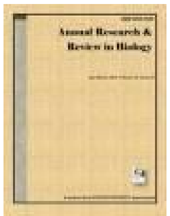

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Research on the Effect of Pigments Rate on Quality and Quantity of Secondary Metabolites in *Camellia sinensis* L. in 13 Experimental Clones and Comparison in Two Times of Spring and Summer

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Author's contribution

This work was carried out by one author. Author PR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Also author PR managed the analyses of the study and managed the literature searches. Author PR read and approved the final manuscript.

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ABSTRACT

The present study was conducted in 2009 in order to investigate the effects of chlorophyll and carotenoid on determining the quality and quantity of dry tea that was obtained from the selective tea clones and was available in research bases supervised by the National Tea Research Center. To carry out the experiment, random blocks were selected and 13 treatments with 3 repetitions were applied twice (once in the spring and another in the summer). Samples were transferred to the laboratories of the national tea research center in order to measure chlorophyll and carotenoid amounts and qualitative and quantitative characteristics such as caffeine, tannin, Theaflavin (TF), Thearobigin (TR), transparency, function, and total colour. Assessing the amount of chlorophyll and carotenoid was carried out by extracting green tea leaves with methanol and reading it with spectrophotometer; wavelengths of 666 nm, 653 nm, and 470 nm were respectively applied for chlorophyll a, chlorophyll b, and carotenoid. Data analysis was conducted using SPSS and MSTAT-C.

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The results of variance analysis showed that there was a significant relation between genotypes and Isolation time. They also indicated that genotype and sampling time had mutual effect on each other. Moreover, mean comparison was conducted through Duncan and Sheffe method, the results of which showed that genotype means place in different groups based on different features. Also, assessing correlation of features indicated that pigments rate had a negative correlation with quality factors such as Theaflavin (polyphenol), Thearobigin (Alkaloid), and tannin (polyphenol). It was also concluded that, pigments like chlorophyll and carotenoid had a significant positive relation with tea quality. According to the results of the study, it seems that colon 100 with higher amount of product, lower rate of chlorophyll and carotenoid pigments, and more polyphenols and alkaloids had better performance.

Keywords: Tea; chlorophyll; carotenoid; thearobigin, theaflavin, transparency, function.

1. INTRODUCTION

Tea is one of the oldest drinks that was first cultivated by China and Japan[1]. Today, it is considered as the national drink in Iran and most countries in the world. Studies conducted by historians indicate that the Chinese knew tea bushes 3000 B.C. Therefore, tea cultivation and consumption were originated in China and goes back about 5000 years. Tea cultivation has developed in Europe since the 15th century. Afterwards, tea cultivation and industry grew fast all over the world [2]. Tea cultivation in Iran was introduced by Haj Mohammad Hossein Isfahani in 1882, contemporary with Nasseraddin Shah's reign. However, it did not develop due to several reasons. Therefore, tea cultivation in Iran was postponed until 1940 and cultivated afterwards following attempts made by a person called Mohammad Mirza nicknamed Kashefossaltaneh who is known as founder of tea cultivation in Iran [3].

Like coffee and cocoa, tea is one of the most common drinks in the world. Second to water, it is the most liked drink in Iran. Iran has about 32 thousand hectares of tea garden along Caspian Sea in Guilan and Mazandaran provinces with an annual capacity of 60,000 tons of tea production. Per capita consumption of dry tea in Iran is estimated to be 27 kg in year. Tea cultivation in Iran, employment of 70,000 families, dozens of tea packaging factories, creating hundreds of other related jobs, and high potential of tea cultivation growth and the industry development have brought this product in the focus of attention [4].

During appropriate seasons on leaf picking, fluctuations in leaf growth depend on photosynthesis stages and the leaf age. Colour change of leaves (paleness) that can be observed during different periods of leaf picking season is a dynamic phenomenon that is reversible through irrigation, rainfall, and fertilization. Colour density of leaves from green to yellow, and vice versa, can be well controlled through complex compounds like chlorophyll, carotenoid, flavonoids, essential anthocyanins which are created in leaf chloroplast on biosynthesis path [5].

The most important components of tea include proteins, carbohydrates, fats and fatty acids, and pigments such as chlorophyll and carotenoid, volatile compounds, phenolic compounds, alkaloids, caffeine and minerals [6].

