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EVALUATION OF SIGNIFICANT PIGMENTS IN GREEN TEAS OF DIFFERENT ORIGIN

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ABSTRACT

This work monitors flavonoid pigments (theaflavins and thearubigins), and chlorophyll in green teas from different growing regions (India, China, Russia, Vietnam). These pigments affect the quality of the finished tea infusion and their quantity is affected by the way of tea processing (fermentation). 15 samples of green loose teas were selected for the analysis. The content of these pigments was determined using UV-Vis spectrophotometry, the concentration of flavonoid pigments was measured at a wavelength of 665 nm (theaflavins) and 825 nm (thearubigins). Concentration of flavonoid pigments was determined using the standard conversion coefficients, which are 38.7 for TRs and 1.4 for TFs. Chlorophyll concentration was measured at 642.5 and 660 nm. Chlorophyll concentration was determined by the conversion using international standard conversion coefficients and calculation of linear regressions. The results were statistically processed and evaluated in the program of UNISTAT version 5.6. The total amount of chlorophyll ranged from 0.20 to 1.33 mg/L of tea. Concentrations of theaflavin ranged from 0.15 to 0.66 g/100 g of tea and TRs from 2.00 to 11.15 g/100 g of tea. The results showed that the amount of theaflavins, thearubigins and chlorophyll in green teas varied ($P < 0,05$), especially in teas from lowlands in Vietnam and Krasnodar (Russia). Statistically demonstrable difference ($P < 0,05$) in the content of pigments was also recorded in a group of Indian teas (from lowlands) and, from a group of Chinese teas, a statistical difference ($P < 0,05$) was demonstrated in the content of pigments in the mountain teas. On the basis of the results it can be concluded that the quality of green tea is mainly influenced by the processing method depending on the processing area (the factory), than by their country of origin.

Keywords: *Camellia sinensis* L.; theaflavins; thearubigins; chlorophyll; UV-vis spectrophotometry

INTRODUCTION

Green tea is produced of tea leaves (*Camellia sinensis L.*) that have not undergone the process of fermentation. Until recently, the world trade in tea focused almost exclusively on black tea. Nowadays, the situation in this respect has changed and tea fans can choose from many types of green tea **(Arcimovičová, Valíček, 2000)**. It is expected that global consumption of green tea will continue to rise, as the trend especially about its medicinal effects is spreading **(Mitscher, Dolby, 2006)**. World tea production had exceeded 4 million tons per year and is continuously growing. Around the world, green tea represents about 20 per cent of production **(Sang et al., 2011)**. Recently, green tea has been valued for its high content of antioxidants, which are positively applied in the prevention of many diseases, including cardiovascular diseases and some cancers.

The aim of this research is to determine the concentration of important pigments of green tea and thus to evaluate its sensory value (colour, taste) and quality of the analyzed teas, while determining the impact of the growing area on their concentration.

The green tea infusion has a pale yellow color, unlike that of black tea, which is reddish brown **(Owuor, 2003)**. The most numerous group of chemicals that significantly affect the color and sensory profile of green tea are components belonging to the group of polyphenols and chlorophyll. These compounds affect the sensory profile of tea, in particular its color and flavor **(Bernegg, 1991)**.

The polyphenolic compounds influencing the tea infusion color include generally defined flavonoid pigments, such as theaflavins (Figure 1) and thearubigins (Figure 2). Concentration of these pigments increases with the increasing degree of fermentation. Polyphenols (mainly catechins) of green tea leaves are oxidized during fermentation by oxidoreductases in enzymatic browning reactions to the respective o-quinones. These condense with the present catechins of green tea leaves to the pigments of black tea - flavonoid pigments. Therefore, the presence of these pigments in non-fermented tea can worsen its sensory quality; in particular indicate some degree of undesirable fermentation **(Velíšek, Hajšlová, 2009; Taylor, 2003)**.

