken leaves, and tea dust in the package. All the leaves are whole, have a light green color, an oblong shape and a slight hairiness. The aroma is pleasant, delicate, and herbal.

The results of a microscopic examination of a silver tea leaf are shown in Fig. 1-2.

As the analysis of the data has shown, the analyzed tea leaf has an oblong shape (the ratio of the length of the leaf to the maximum width is bigger), it is limited by saw toothed edges (smooth at the base), the teeth are provided with rounded glandular hairs characteristic for silver tea tips.

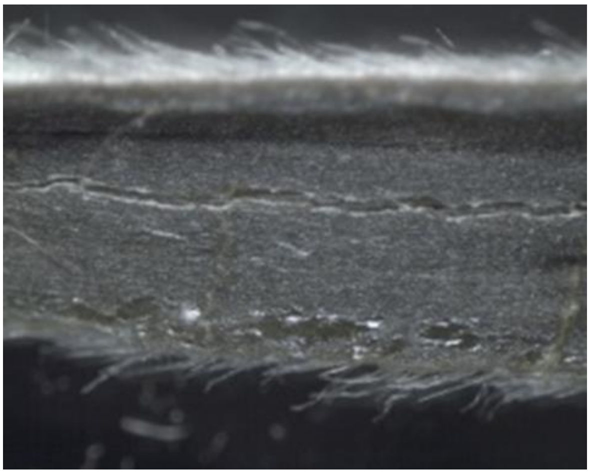

Fig. 1. Micrograph of a silver tea leaf. Increase x100.

Fig. 2. Micrograph of a silver tea leaf. Increase ×60.

The epidermis of the underside of the leaf covered with characteristic silvery white rigid unicellular hairs. They can easily be seen with the naked eye on the surface of the unblown buds and the upper leaf. The epidermis of the upper side of the leaf is devoid of hairs and stomata. Between the upper and lower epidermis of the leaf are palisade tissue (single-layered, loose, consisting of large, strongly elongated cells) and spongy mesophylls. Between the mesophyll cells there are branched giant cells - idioblasts, characteristic for the tea leaf, since they do not exist in other plants.

These morphological features may be reliable criteria for the identification of the nature of tea raw materials.

Based on the study of the microstructure, tea labeled Silver Tea (Nandana Tea Factory) is an example of a white (silver) tea.

Qualimetric identification of tea aims to establish compliance of the actual commercial brand with the information indicated in the labeling. Currently, for these purposes, HPLC with UV detection is the most commonly used method. This method allows the simultaneous determination of the content of existing substances in tea. The complex of these indicators is very specific for each brand of tea.

Analysis of the chromatogram (Fig. 3) of the chloroform extract of tea leaves mainly indicates the presence of signals of low polar components. None of the extract components could match the existing standard. Components 12 and 15 have similar absorption spectra, which gives reason to assume that these compounds may be glycosides differing in the number of carbohydrate units and / or their substitution in other positions of the aglycon.

A chromatogram of the alcohol extract of tea leaves is shown in Fig.4. Comparing the chromatograms of these two extracts, it can be concluded that when extracted with more polar ethanol, the output of the more polar components of tea leaves naturally increases as compared to chloroform. It was not also possible to identify any known component in this extract. Absorption spectra of the components 5-10 and 13-15 are practically identical, in components 11 and 12, a pronounced local maximum in the visible region is added to the UV-visible spectrum profile. Another group of closely related absorption spectra is formed by components 16 and 17.

