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INFLUENCE OF THE ADDITION OF CHAMOMILE ON THE CONTENT OF TANNINS IN TEA

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Abstract: *In this work, the influence of chamomile on the content of tannin in the blended green and black teas is investigated by the titrimetric method, which relies on the assessment of tannin oxidation by potassium permanganate with the indicator indigo carmine. The standard procedure of titration was altered in such a way that the dilution of filtrate used during the investigation was decreased by 10 times. This modification made it possible to use the regular 250 ml flasks instead of big and inconvenient porcelain bowls. This way, identification of the point of equivalence became easier and more accurate. Application of this modified method to the determination of tannin in a series of blended teas showed results that are in good agreement with those obtained by the standard methods. They proved that the content of tannin in tea does not depend on the added chamomile and, therefore, this herb does not depress the medical value of the investigated blended teas.*

Keywords: *tannins, blended tea, chamomile, medical values, modified titration, permanganatometry*

1. Introduction

Tea is one of the most common beverages in the world, which has unique taste and specific organoleptic properties. It relieves fatigue and headaches, increases mental and physical activity, adsorbs harmful substances and removes them from the body [1, 2]. The chemical composition of tea leaves is very diverse and according to the latest data [3], tea contains about 500 different substances. Tea leaves polyphenols have antiviral, antitumor and anti-oxidant activity. They are characterized by anti-inflammatory, antimicrobial, hemostatic, antispasmodic, antioxidant properties. Having the ability to balance human blood sugar levels, they can be used for the prevention and treatment of type II diabetes [4, 5]. There is no universal method of quantitative analysis of tannins, because tannins are a mixture of different classes of polyphenols that have different structures. The basic methods, which often used for quantitative determination of tannins are the next – gravimetric, photocolorimetric, spectrophotometric, titrimetric, nephelometric, tensiometric and others [3, 5–14]. Determination by gravimetric analysis is often performed by deposition. Quantitative determination of tannins by gravimetric method is based on their precipitation by gelatin, heavy metal ions, skin powder. The disadvantage of this method is the long-term definition. The titrimetric method is based on the oxidation of tannins by potassium permanganate in the presence of the indigo carmine indicator [15]. The disadvantage is the use of a large volume of water during the titration, which is carried out in a porcelain cup for evaporation and mixing – with a glass rod creates signifi-

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cant inconvenience of fixing the equivalence point. Using various additives – berries, leaves, herbs, etc., tea can be enriched with vitamins and beneficial properties. Chamomile is one of the most valuable medicinal plants, which constantly attracts the attention of researchers. To date, scientists have substantiated the possibility of using herbal collection, one of the components of which is chamomile, in anticancer therapy [16]. Tannins and catechins, as well as rutin (vitamin P) regulate the process of collagen formation in blood vessels, which helps to normalize blood pressure. Tea is the main source of these substances for the human body [7, 17]. Therefore, the study of the content of tannins in various teas, in particular blended with medicinal plants, is quite relevant. The purpose of the work is to improve the standard method of titrimetric determination of tannin content in tea; determine how the different content of chamomile supplement in blended teas based on black / green will affect the concentration of tannins in them.

2. Materials and methods

The tea samples which were used for research were: Greenfield Golden Ceylon (1st grade, black leaf tea, loose, origin – Ceylon, country of origin – Ukraine) and green Ahmad Tea Chinese Green Tea (leaf tea, Chinese). As a supplement, medicinal chamomile was used – PJSC "Liktravy", Ukraine. For the experiments, 2 series of blended samples based on only black or only green (black / green) tea with different wt. % of chamomile additive were prepared in parallel (Table 1).

Black / green tea without the addition of chamomile and chamomile without tea were used as control samples. To determine the content of tannins in the studied samples of blended teas, a standard method of permanganotometric titration in the presence of an indigo carmine indicator was chosen [15].

Table 1

Test samples

№ sample	1	2	3	4	5	6	7	8
Tea (black / green) wt. %	100	90	80	60	50	40	20	0
Chamomile wt.%	0	10	20	40	50	60	80	100

Preparation of 0.1 % solution of indigo carmine: 0.5 g of finely ground pure indigo carmine was dissolved in 25 cm^3 of chemically pure concentrated sulfuric acid (ρ = 1.84 g/cm^3); the volume was adjusted to 500 cm^3 by gradually pouring the solution into distilled water. The resulting solution was filtered through a pleated filter.