Flavonoid pigments are responsible for the distinctive dark color and flavor of the tea **(Halder et al., 2005)**. Theaflavins give the tea infusion golden-yellow color and a characteristic brightness and freshness. Thearubigins produce red to brown infusion color and cause astringent taste **(Bhuyan et al., 2009)**. Thearubigins also negatively affect the brightness of tea. Although the concentrations of other chemicals contribute to the quality in the finished tea, it is the concentration of theaflavins and thearubigins that has a great effect on the brightness and color density, astringency, and the overall quality of tea **(Ghosh et al.,**

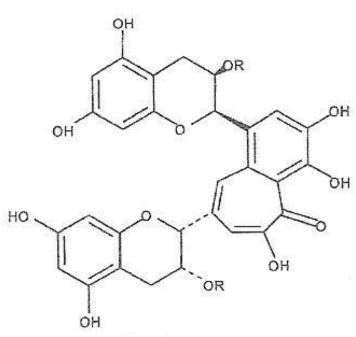

Figure 1 Structure of theaflavin

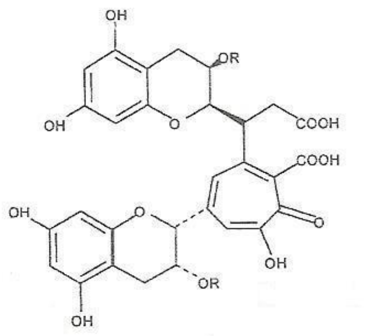

Figure 2 Structure of thearubigin

2012). Theaflavins (hereinafter TFs) are soluble dimeric flavonoids containing a seven-membered tropoline ring **(Velíšek, Hajšlová, 2009)**. TFs are formed by oxidative linking by dihydroxybenzene and trihydroxybenzene rings forming the respective pairs of flavan-3-ols (epicatechin and epigallocatechin respectively) in benzotropolone ring **(Wang, Ho, 2009)**. TFs cause bright red-orange color **(Menet, 2004)**. In black tea, the main theaflavin derivatives are theaflavin (TF_1), theaflavin-3-gallate (TF_2A), theaflavin-3´-gallate (TF_2B), and theaflavin-3,3´digallate (TF_3) **(Liu et al., 2005)**. Thearubigins (hereinafter TRs) are a very heterogeneous mixture of soluble to insoluble oxidation products **(Velíšek, Hajšlová, 2009)**. TRs show a reddish-yellow, orange-brown, reddish-brown to dark brown color **(Menet, 2004)**. Unlike TFs, TRs have not been previously described and are continually under research **(Singh, 2011)**. TRs have a higher molecular mass (700-40000) than catechins and TFs, and are thus ill-defined in terms of chemical structure and biological activity **(Babich et al., 2006)**.

Besides these two pigments, colorless bisflavanols, red theaflavin acids, yellow theacitrins, and brown theafulvins are also formed. During fermentation, about 20 per cent of flavonoids in tea leaves turn into insoluble compounds (apparently they are covalently bound to proteins via their thiol groups) which remain in the remnants of tea leaves when the infusion is prepared **(Velíšek, Hajšlová, 2009)**.

Chlorophyll pigments (chlorophylls) are a group of green pigments which are found in the tissues providing photosynthesis. Among them chlorophyll are the most abundant pigments on the earth. It is a key component of photosynthesis necessary to absorb sunlight **(Hörtensteiner, Kräutler, 2011)**. Originally, chlorophyll only referred to green pigments involved in photosynthesis in higher plants. Later, this term was extended to all photosynthetic porphyrin pigments **(Velíšek, Hajšlová, 2009)**. From the viewpoint of food industry, the greatest importance is ascribed to chlorophylls (chlorophyll *a*, chlorophyll *b*) which are contained in higher plants in a ratio of approximately 3:1. Chlorophyll *a* is present in all plants, the occurrence of the other ones is limited. The difference between them is very small. Chlorophyll *a* is blue-green and it has a methyl group located at the second pyrrole ring. Chlorophyll *b* is yellow-green and it has an aldehyde (formyl) group at the said pyrrole ring **(Ustin et al., 2009; Heaton, Marangoni, 1996)**. Chlorophyll is a very important pigment in tea leaves and unfermented tea, because its concentration influences the final color of the infusion so typical for green (unfermented) tea. During tea fermentation, chlorophyll produces pheophorbines and phaeophytins that contribute to the dark color of black tea **(Harbowy, Balentine, 1999)**. Pheophorbines and phaeophytins are the main degradation products of chlorophyll **(Cartaxana et al., 2003)**.

