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Investigation, identification and determination of natural coloring compounds in black tea

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Abstract

In this study, in order to investigate the natural colorants of black tea, various test methods were developed and set upped. For this purpose, methods for measuring natural tea pigments, polyphenols, synthetic dyes, some metals and the pH of the extract were developed. The results of measuring natural tea pigments showed that Iranian tea has higher natural pigments (chlorophyll, pheophytine and their derivatives) than foreign tea. The results of analysis of polyphenols showed that foreign tea compounds have higher polyphenolic compounds and therefore the colour of the extract was much richer than the colour of Iranian tea extract. Examination of synthetic colours also showed that there is no synthetic colour in any of the foreign or Iranian tea samples. Heavy metal testing with ICP-OES also showed that foreign tea has more metals, especially aluminium, than Iranian tea. Also, measuring the pH of Iranian and foreign brewed teas showed that the extract of foreign teas is more acidic than the extract of Iranian teas. According to the results, it can be concluded that the origin of the difference in the colour of brewed tea extract is primarily due to the variety of tea itself and its proper processing and fermentation process, which can lead to higher formation of theaflavins and thearubigins (natural red pigments). On the other hand, high concentrations of aluminium and polyphenolic compounds in foreign tea can also lead to the formation of coloured complexes (orange to red) and more colourful tea colour.

Keywords Black tea, Natural pigments, Synthetic dyes, Pheophytin, Polyphenolic compounds, Aluminium, Liquid chromatography

Introduction

Tea is a product that is obtained from buds, leaves and young crisp stems of varieties of the tea plant with the scientific name Camellia sinensis (L) O. Kuntze and is available in fermented (black tea), non-fermented (green tea) and semi-fermented forms. (Oolong) is consumed. Black tea is the main form of tea, which is obtained by using an accepted method of tea making, which is based on the plus process, rubbing, oxidation (fermentation) and drying.

Tea processing plays a very important role in creating the final quality of black tea. Among the various stages of tea processing, the oxidation stage is more important than the other stages, and it is in this stage that many quality characteristics of dry tea such as color, aroma and taste are created [4]. The color compounds that are naturally present in tea are: theaflavin (yellow), theaflavin gallates (golden yellow), theorubigin (red-brown), flavonol gly-

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cosides (pale yellow), pheophorbide (brown), pheophytin (black), and carotene (yellow).

During oxidation, the biochemical substances of green leaves, including polyphenols, amino acids and other substances, are transformed into compounds that create the quality characteristics of extracted black tea during enzymatic or non-enzymatic reactions. The main compounds that are formed during oxidation are theaflavins (TF) and thearubigins (TR). These compounds have an important effect on the quality characteristics of black tea [4]. Theaflavin can be seen in yellow-orange color in the solution and plays a role in measuring clarity, which is a desirable characteristic of tea. The total concentration of theoflavins in black tea is about 1 to 3%. Theorubigin appears red to brown in solution. Thearobigins play an important role in creating total color, which is a quality indicator in tea [11]. Factors that affect the formation and decomposition of theaflavins and thearobigins also affect the final quality of tea. The amount of production of theaflavins and thearobigin in different cultivars during the fermentation process is different. Also, each cultivar produces different amounts of theaflavin and thearobigin at different harvest times. The amount of theoflavins and thearobigins also changes under the influence of temperature and duration of fermentation [4].

Deka and Bhattacharya [2] by examining the quality indicators of black tea at different harvest times of green tea leaves concluded that the fermentation time in the same group is different at different harvest times. Also, Obanda et al. [11] investigated the changes of theaflavins and thearobigins during the fermentation process and reported that with the increase in the fermentation time, the amount of theaflavins and transparency decreased, but the amount of thearobigins and the total color increased. Finds. These reports and other similar reports show the effect of different factors on the amount of theoflavins and thearobigins in black tea and its effect on the clarity and overall color.

In this study, it has been tried to investigate the factors affecting tea color change as an important quality index. Despite the fact that Iran is one of the important tea-producing countries and the produced tea is of high quality, but due to its low coloring and late brewing, it has less acceptability and consumption in the society than imported foreign teas. The high consumption of foreign tea in Iran is in a situation where the society considers foreign tea to have artificial color, despite the fact that based on the characteristics listed in the national standard No. 623 "Black tea - characteristics and test methods" and the test of imported samples Such a thing has not been proven. Therefore, the purpose of this research is to investigate, identify and measure the natural coloring compounds in black tea in order to prove or disprove this hypothesis that the reason for the high coloring of black tea in a very short time is the presence of natural colorants in all types of tea. It is black or the addition of artificial dyes or its quality.