Preparation of 0.1 mol/dm^3 of potassium permanganate solution: $KMnO_4$ weighing 1.58 g was weighed on technical scales. After complete dissolution of the drug, the contents of the flask (500 cm^3) were adjusted to the mark with distilled water. The normality of the prepared solution was established after 10 – 12 days.

Preparation of exactly 0.1 mol/dm^3 of oxalic acid $H_2C_2O_4$ solution: on analytical scales weighed the exact portion of recrystallized oxalic acid weighing 1.5750 g, transferred to a volumetric flask with a capacity of 250 cm^3, dissolved it in distilled water, adjusted to the mark with distilled water.

Standardization of $KMnO_4$ solution: to 10 cm^3 of freshly prepared oxalic acid solution was added 15 cm^3 of sulfuric acid solution (1 part of concentrated H_2SO_4 : 5 parts of H_2O), heated to a temperature of 70 – 80 °C, immediately titrated. At the end of the titration, the solution turns pink, which does not disappear for 1 – 2 minutes.

Titration was performed three times, finding the arithmetic mean value of the vol-

ume of titrant consumed. The normality of the $KMnO_4$ solution (N_{KMnO_4}) was calculated by the formula:

$$N_{KMnO_4} = \frac{N_{H_2C_2O_4} \cdot V_{H_2C_2O_4}}{V_{KMnO_4}},$$

where $N_{H_2C_2O_4}$ – normality of the $H_2C_2O_4$ solution, mol/dm^3; $V_{H_2C_2O_4}$ – volume of 0.1 mol/dm^3 of $H_2C_2O_4$ solution (10 cm^3); V_{KMnO_4} – volume of $KMnO_4$ solution used on titration, cm^3.

According to the standard method, the determination of the content of tannins is carried out starting with the grinding of a tea sample in a porcelain mortar; 2.5 ± 0.0002 g of crushed tea is weighed on clear glass and transferred to a 250 cm^3 flask. Then 200 cm^3 of boiling distilled water is poured into the flask and placed in a water bath for 45 minutes. The resulting extract is filtered into a volumetric flask with a capacity of 250 cm^3. The content of the flask is cooled to 20 °C and made up to the mark using distilled water. Using a pipette, 10 cm^3 of the filtrate is taken and placed in a cup for evaporation, which is pre-poured with 750 cm^3 of water and 25 cm^3 of 0.1 % solution of the indigo carmine indicator. Potassium permanganate is titrated using the 0.1 mol/dm^3 solution with constant stirring with a glass rod. During this, the blue color of the liquid gradually changes through blue-green, dark green, light green, yellow-green, and turns yellow with a golden tinge. Completion of titration is determined by the disappearance of the green hue and the appearance of a pure stable yellow color. The volume of 0.1 mol/dm^3 of potassium permanganate consumed solution is fixed in cm^3. Titration is performed three times and the arithmetic mean of the consumed titrant volume is calculated. Similarly, the blank titration of water with indigo carmine 0.1 mol/dm^3 solution of potassium permanganate is performed. The content of tannins (X) in % is determined by the formula:

$$X = \frac{(a - a_1) \cdot 0{,}004157 \cdot V}{V_1 \cdot m} \cdot 100\%;$$

where X is the tannins content, %; a – volume of 0.1 mol/dm^3 of $KMnO_4$ solution, used on titration of prepared of tea extract, cm^3; a_1 – volume of 0.1 mol/dm^3 of $KMnO_4$ solution used on titration of water with indigo carmine, cm^3; 0.004157 – the amount of tannins that is oxidized 1 cm^3 0.1 mol/dm^3 solution $KMnO_4$, g; m – the weight of a portion of dry tea, g; V – volume of the obtained tea extract, cm^3; V_1 - volume of tea extract taken for research, cm^3.

3. Results and discussion

As mentioned above, the standard method for the determination of tannins in tea by the permanganate method is not very convenient due to such features as the addition of 750 cm^3 of distilled water, use in the titration of large porcelain cups and mixing their contents with a glass rod. We modified the methodology. In particular, it is proposed to reduce the volume of distilled water by 10 times, which allows using ordinary titration flasks with a capacity of 250 cm^3 instead of large and uncomfortable porcelain cups and eliminates the need to mix the contents of the flask with a glass rod.The results of titration of extracts of some test samples obtained by standard and modified methods are presented in Table 2.

