Agronomy Research **19**(X), xxx–ccc, 2021
https://doi.org/10.15159/AR.21.126

Morphology of *Camellia Sinensis* L. leaves as marker of white tea authenticity

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Received: February 1st, 2021; Accepted: April 24th, 2021; Published: August 18th, 2021

Abstract. Tea is one of the most common drinks in the world. Classic tea is obtained by brewing the leaves of the Camellia sinensis L plant in hot water. However, even the leaves collected from the same branch of the same tea bush can have completely different anatomical, biochemical and taste characteristics. White tea is the youngest, immature apical leaves of the tea bush (fleshes) together with leaf buds (tips) which are is considered the most valuable parts of teaplant.
The chemical composition of tea is studied in sufficient detail, however, there are still no uniform criteria for determining the authenticity of white tea leaves, which creates great preconditions for falsifying this most valuable type of raw material. The aim of this study was to study the macro- and microstructure of white tea leaves from different manufacturers and to determine the morphological markers of the authenticity of white tea leaves.
The objects of research were white tea from the Nandana Tea Factory (Sri Lanka) and white tea from an unknown manufacturer, purchased from a local tea shop.
The study of raw materials was carried out in accordance with the requirements of GF XIV OFS 1.5.1.0003.15 'Leaves' and OFS 1.5.3.0003.15 'Technique of microscopic and microchemical examination of medicinal plants and herbal medicinal products.'
The work was carried out on the basis of the laboratories of the Department of Food Technologies of FGBOU VO Saratov GAU named after N.I. Vavilov, and the Department of General Biology, Pharmacognosy and Botany, Saratov State Medical University named after V.I. Razumovsky Ministry of Health of Russia.
Studies of the structure of white tea leaves from various manufacturers have shown that the structure and presence of morphological elements of leaves, such as hairs, stomata, leaf edge, druses, sclereids, differ markedly and can serve as reliable markers for identifying the variety of tea.

Key words: white tea, morphological signs, tea grade, tips.

INTRODUCTION

Tea is one of the most common drinks in the world. Classic tea is obtained by brewing the leaves of *Camellia sinensis* L. in hot water. However, even leaves harvested

from the same branch of the same tea bush can have completely different anatomical, biochemical and taste characteristics. The most valuable is white tea made from the youngest, immature apical leaves of a tea bush (fleshes) together with leaf buds (tips). Different studies have found many health beneficial propertiesof white tea extract such as antioxidant (Dias et al., 2013), antimutagenic (Rusak, 2008; Mao et al., 2010), hypolipidemic (Söhle et al., 2009), antidiabetic (Anderson & Polansky, 2002), vasoprotective and antihypertensive (Curin & Andriantsitohaina, 2005), antimicrobial (Chou et al., 1999). Such an impressive list of positive effects on the human body greatly increases both the value of white tea among consumers and the interest of producers in its procurement, despite the labour-consuming nature of this process.

White tea represents unexpanded leaf buds or buds with 1–2 top young leaves. It is called white because of the large number of hairs that give the leaves a characteristic shade. While being processed, white tea undergoes only 2 technological steps: withering and drying. This allows one to maximally preserve the original chemical composition of the leaves and determines a large number of medicinal effects of white tea (Yoshino et al., 1994; Chou et al., 1999; Anderson & Polansky, 2002; Curin & Andriantsitohaina, 2005; Gramza et al., 2005; Evstigneeva et al., 2016).

G. Santana-Rios et al., (Santana-Rios et al., 2001) noted the similarity of the chemical composition of white and green tea, namely, nine main components of white tea (catechin, epicatechin, epigallocatechin, epicatechingallate, epigallocatechin-3-gallate, theobromine, theophylline, caffeine, gallic acid) are also found in green tea, and the total amount of catechins in white and green tea is about the same. At the same time, the research results show that white tea is superior to green tea in terms of its antimutagenic activity (Santana-Rios et al., 2001).

Similar results were obtained by T. R. Dias et al (Dias et al., 2014). It was found that the main component that determines the antioxidant potential of white and green tea is epigalocatechin-3-gallate (EGCG). Its content in white and green tea from the same manufacturer is very different - in white tea it is twice as much EGCG as in green tea. Accordingly, the antioxidant activity of white tea is more pronounced.