Materials and Methods

32 samples of domestic and imported tea from different brands available in the market were prepared and the measurement of artificial colors, polyphenols, chlorophyll pigments and heavy metals was done on them.

Artificial color identification test was performed by thin layer chromatography (TLC) method with a little modification according to the method of Kusmita et al. [9]. The pigment composition of tea extract was analyzed using silica gel GF254 as the stationary phase and hexane: diethyl ether: acetone (2:3:6, volume/volume) as the mobile phase. The color of each spot on the TLC plate was observed and the Rf value was calculated.

Extraction and measurement of chlorophyll pigments with the help of KNAUER Euro Chrom high performance reverse phase liquid chromatography device, made in Germany, equipped with K1100 four-channel pump and K-2600 UV-Vis detector, with a little modification according to the method of Hornero-Méndez et al. and INSO 14838 completed [5 & 6]. Separation was done using C18 column. Pigment washing was done using the mobile phase of acetone: methanol: water (4:36:60, volume/volume percentage) in an isocratic manner and with a flow rate of 1.5 mL min-1. The injection volume was 20 microliters and the detection wavelength was 410 and 660 nm.

Extraction and measurement of polyphenolic compounds by reverse phase high performance liquid chromatography KNAUER model EuroChrom made in Germany equipped with four channel pump model K1100 and UV-Vis detector model K-2600 with a little modification according to He et al.'s and INSO 8986-2 methods [3 & 7]. The separations were done in a C-8 chromatographic column with a length of 20 cm, an inner diameter of 4.6 mm and filled with particles of 5 micrometers (25mm × 4.6mm, 5µm). Separation was done using C18 column. Gradient elution using 3 mobile phases (according to Table No. 1) was used to separate polyphenolic compounds.

Table 1 Gradient washing conditions of polyphenolic compounds in tea extract

Time	Mobile Phase A	Mobile Phase B	Mobile Phase C
0	100	0	0
10	100	0	0
25	68	32	0
35	68	32	0
36	0	100	0
46	0	100	0
47	0	100	100
73	0	100	100
74	100	0	0
85	100	0	0

ICP-OES device was used to identify and determine the amount of aluminum, iron, copper, cobalt, nickel, manganese, chromium, magnesium, calcium and sodium metals according to Ahmadi Moghaddam et al.'s [1].

Results and Discussions

pH of brewed tea extract

In order to investigate the possibility of adding alkaline compounds to the tea sample to intensify the color of the brewed tea, the pH of the tea samples was measured. The results are reported in Table 2.

Table 1 Gradient washing conditions of polyphenolic compounds in tea extract

Foreign tea		Domestic tea	
Sample Number	**pH of extract**	**Sample Number**	**pH of extract**
1	5.325	9	5.625
3	5.315	2	5.621
4	5.341	23	5.611
5	5.324	24	5.612
6	5.313	25	5.609
7	5.355	26	5.617
8	5.346	27	5.619
10	5.324	28	5.621
11	5.322	29	5.628
12	5.327	30	5.625
13	5.326	31	5.622
14	5.317	32	5.626
15	5.318		
16	5.327		
17	5.323		
18	5.324		
19	5.333		
20	5.327		
21	5.329		
22	5.324		

The results show that the pH of domestic and foreign tea samples is in the acidic range. However, the Lunkes & Hashizume [10] studied the pH of teas commercially available in Brazilian market and they found that the mean pH values for the brewed teas ranged between 6.75 and 7.89 (alkaline range), which can be attributed to the difference between tea varieties.

Also, the results indicate that the pH of the brewed extract of domestic tea samples is about 0.3 units higher than that of foreign tea samples. This result shows that the addition of alkaline compounds such as sodium bicarbonate, etc., is not possible to intensify the color, and the low pH of foreign samples can be attributed to the production process and better fermentation process of foreign samples.

Identification of artificial colors in tea samples

The test to identify artificial colors (acidic or basic) in domestic and foreign tea samples showed that there is no artificial color in any of the tea samples. The results of the investigation and identification of artificial colors show that the origin of the difference in color intensity of foreign tea samples compared to domestic tea cannot be caused by the addition of artificial colors and the origin of this color difference is dependent on other factors that will be investigated further.