Table 2
The volume of 0.1 N $KMnO_4$ solution (ml) used during the titration of some test samples by standard and modified methodology

Sample	№1	№3	№ 4	№ 5
Standard methodology	2.6	2.3	1.9	1.7
Modified methodology	2.6	2.3	1.9	1.7

Comparative analysis of the results (Table 2) shows that the values of the volumes of titrant spent during titration by standard and modified methods are absolutely the same. In addition, as the water volume decreases, it is easier to observe a clear change in color from blue to straw yellow, i.e. the identification of the equivalence point is more accurate. Determination of tannins content in the studied samples was performed using a modified method. The obtained experimental results are presented in Fig. 1. The data show that with decreasing wt. % of green / black tea in blended samples with chamomile medicinal content of tannins decreases. In samples № 5 (50 wt. % Green tea and 50 wt. % Chamomile) and № 6 (40 wt. % Green tea and 60 wt. % Chamomile) the content of tannins is the same. Test sample № 7 (20 wt. % Green tea and 80 wt. % Chamomile) and control sample № 8 (100 wt. % Chamomile) contain 2.9 wt. % tannins (Fig. 1, curve 1). In samples № 6 (40 wt. % Black tea and 60 wt. % Chamomile), № 7 (20 wt. % Black tea and 80 wt. % Chamomile) and in the control sample № 8 the tannins content is almost the same and equal 2.6 – 2.7 wt. % (Fig. 1, curve 2). There is a pattern: in blended samples with a low content of green / black tea, the value of the tannin content is approximately 3 wt. %.

In general, regarding the effect of the addition of chamomile on the content of tannins in blended black / green teas, it can be stated that it does not depend on the content of chamomile. Approximation of experimental data shows a linear dependence of tannin content on wt. % of tea in blended samples $y = kx + a$, where $a = 0$ (Fig. 1): based on green tea $y = 0{,}104x$; based on black – $y = 0{,}065x$. However, when the content values are less than 30 wt. % of black / green tea, there is a deviation from these linear dependences in the form of some "background values" of tannin content in the range of 2.6 – 2.9 wt. %. If these background values were characteristic of chamomile, they should be manifested at low wt. % chamomile in blended samples. But under such conditions, the linear approximations would be different from the zero value of the parameter *a*, in particular: on the basis of green tea $y = 0.074x + 2.92$; based on black – $y = 0.039x + 2.6$. According to the literature on the chemical composition of chamomile [3, 9], tannins are absent. Therefore, in our opinion, it is incorrect to list the amount of potassium permanganate used by wt. % tannins in blended samples with a content of chamomile drug more than 70 wt. %.

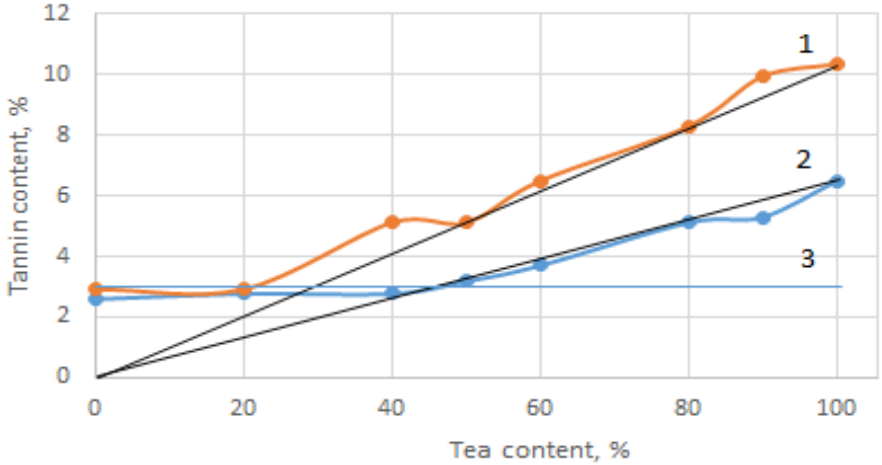

Fig. 1. Dependence of tannins content in blended teas on the wt. % of tea: 1 – green tea; 2 – black tea; 3 – sensitivity limit of the method; lines on experimental graphs – linear approximation of theoretical models