MATERIAL AND METHODOLOGY

To compare the selected pigments, 15 types of loose green tea were selected from India, China, Vietnam, and Russia. All the analyzed samples are presented in Table No. 1.

Method for determination of flavonoid pigments - theaflavins and thearubigins

The principle of the method was extraction of theaflavins and thearubigins in aqueous solution for a period of 15 minutes and then measuring their absorbance at a wavelength of 665 nm for TFs and 825 nm for TRs compared to water as a blank sample **(Ošťádalová et al., 2010; AOAC, 2009; Perkampus, 1992)**. Concentration of flavonoid pigments was determined using the standard conversion coefficients, which is 38.7 for TRs and 1.4 for TFs. The conversed values of TFs and TRs were expressed as g per 100 g of tea **(USDA database; Bhagwat et al., 2010)**.

*Workflow***:** 3,0 g tea sample was weighed and bathed in 100 ml water at 85 °C. The samples prepared in this way were being extracted for 15 minutes. After cooling down to room temperature (20 °C), the absorption of the samples was measured. In all the cases, the measurement maximum absorption of the absorption spectrum was checked in a broader wavelength range **(Ošťádalová et al., 2010; AOAC, 2009; Perkampus, 1992)**.

Method for determination of chlorophyll

The principle of the method was the extraction of chlorophyll in diethyl ether for a period of 15 minutes and then measuring the absorbance at a wavelength of 500-900 nm in contrast to diethyl ether as a blank sample **(Ošťádalová et al., 2010; AOAC, 2009; Perkampus, 1992)**.

Table 1 Summary of all the analyzed samples of green tea

Sample Number	Tea Name	Origin
1	Darjeeling Green Soureni FTGFOPI	India (Darjeeling Province - Mirik; mountains)
2	Assam Green Tea OP	India (Assam Province; lowlands)
3	Ceylon Sencha	India - Sri Lanka (lowlands)
4	Darjeeling Green Hilton SFTGFOPI	India (Darjeeling Province - Ambiok; mountains)
5	En Shi Yu Lu "Green Dew"	China (Hubel Province; mountains)
6	Gunpowder Temple of Heaven	China (Zhejiang Province; mountains)
7	Yunnan green	China (Yunnan Province; mountains)
8	China Sencha	China (En Shi Province; mountains)
9	Green tea	Russia (Sochi region; lowlands)
10	Green tea "Class 1"	Russia (Krasnodar region; mountains)
11	Green tea "Extra"	Russia (Krasnodar region; mountains)
12	Green tea - hand made	Russia (Dagomis region; lowlands)
13	Vietnam Tea	Vietnam (lowlands)
14	Vietnam Ché ngon So	Vietnam (mountains)
15	Ché uốp hoa nhai with jasmine - hand made	Vietnam (lowlands)

Chlorophyll concentration was determined by the conversion using international standard conversion coefficients and calculation of linear regressions:

$\mathbf{ch_c = 7.12 \times A_{642.5} + 16.8 \times A_{660}}$, where
ch_c….. concentration of total chlorophyll in tea infusion [mg/g of tea]
A_{660nm} … absorbance value measured at 660 nm
$A_{642.5nm}$ … absorbance value measured at 642.5 nm

Workflow: 1 g tea samples were weighed and bathed in 10 ml diethyl ether. The samples prepared in this way were being extracted for a period of 15 minutes and then their absorbance was measured. In all the cases, the measurement maximum absorption of the absorption spectrum was checked in a broader wavelength range (Ošťádalová et al., 2010; Britton et al., 1995).