Identification and measurement of natural chlorophyll pigments

The extracts obtained from the extraction of chlorophyll pigments were injected into a liquid chromatography machine in order to identify and compare this group of compounds in foreign tea and domestic tea under the same conditions as the Iranian national standard number 14838 [6]. At first, in order to identify the peak location of chlorophyll and pheophytin, chlorophyll was extracted from the spinach sample and injected into the device. Next, the extract was acidified to convert chlorophyll into pheophytin to determine the inhibition time of pheophytin.

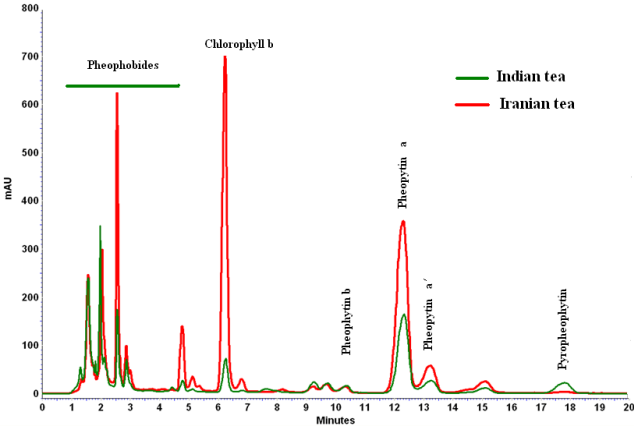

Fig.1 Comparative chromatogram of domestic and foreign tea samples

Chlorophyll pigments include pheophobides, pheophytin a, chlorophyll b, had the highest amount in Iranian tea samples.The results show that the chlorophyll pigments of all domestic tea samples have much higher values than foreign tea samples. In Figure 1, a comparative chromatogram of domestic and foreign tea samples is given. The results indicate that the brewing process leads to the destruction of chlorophyll dyes and their complete metabolism, and in such a way that no peak can be seen in the chromatogram of these compounds at the indicated inhibition times. Yu et al.'s studies showed that chlorophyll decomposition was found closely related with chlorophyllide, pheophorbide, and pheophytin [13]. Therefore, it was concluded that the origin of the difference in the color of Iranian and foreign brewed tea is not related to chlorophyll pigments and we should have searched for other colored compounds in brewed tea.

Identification and measurement of polyphenol compounds

Considering that the chlorophyll compounds were not the source of the color in brewed tea, therefore according to the reports in the sources that also reported the presence of natural polyphenolic pigments in tea, therefore the identification and determination of the amount of polyphenolic compounds in tea was investigated. Polyphenol compounds in black tea include three main categories of catechin compounds, theoflavin compounds and thearogbin. Therefore, it was tried to set up a separation method based on liquid chromatography to separate and measure these compounds. By making modifications in the Iranian national standard 8986 -2 gradient washing program [7], it was possible to separate these three groups of compounds. In Figures 2-3, the chromatograms of two samples of foreign and Iranian teas at two wavelengths of 275 nm and 500 nm are superimposed. The results of examining the profiles of foreign and Iranian tea samples show that, in general, the polyphenolic compounds in foreign tea are more than in Iranian tea. Especially, the thearogbin compounds, which are another category of natural tea pigments, are more in foreign tea than in Iranian tea, which can be concluded that one of the main reasons for the color difference in foreign tea and Iranian tea is related to this category of compounds.