Most likely, the reason for such results is the relatively low sensitivity of the permanganatometric method to determine the content of tannins in tea, which, according to the literature, is equal to 3 wt. % (Fig. 1, line 3). The content of 2.9 wt. % tannins in the control sample № 8 can be explained by the fact that potassium permanganate oxidizes not only polyphenols, but also simple phenolic compounds and other reducing agents of non-phenolic nature, in particular organic acids, vitamin C and a number of compounds contained in chamomile. Theoretical modeling and quantitative evaluation of the content (wt. %) of tannins in samples of black or green tea without the addition of chamomile were performed to test this hypothesis. The theoretically calculated values correlate well with the values of tannins content found experimentally in the blended samples (Fig. 2, Fig. 3).

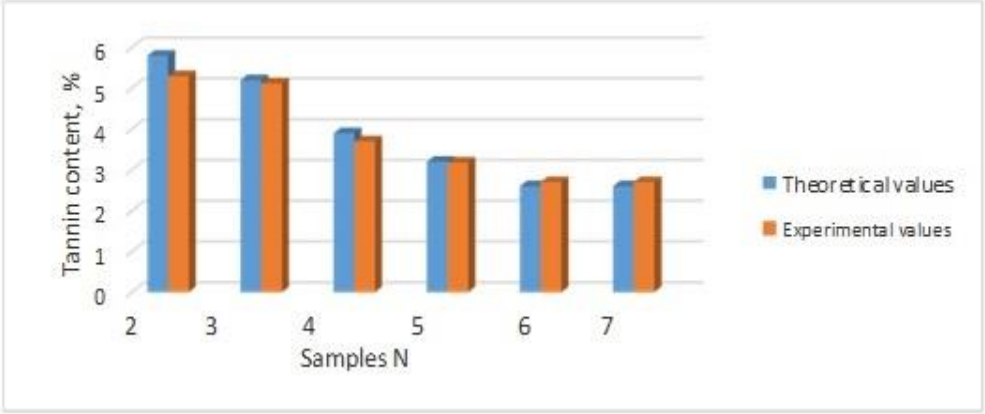

Fig. 2. Comparative diagram of tannins content in samples № 2–7 (blended black tea), calculated theoretically and found experimentally

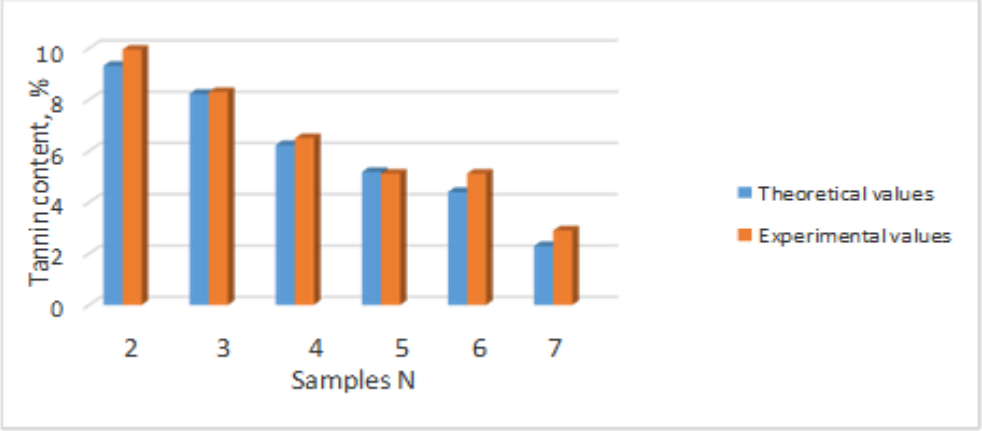

Fig. 3. Comparative diagram of tannins content in samples № 2–7 (blended green tea), calculated theoretically and found experimentally

4. Conclusions

It was found that the addition of chamomile to black or green tea does not affect the tannin content in them. Therefore, it is possible to make blended teas with different content of chamomile, which will have

medicinal properties without losing their properties, i.e. to expand the range of teas.
The standard procedure of titration was altered in such a way that the dilution of filtrate used during the investigation was decreased by 10 times. This modification made it possible to use the regular 250 ml flasks instead of big and inconvenient porcelain bowls. This way, identification of the point of equivalence became easier and more accurate.
The proposed method for determining the tannin content in blended black and green teas has been tested. The results obtained by standard and modified methods are exactly the same.

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