In the research work by U.J. Unachukwu et al., (Unachukwu et al., 2010) contradictory data were obtained: the amount of total catechins in white tea, depending on the variety, can be less, more or comparable to different varieties of green tea. Moreover, even within one type of white tea, produced by different companies, the content of common catechins can be more than 10 times as different.

Such significant differences in the chemical composition of white tea can be associated, among other factors (like climate, soil composition, intensive or organic agriculture, altitude, even the southern or northern part of the slope), with the lack of markers of the authenticity of the tea leaf. The chemical composition of tea has been studied in sufficient detail, however, there are still no uniform criteria for determining the authenticity of white tea leaves, which creates great preconditions for the falsification of this most valuable type of raw material. Thus, the aim of this research article was to study the macro- and microstructure of white tea leaves from different manufacturers to determine the morphological markers of authenticity of white tea leaves.

The objects of research were white tea from the Nandana Tea Factory (Sri Lanka) and white tea purchased from a tea shop in Saratov. It should be clarified that white tea from the Nandana Tea Factory was produced, packaged and purchased at the factory, the labeling corresponded to the requirements of TR CU 022/2011 Technical

Regulations of the Customs Union ‘Food products in terms of their labeling’. Tea bought in a teashop in Saratov was sold by weight, there was no labeling.

MATERIALS AND METHODS

The study of raw materials was carried out on a Carl Zeiss Primo Star microscope in accordance with the requirements of GF XIV OFS 1.5.1.0003.15 ‘Leaves’ and OFS 1.5.3.0003.15 ‘Technique of microscopic and microchemical study of medicinal plant materials and herbal medicines.’

For the analysis, whole leaves or pieces of a leaf blade with an edge and a vein, pieces of a leaf from the base and apex, and pieces of a petiole (if the leaf has a petiole) were taken.

They were bleached in the following way: several pieces of raw material were placed into a flask or a test tube, a sodium hydroxide solution of 5% diluted with water (1:1) was added and boiled for 2–5 min, depending on the thickness and density of the objects, avoiding strong softening. Then the contents were poured into a glass, the liquid was poured through 2–4 layers of gauze, which was used to cover the glass, and the raw materials were thoroughly washed with water, each time the water was drained through the same gauze. The content of the glass was transferred in a small amount of water into a Petri dish. The particles of raw material remaining on the gauze were washed off into the same Petri dish. The pieces were removed from the water with a scalpel or spatula and placed on a glass slide in a drop of a 33% glycerin solution. Pieces of raw material, bleached and placed on a glass slide, were divided into two parts with a scalpel or dissecting needles, one of them was carefully turned over. A piece of petiole was placed on a glass slide. The thin petioles were crushed with a scalpel or the reverse end of a dissecting needle to release the epidermis. The epidermis was removed from the thick petioles using dissecting needles or a razor, removing the coarse inner parts of the petiole, which prevented us from obtaining a good epidermal micropreparation. The object was covered with a cover glass, if necessary, slightly pressed down from above with a clean reverse end of the dissecting needle and slightly heated until air bubbles were removed, after cooling, the leaf and the epidermis of the petiole were examined from both sides under a microscope, first at low, then at high magnification. The upper and lower epidermis, as well as deep leaf structures located under the epidermis (parenchyma, inclusions, vessels, etc.) were examined at different magnifications, using a macro- and microscrews.

The preparation of cross-sections of petioles was carried out as follows. Slices were made from the soaked petioles, by clamping pieces of the petiole into a bottle cork or elderberry core. When using a bottle cap, it was preliminarily boiled in water for 15 minutes. A piece of elderberry or bottle cap was cut into halves and a piece of leaf was clamped between the two halves. To make cross sections, the surface of the piece should be prepared so that it is strictly perpendicular to the axis of the petiole or leaf vein. The finished sections were placed in a Petri dish with water, from where the sections were taken out and examined under a microscope; the most relevant samples were selected.

RESULTS AND DISCUSSION

Dried raw white tea A (tea sample from the factory) is represented by whole leaves of a silvery-green color with a petiole, with abundant pubescence on the underside of the leaf. The leaf blade (lamina) is turned inward from the edges through the ventral side. A leaf measuring 1.2–3.0 cm in length and up to 0.5 cm in width has a dorsal crescent-shaped bend. The main vein on the underside of the leaf is absolutely visible. Broken leaves are rarely found. Inside some leaves, there are tips that are morphologically similar to the rest of the leaves, but smaller and coiled into a tube (Fig. 1).