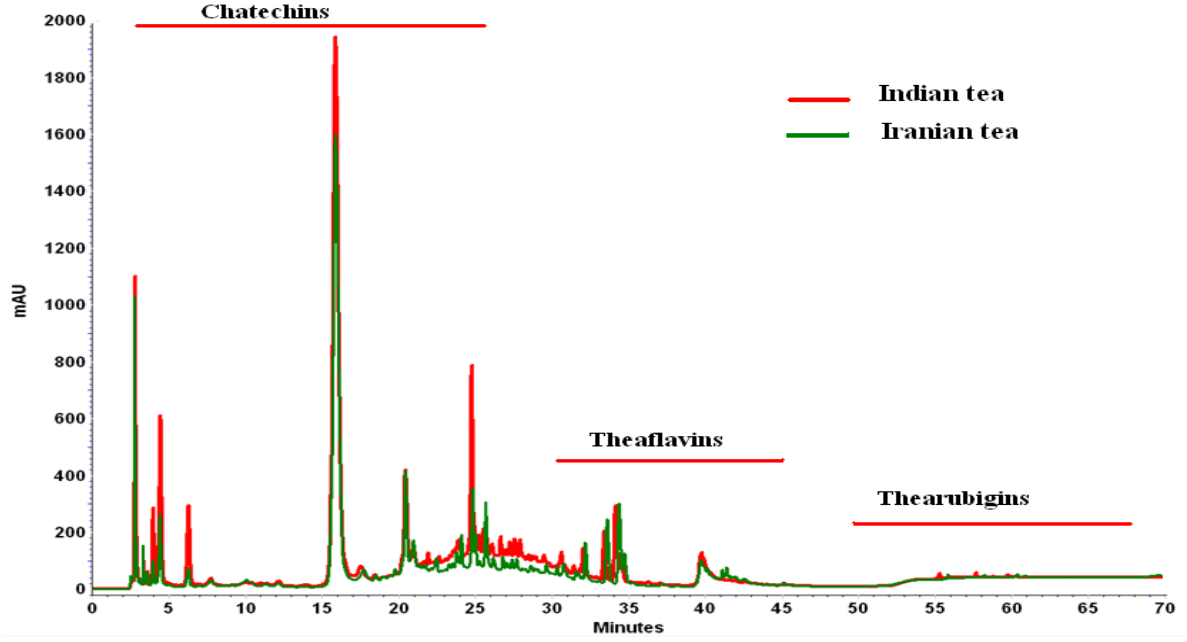

Fig.2 Chromatogram comparison of polyphenol compounds of foreign and domestic tea samples at 275 nm

Our results were in agreement with the findings of Karori et al [8] as their studies showed that the cultivar type is critical in determining the polyphenol compound of black teas.

Investigating the amount of metals in tea samples

Some metals such as aluminum and iron can interact with tea constituents such as polyphenols and thus affect the color of tea extracts. In this regard, there are several reports regarding the formation of colored complexes between catechins and aluminum [1 & 12]. Therefore, various metals such as aluminum, iron, cobalt, nickel, copper, manganese, chromium, zinc, calcium and sodium were measured in foreign and domestic tea samples. The results of two foreign and Iranian tea samples are given in Table 3.

The results showed that the concentration of metals such as aluminum, iron and manganese in tea samples is very high, which requires serious investigation and determination of the permissible limit for it. On the other hand, contrary to the initial assumption, the amount of aluminum in domestic tea samples is higher than that of foreign tea samples, and the hypothesis of intensifying the color of foreign tea due to the formation of a complex of catechins with aluminum is reduced.

Table 3 Test results of metals in tea samples

Metals	Concentration of metals in tea samples (mg Kg^{-1})			
	Domestic tea		Foreign tea	
	Case 1	Case 2	Case 1	Case 2
Al	664	791	411	611
Fe	212	256	99	138
Co	<LOQ	<LOQ	<LOQ	<LOQ
Ni	<LOQ	<LOQ	<LOQ	<LOQ
Cu	<LOQ	<LOQ	<LOQ	<LOQ
Mn	926	1078	634	769
Cr	<LOQ	<LOQ	<LOQ	<LOQ
Zn	<LOQ	<LOQ	<LOQ	<LOQ
Mg	1918	1823	1897	1807
Na	481	329	301	340