In the IR spectrum, absorption bands, corresponding to the vibrations of the main functional groups of the compounds that make up tea, mainly polysaccharides, are observed (Fig. 5). A wide absorption band at 3200-3400 cm^{-1} is characteristic for valence vibrations of hydrogen-bonded OH groups. The absorption bands,

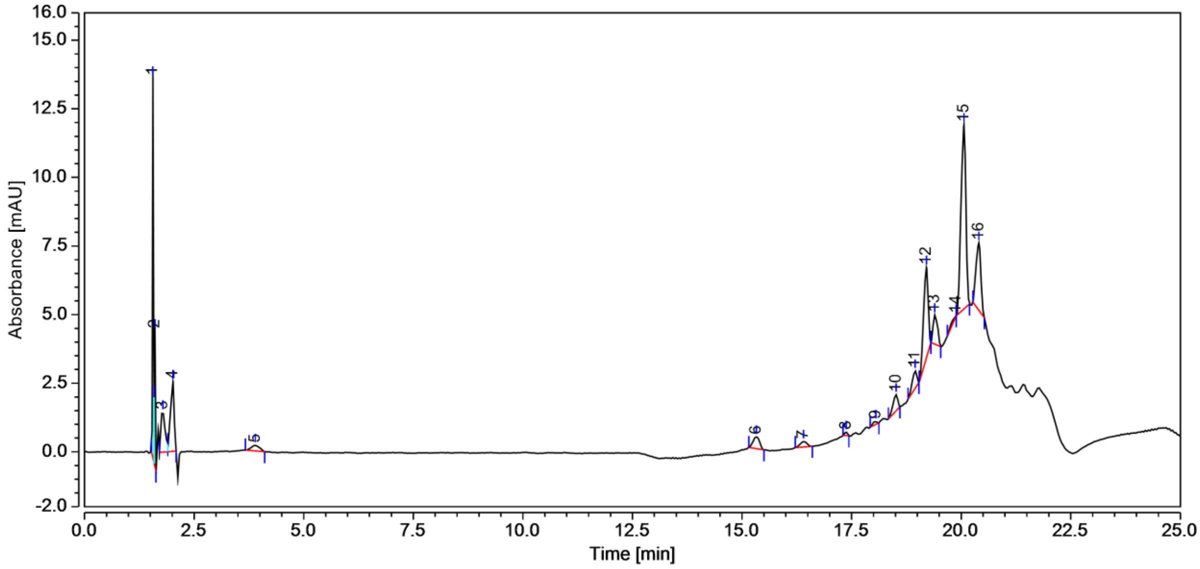

Fig. 3. Chromatogram sample of chloroform extract of tea leaves.