Statistical analysis: All measurements were performed 3 times for each analyzed tea sample, and then average value and standard deviation were calculated. Parametric double-sided Student's test (P <0.05) was used for the evaluation of the measured data. The calculations were computed by rational calculations using the UNISTAT software, version 5.6. Light.

RESULTS AND DISCUSSION

The average concentrations of flavonoid pigments, in particular theaflavins and thearubigins, in individual types of green teas analyzed, including standard deviations, are listed in the following Table No. 2. The lowest values of chlorophyll are highlighted therein.

As i tis shown in Tab. No. 2, theaflavin concentrations ranged from 0.15 to 0.66 g/100 g of tea and TRs from 2.00 to 11.15 g/100 g of tea. In accordance with **You et al. (2011)**, the average content of TFs in green teas should be about 0.34 per cent and, as stated by **Wang (2010)**, the concentration of TRs in green tea should not increase the average to 8 per cent, as this may impair the quality. **You et al. (2011)** further report that higher concentrations of TFs in green teas may indicate their partial fermentation, which points to their imperfect processing (heat treatment) or a long period of storage. This value in green tea is sensory acceptable, but the quality is not very good, as stated **Owuor (2003)**.

An interesting finding is that the highest values (P <0.05) of both pigments were recorded in the same teas, namely in tea No. 3 originating from India and in Vietnamese tea No. 15. Compared to other samples of green tea, statistically proven (P <0.05) higher TFs values were identified for the Chinese tea No. 7, Russian teas No. 9 and 11 (from the Russian region of Krasnodar), and Vietnamese tea No. 13. For all the above-listed tea samples, pigment content was found in higher values (then published in above mentioned literature, which is higher than 0.34 per cent for TFs and 8 per cent for TRs). According to proportional values of TFs and TRs, it can thus be assumed that undesirable fermentation had occurred in these teas. Furthermore, the above can also be explained by the fact that both Russian and Vietnamese teas came from the same area and were processed in the same factory, therefore, under the same conditions, the so-called tea formulas.

As stated by **Obanda et al. (2001)**, the amount of flavonoid pigments depends on the original content of catechins in green tea leaves and subsequent fermentation. Higher concentrations of catechins in tea plants come from higher elevations and, as reported by **Brown et al. (2003)**, a higher concentration of flavonoid pigments may be caused by a higher content of catechins in the original green tea leaves. However, this is in contradiction to our results, because teas from mountain areas have a lower content of flavonoid pigments (approximately 3 g/100 g of tea; total amount TFs and TRs). According to our results, it cannot therefore be assumed that the contents of TFs and TRs is influenced by the tea growing regions, but by the processor.

In the further research, we focused on the analysis of chlorophyll as an important pigment of green tea. The results are presented in Table 3. The lowest values of chlorophyll are highlighted therein.

The total amount of chlorophyll ranged from 0.20 to 1.33 $mg.L^{-1}$ of tea. In his work, **Wolf (1959)** reported the average chlorophyll content in green tea in the amount of 1.39 mg per 1 g of dry leaves. Similar data have recently been published also in the works by **Wright (2005)** and **Lornaty et al. (2010)** who found in unfermented tea leaves an average of 1.2 mg of chlorophyll per 1 g of dry matter.

Except for samples No. 3 (India), 7 (China), 9, 11 (Russia), 13, and 15 (Vietnam) where the lowest chlorophyll amounts of all the analyzed green teas were found ($P < 0.05$), teas in our research corresponded to the above values.

The samples of green teas included in our analyses with the low total chlorophyll results rather correlated with the values of chlorophyll corresponding to partially fermented or fermented teas. Chlorophyll content in the fermented tea ranges from 0.9 mg to 0.1 mg per g of dry leaves, as stated by **Lornaty et al. (2010)**. Moreover, these are the same samples of green teas, in which statistically significantly higher values of flavonoid pigments were found (concentrations rather equivalent to fermented tea). Thus it can be assumed that these teas underwent a process of fermentation which is undesirable for green tea. Which can also be confirmed based on the results by **Khamessan, Kermasha (1995) and Daooda (2003)**.