The raw material of tea B (tea sample from the store) contains less than half of these leaves. Most of them are almost whole leaves up to 5 cm in length and parts of leaf blades (lamina) are quite large (up to 1.5×1.5 cm), unfolded. The upper side of the leaf blade is black and almost without hairs, the lower side is similar in appearance to tea A. There are also a large number of small parts of leaves up to 2×2 mm in size, sometimes branches up to 2 cm long are found (Fig. 2).

Figure 1. White tea A.

Figure 2. White tea B.

Raw tea A does not change its shape after brewing, but the color of the leaves changes from silvery green to dark green, almost black. Tips are clearly visible. The leaf is rather thick and fragile; when we try to take a sample for a micropreparation, the upper and lower epidermis are easily peeled off, and the leaf itself breaks apart (Fig. 3).

After boiling, tea B leaves unfold, become slightly transparent, green in color. It should be noted that the leaves strongly stain the water even after several washes. Quite a large number of branches are also visible (Fig. 4).

Figure 3. Tea A after boiling.

Figure 4. Tea B after boiling.

Figs 5 and 7 show a very high density of hairs and numerous outgrowths of the edge of the leaf blade, which are not easily visible.

In Figs 6 and 8, a much smaller number of hairs are noted, and the outgrowths of the leaf edge are filled with extractive substances or are absent (the points of their attachment are visible).

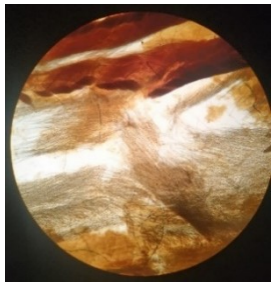

Figure 5. Tea A leaf plate. Magnification 10×4.

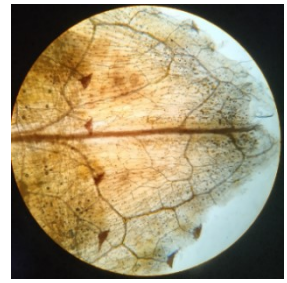

Figure 6. Tea B leaf plate. Magnification 10×4.

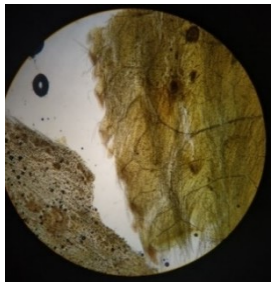

Figure 7. Tea A leaf plate. Magnification 10×4.

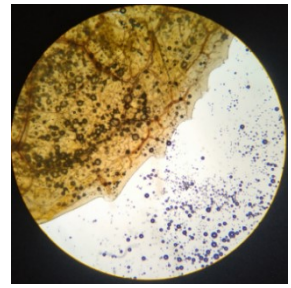

Figure 8. Tea B leaf plate. Magnification 10×4.

The photograph of the lower epidermis of tea A (Fig. 9) shows such a high density of hairs that they completely block the view of the underlying structures. Fig. 11 shows where the hairs are attached to the epidermis. Epidermal cells with some cellular structures and hair attachment points are clearly visible. You can see that between the adjacent hairs there are from 1 to 3 cells of the epidermis.

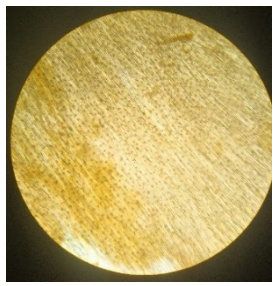

Figure 9. Tea A trichomes. Magnification 10×10.

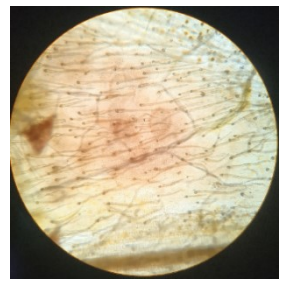

Figure 10. Tea B trichomes. Magnification 10×10.

Tea B is characterized by a significantly lower hair density (Fig. 10). Moreover, the length of the hairs B is shorter (Fig. 12).