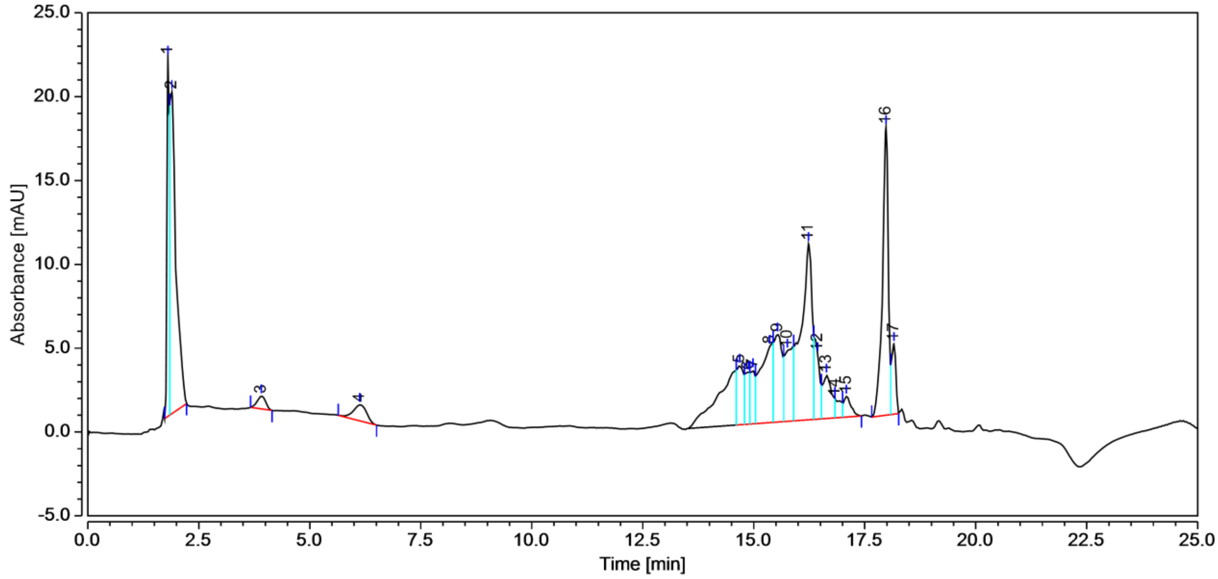

Fig. 4. Chromatogram sample of alcohol extract of tea leaves.

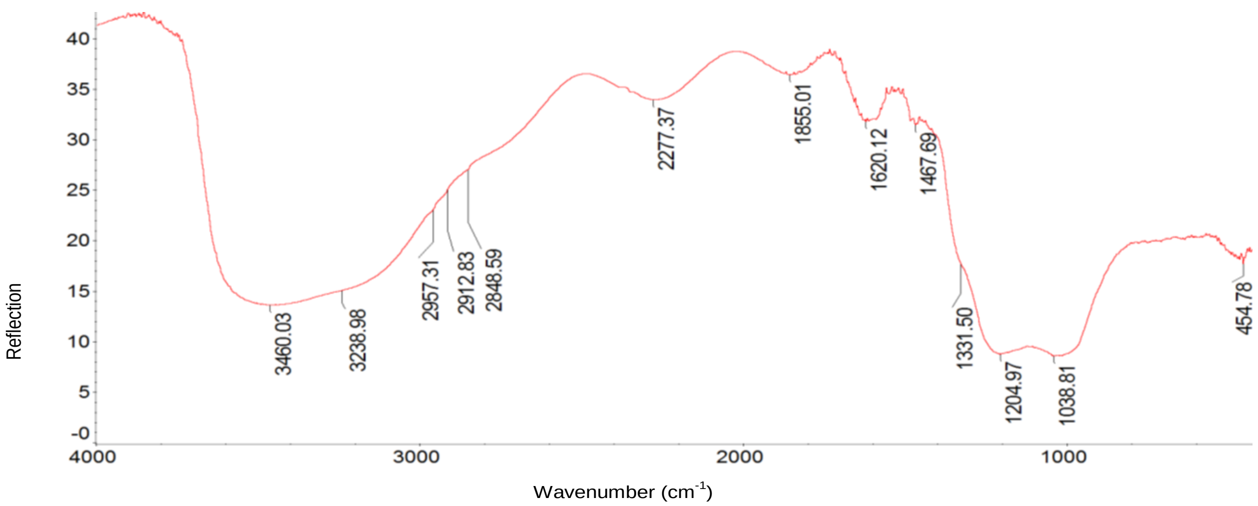

Fig. 5. A sample of the IR spectrum of the extract of tea leaves.

corresponding to the valence vibrations of the aliphatic CH groups, are observed at 2957, 2913 and 2849 cm^{-1}. The bands at 2929, 1700-1460 and 1400-1200 cm^{-1} is considered characteristic for polysaccharide containing tea samples. Thus, a wide band with a maximum of 1620 cm^{-1} can be attributed simultaneously to valence vibrations of carbohydrate rings, and to deformational vibrations of water molecules, as well as to deformational NH vibrations or antisymmetric vibrations of carboxyl groups. The bands that appear in the form of low-intensity shoulders at 1431 and 1331 $см^{-1}$ correspond to weak deformational vibrations of CH. The bands of 1200-1000 cm^{-1} are characteristic for O-substituted glucose residues of tea polysaccharides. In general, it can be concluded that the IR spectrum profile of the tea extract is similar to that of polysaccharides that indicates their high content in the extract.

According to literary data, it is from sugars that catechins are formed, which represent the greatest value of tea. Catechins account for 70-80% of flavonoids. Fresh sheet catechins are oxidized and reacted with other substances during treatment, such as esters or proteins, and transformed into more complex ones, such as tannin, thioflavins and others [3].