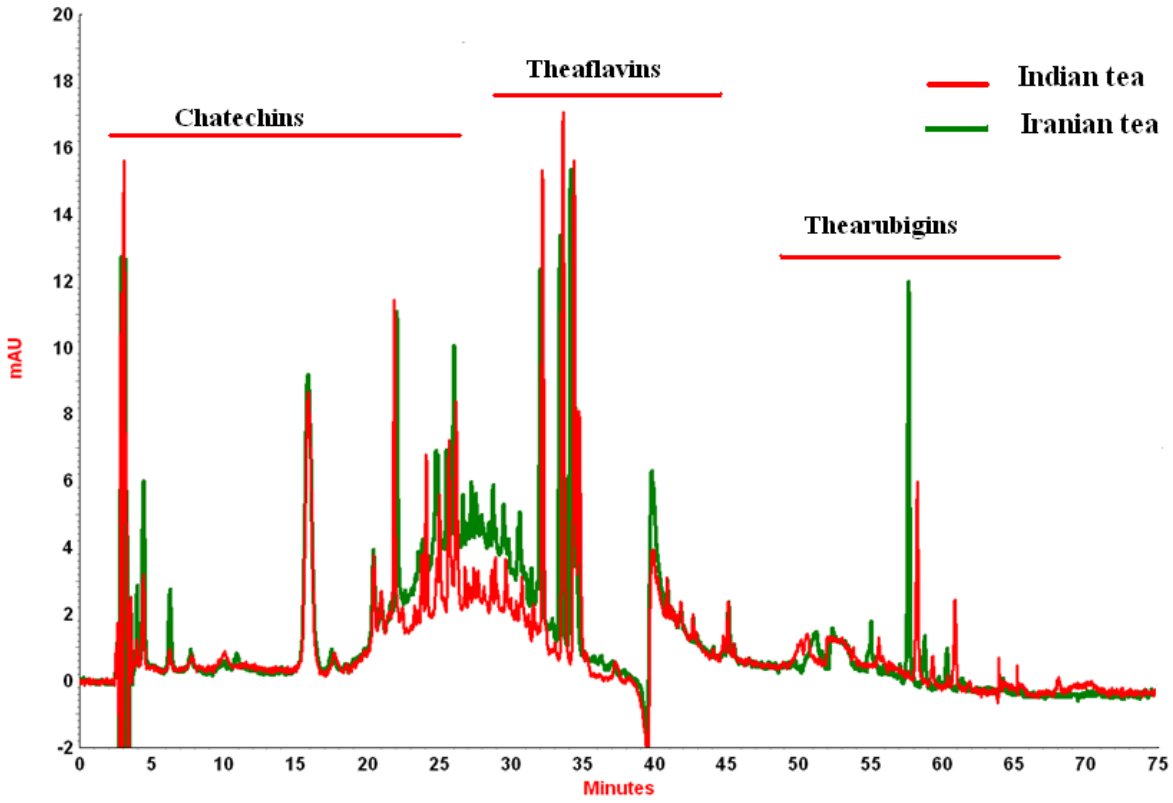

Fig.3 Chromatogram comparison of polyphenol compounds of foreign and domestic tea samples at 500 nm

Conclusions

According to the various doubts regarding the quality of Iranian and foreign teas, various parameters that can affect the color intensity of tea such as the investigation of natural chlorophyll pigments, polyphenolic compounds, metals, the presence of artificial colors and also the pH of the extract Brewed tea was studied and reviewed. The analysis of foreign and domestic tea samples for the presence of artificial color showed that none of the foreign and domestic tea samples contain artificial color. The investigation of natural chlorophyll pigments showed that Iranian tea has higher chlorophyll pigments than foreign tea, but the brewed extract of both Iranian and foreign teas did not contain any chlorophyll pigments. Also, the examination of polyphenolic compounds of tea shows that the amount of these compounds in foreign tea samples is higher than Iranian tea and thearogbinic compounds (red pigments) in foreign teas are much higher than Iranian tea. Also, the oxidation process revolves around polyphenols, particularly the enzymes polyphenol oxidase and peroxidase. When the cells inside tea leaves are damaged and the components inside mix and are exposed to oxygen, a chemical reaction begins. This reaction converts the polyphenols known as catechins into flavanoids called theaflavins and thearubigins (which are also polyphenols). Theaflavins provide tea with its briskness and bright taste as well as its yellow color, and thearubigins provide tea with depth, body and its reddish color. Chlorophylls are converted to pheophytins and pheophorbides (pigments that lend to the black/brown color of dry oxidized tea leaves) during oxidation. Therefore, one of the reasons for the difference in the color of Iranian and foreign teas can be attributed to the oxidation process in tea leaves, so that the intensity of the oxidation process in the production process of black tea is effective in its coloring.

On the other hand, the analysis of metals in domestic and foreign tea samples shows that the amount of metals such as aluminum, manganese and iron in the tea samples is very high, which requires further investigation regarding the permissible limit for this category of metals. In general, according to the results obtained and the results available in the sources, it can be concluded that the origin of the difference in the color of the brewed tea extract is primarily caused by the variety of the tea itself and its proper processing and oxidation process, which can lead to higher formation of theoflavins and thearogbins (natural red pigments). On the other hand, the high concentration of aluminum and polyphenolic compounds in foreign tea can also lead to the formation of colored complexes (orange to red) and the color of the tea becomes more colorful. Also, the presence of turbidity in the cooled foreign tea extract can be attributed to the low solubility of heavy polyphenolic compounds (theaflavins and thearogbins).

Funding
This project was done with the financial support of the National Standard Organization (Project ID 98028).

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: Oct. 2022 Accepted: Jan. 2023
Published online: Apr. 2023

DOI: 10.22034/ASAS.2023.359580.1012

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