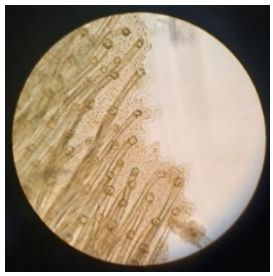

Figure 11. Tea A trichomes. Magnification 10×40.

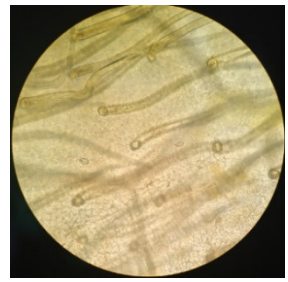

Figure 12. Tea B trichomes. Magnification 10×40.

The stomata on tea A could not be seen due to the very high density of hairs.

The stomata on tea B are clearly visible (Fig. 13). In addition, cuticle folds are visible in Fig. 14.

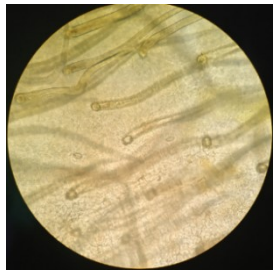

Figure 13. Tea B stomata. Magnification 10×40.

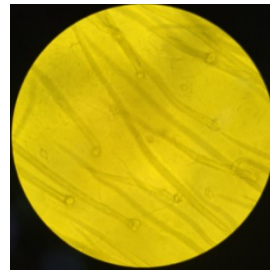

Figure 14. Tea B cuticle. Magnification 10×40.

Fig. 15 shows conductive bundles (fasciculus) of white tea A, which have a typical structure. The spiral thickening of the vessel wall is clearly visible. Thicker vessels are located in the center of the vascular bundle, the diameter of the vessels decreases towards the periphery.

Conductive bundles of tea B have an identical structure, with the exception of the central vein - sclereids are randomly scattered around it (Fig. 16).

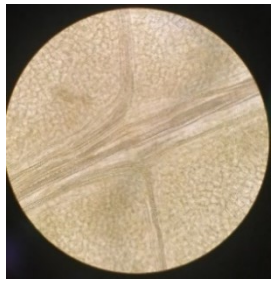

Figure 15. Tea A conductive bundles. Magnification 10×40.

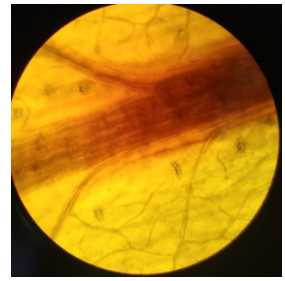

Figure 16. Tea B central vein. Magnification 10×10.

Along the entire edge of the leaf of tea A, in the middle part of the leaf blade, there are outgrowths of the leaf edges closely spaced to each other with vessels approaching them. Morphologically, these outgrowths are similar to the top of the leaf blade. It is noticeable that the formations of the leaf edge can be heterogeneous in size and shape, but have the same general structure (Figs 17, 19, 21).

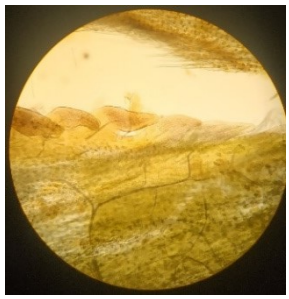

Figure 17. Tea A leaf edges outgrowths. Magnification 10×10.

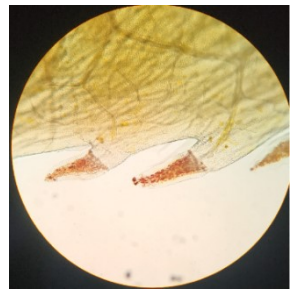

Figure 18. Tea B leaf edges outgrowths. Magnification 10×10.

Figure 19. Tea A leaf edges outgrowths. Magnification 10×10.

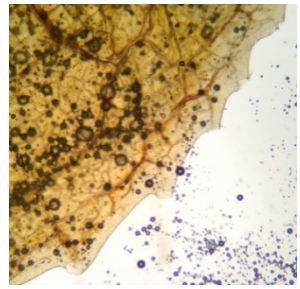

Figure 20. Tea B leaf edges outgrowths. Magnification 10×10.