Thus, as the spectral characteristics of the leaves of silver tea show, this sample has its own unique spectral profile and is not comparable with known standards.

Studies of the qualitative composition of tea have revealed that polysaccharides predominate in spectral characteristics.

Aroma, taste, color (intensity, brightness) and transparency of the infusion after tea brewing, the color of the brewed leaf, and the appearance of the tea are attributed to the organoleptic parameters of the quality of tea drinks.

The quality criteria for the drink vary depending on the multiplicity and brewing temperature. As it can be seen from the data in Table 1, during the first brewing, the tea leaf gradually begins to give off flavoring and flavoring substances.

The extraction of water-soluble substances in silver tea with each brewing was 0.4%, which was noted in the organoleptic analysis.

The most distinct aroma and flavor characteristics of the drink are provided by the second and third brewing.

With subsequent brewing, it is not possible to extract the same intense flavor and aroma, even with increasing infusion time (fourth, fifth brewing). By the sixth, seventh brewing, tea gives away a greater amount of flavoring substances and the astringency of tea appears which was absent before, the drink acquires a light shade.

Based on the organoleptic analysis, if the parameters of the brewing process are observed, silver tea brewing can be used up to 7 times, without loss of sensory properties. Moreover, with each subsequent brewing, new flavors are revealed.

The study of dry substances in a sample of silver tea showed their content of 93.3%, which complies with international and domestic standards.

IV. Conclusion

Thus, there is a possibility of silver tea identification on the basis of the anatomical and morphological features of the leaves, which have an elongated shape, and are limited by saw-toothed edges, the teeth are provided with rounded specific glandular hairs.

TABLE I. ORGANOLEPTIC EVALUATION OF SILVER TEA QUALITY WITH THE USE OF THE “SPILLAGE” METHOD

The name of indicators	***Brewing***						
	1st brewing	***2nd brewing***	***3rd brewing***	***4th brewing***	***5th brewing***	***6th brewing***	***7th brewing***
Temperature Brewing time	t = 60°C 1 min	t = 60°C 1 min 30 sec	t = 60°C 1 min 50 sec	t = 60°C 2 min 30 sec	t = 60°C 2 min 30 sec	t = 60°C 2 min 30 sec	t = 60°C 3 min
Intensity of flavor	Gentle, delicate	Distinct	Delicate floral	Delicate floral	Delicate honey	Light	Absent
Characteristic of aroma	Floral, delicate, well perceptible	Complex floral intense	Complex delicate floral honey	Light	Light herbal	Herbal	Subtle herbal
Presence of flower taste	Intense	Distinct	Intense	Light, fresh	Sugary sweetish penetrating	Complex penetrating	Sweetish tart herbal
Presence of sweet taste	Perceptible	Distinct	Intense	Light	Floral, honey	Light	Subtle
Aftertaste	Subtle, pleasant	Perceptible floral	Soft sweetish	Light	Soft sweetish	Velvety	Tart
Infusion color	Transparent, white	Transparent, white	Transparent with a light tinge	Transparent	Transparent	Light yellowish greenish	Transparent slightly greenish
Flavor	Not distinct, delicate exquisite	Enveloping sweet	Enveloping	Enveloping, vegetable	Floral, honey	Distinct herbal, vegetable	Herbal tart sweetish
The presence of velvety	Absent	Light appearance	Distinct	In the entire oral cavity	Enveloping soft	Light	Absent

By HPLC method with UV detection, it was found that silver tea has its own unique spectral profile and is not comparable with known standards. There is a need to develop and apply methods of quick authentication of white tea varieties.

The criteria for sensory evaluation of the quality of silver tea drinks have been developed, showing that when it is brewed by the “spillage” method, new tastes and aroma variations are revealed with each subsequent brewing, while extraction of water-soluble substances of extractive substances is 0.4%, which indicates their gradual extraction

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