Table 2 Average amounts of TFs and TRs [g/100g of tea] in individual samples of the analyzed green teas

Tea Samples	TFs	TRs
India		
1	**0.23** ±0.20	**2.34** ±0.75
2	**0.30** ±0.10	**4.31** ±0.88
3	**0.66** ±0.15	**10.49** ±0.05
4	**0.35** ±0.25	**5.86** ±0.09
China		
5	**0.21** ±0.12	**3.51** ±0.07
6	**0.45** ±0.07	**6.80** ±0.69
7	**0.50** ±0.16	**8.31** ±0.10
8	**0.34** ±0.12	**5.13** ±1.2
Russia		
9	**0.54** ±0.08	**10.20** ±0.33
10	**0.15** ±0.10	**2.00** ±0.09
11	**0.54** ±0.25	**10.49** ±0.21
12	**0.21** ±0.36	**3.27** ±0.29
Vietnam		
13	**0.58** ±0.22	**10.01** ±0.22
14	**0.25** ±0.21	**3.12** ±0.19
15	**0.64** ±0.11	**11.15** ±0.05

Table 3 Average amount of chlorophyll [mg.L-1g of tea] in individual samples of the analyzed green teas

Tea Samples	Chlorophyll
India	
1	1.15 ±0.57
2	1.22 ±0.12
3	0.20 ±0.36
4	1.28 ±1.25
China	
5	1.28 ±1.01
6	1.18 ±0.99
7	0.68 ±0.12
8	1.33 ±0.33
Russia	
9	0.28 ±0.18
10	1.13 ±0.25
11	0.31 ±0.52
12	0.88 ±0.10
Vietnam	
13	0.58 ±0.08
14	1.22 ±0.09
15	0.62 ±0.02

Changes in chlorophyll concentration in different varieties of tea are explained by the fact that, after incomplete heat treatment of tea leaves and their subsequent fermentation, enzymes of chlorophyllase and chlorophyll degrading enzymes remain active, which results in chlorophyll degradation to pheophorbines and phaeophytins, their compounds play an important role in providing the typical dark color of fermented tea, during the processing.

In view of the growing area, its effect on the concentration of chlorophyll cannot be directly determined. No differences in chlorophyll content among the samples of green teas originating from lowlands and mountainous areas of the same origin were statistically demonstrated ($P < 0.05$). As stated by **Wei et al. (2011)**, although the amount of chlorophyll is affected by the cultivation conditions when its quantity increases in tea plants grown at lower temperatures and higher relative humidity, its production is, however, most affected by photosynthesis which is the most intense in young leaves **(Khamessan, Kermasha, 1995)**. Thus it can be concluded that the content of chlorophyll is mainly influenced by the way of tea leaves processing.

CONCLUSION

The aim of the study was to evaluate the content of major pigments (theaflavins, thearubigins, chlorophyll) in green tea affecting the sensory profile and quality of teas. It was found that teas of the same origin from different provinces differ statistically ($P < 0.05$) in their content of flavonoid pigments and chlorophyll. Specifically, it was the Russian tea from the mountainous region of Krasnodar and Vietnamese teas grown in the lowlands. Statistically demonstrable difference ($P < 0.05$) in the content of pigments was also recorded in a group of Indian teas (from lowlands) and, from a group of Chinese teas, a statistical difference ($P < 0.05$) was demonstrated in the content of pigments in the mountain tea En Shi Yu Lu “Green Dew”. Other analyzed samples of green tea reached the values of pigments in similar concentrations typical for unfermented tea. Therefore, it cannot be directly determined whether the altitude of the growing region affects the final content of sensory important pigments. On the basis of the results it can be thus concluded that the quality of green tea is mainly influenced by the processing method depending on the processing area (the factory), than by their country of origin. Based on the total evaluation, it can also be noted that a tea from the retail market does not always match the quality standards and its sensory value (color and taste) does not always have to be constant.

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