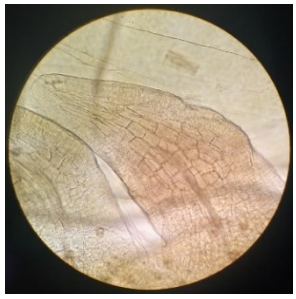

Figure 21. Tea A leaf edges outgrowths. Magnification 10×40.

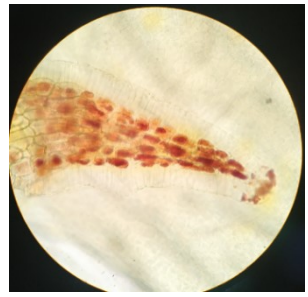

Figure 22. Tea B leaf edges outgrowths. Magnification 10×40.

In tea B, these outgrowths are located slightly less densely (Fig. 18). They, as was previously mentioned, are filled with extractive substances and come off, and the edge of the leaf in the points of their attachment gradually overgrows and levels out (Figs 20, 22).

Sclereids in the leaves of tea A are found near the top of the leaf; in the rest of the leaf blade, they are not found. The leaf edge near the top is solid. Druses are also found only near the top and are concentrated at the edge of the leaf (Fig. 23).

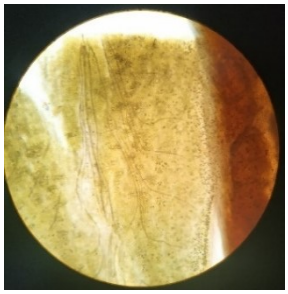

Figure 23. Tea A sclereids and druses. Magnification 10×4.

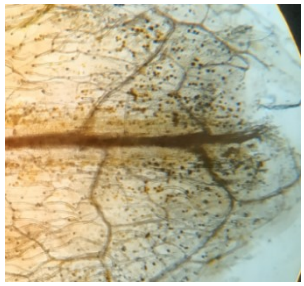

Figure 24. Tea B sclereids and druses. Magnification 10×4.

Druses in tea leaf B are found not only at the top of the leaf blade, but also along the edge of the leaf. Sclereids are not concentrated at the apex either, but begin to spread along the central vein into the main part of the leaf blade (Fig. 24).

CONCLUSION

Studies of the structure of white tea leaves from various manufacturers have shown that the structure and presence of morphological elements of leaves, such as hairs, stomata, leaf edge, druses, sclereids, differ significantly and can be markers of tea raw materials (Table 3).

Table 3. The results of the study of the tea raw materials

Characteristic	Tea A	Tea B
Appearance	Silver-green folded dryish fleshes and individual tips	Silver-green below, black above, partially unfolded leaves and small pieces of leaves
Hairs	Long, very high density	Short, low density
Stomata	Not detected	Found everywhere
Conducting bundles	Without sclereids on the periphery	With sclereids around the central vein
Leaf edge	Living outgrowths, high density	The number of outgrowths is reduced, the initial stage of lignification or absence
Sclereids	At the top of the leaf blade	At the top of the leaf blade and along the central vein
Druses	At the top of the leaf blade	At the top of the leaf blade and along the edge of the leaf

It should be noted that in the works of various scientists, unfortunately, no attention is paid to the morphological characteristics of tealeaves. However, there is a sufficient number of scientific studies, difficult to carry out in certain conditions, on the chemical composition of tea and its effect on the body (Yi Chen et al., 2014; Dias et al., 2016; Pastoriza et al., 2017; Junjie Zhang et al., 2019).

Nowadays, the development of white tea authenticity markers is a relevant topic. The Covid-19 pandemia makes it even more topical, since it raises the issues of food quality and safety. Consumers must be confident not only in the quality, but in the authenticity of food products, especially those that have a beneficial effect on the immune system of the human body.

Taking into account the results of the previous study of the structure of the leaves of white and black tea (Isaev et al., 2020), it can be noted that white tea bought in a store by weight has an intermediate structure between a young and a mature leaf, which indicates a greater age and, thus, such tea cannot be called white.

ACKNOWLEDGEMENTS. The authors express gratitude to the Nandana Tea Factory and personally to Mr. Gunasoma Wanigasekara for the tea samples provided for the study.

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