Owing to containing various phenolic compounds, tea has numerous antioxidant properties [7]. The most prominent compounds existing in tea are polyphenols which include flavonols, flavonoids, flavone DNDLs, and phenolic acids. Compared to old tea leaves, young ones contain more polyphenol. Tannins form about 90% of polyphenolic compounds available in tea, which include catechins of green leaves and their oxidation products such as Teaflavin and Thearobigin. Theaflavin is one of the components of tea that plays an important role in better quality of the tea, is considered as one of the light red pigments of green tea, and is one of the polyphenolic compounds of tea. Theafelavins are antioxidant polyphenols, which are produced from flavone 3Ls of green tea leaves during enzymatic activities (fermentation). Epigallocatechin gallates in green tea are like these compounds [8].

Thearobigins are also polyphonic compounds. The color of black tea depends to a large degree on the amount of Theaflavin, Thearobigin, and the other previously mentioned compounds. Tea contains important alkaloids such as caffeine and theanine that improve nervous system. Caffeine has stimulating effects and is the main cause of relaxing property of tea. It also plays role in quality properties of tea such as pungency, bitterness, and taste [9]. Essences existing in tea also have antioxidant properties [10].

A good tea will be considered as favorable product if its phenolic color components have changed during different stages and given it favorable color and taste [11]. Undoubtedly, more information is needed on the biochemical and physiological role of pigments in tea quality and more research studies need to be conducted. The aims of the present study were investigate of effect of pigments rate(chlorophylls and carotenoid) and another characteristics quality and quantity in 13 clones of tea.

2. MATERIALS AND METHODS

The present study was conducted at Lahijan's Tea Research Station in 2009. A split-plot experiment per unit time in the form of random blocks with 13 treatments (Table 1) and 3 repetitions was conducted twice (one in the spring and another in the summer). Treatments included the selective tea clones. Clones were obtained from tea plantations of Guilan and Mazandaran. In so doing, tea bushes with superior morphological properties were selected and proliferated. After the responses of the plants were observed in circumstances of plantation and store, those plants that had survived and grown better were studied. The amount of fertilizers, weed control, and kind and amount of irrigation were constant for all treatments in question.

2.1 Measuring Carotenoid and Chlorophyll Using Taylor Method (Taylor S, 1992)

Taylor method was utilized to analyze chlorophyll and carotenoid pigments. In so doing, leaves were picked from the pushes through a standard method (i.e., two leaves and one bud were picked). And after they were weighed in order to measure the pigments, they were transferred to the laboratories of the tea research center. After nervures were separated, 0.5 g leaf was weighed and in dark condition abraded in a mortar containing 25 ml pure methanol and finally filtered using a Büchner funnel with the help of a discharge pump. Afterwards, the 2 ml resulted solution was thinned with 8 ml pure methanol, which caused the solution to become 10 ml. Absorption of the resulted extraction in 3 wavelengths of 470 nm for carotenoid, 653 nm for chlorophyll b, and 666 nm for chlorophyll a was read through the model spectrophotometer (ECIL). The amounts of chlorophyll and carotenoid were measured using the following formula:

$$Chla = 15.65A_{666} - 7.340A_{663}$$
$$Chlb = 27.05A_{653} - 11.21A_{666}$$
$$car = 1000A_{470} - 2.860chla - 12.92chlb/245$$

2.2 Measuring Caffeine Using Lakin Method (Lakin A, 1989)

The dried green tea leaves and a certain amount of clone leaf were put is a separatory funnel and normalized ammonium solution 0.88 was added. Ammonium solution created an alkaline environment and caused caffeine extraction to take place better. During the four stages, a certain amount of chloroform was added to the separatory funnel and was stirred slowly. After a while, 2 phases were created. The lower phase (chloroformic) was put in another separatory funnel to which alcoholic potassium was added in order to separate impurities. The chloroformic section contains caffeine. The contents of the separatory funnel were passed through filter paper containing dry sodium sulfate. Caffeine rate was obtained through standard density curve and read at wavelength of 276 nm using spectrophotometer. Caffeine rate was calculated using the following formula:

Caffeine (g per 100 g) = [(sample density according to the standard curve × 100 × inverse dilution factor) ÷ sample weight]

Table 1. The names of the clones tested

Treatments	1	2	3	4	5	6	7	8	9	10	11	12	13
Clones	444	703	548	455	591	468	440	791	2023	100	416	581	578

2.3 Measuring Tannin Percentage Using Gravimetric Method (Smiechowska & Dmowski, 2006) [22]

The sample of dried green tea leaves were used to measure tannin percentage. In so doing, some amount of tea leaf was added to boiling distilled water and kept boiling for 30 minutes. The resulted solution was filtered using filter paper. The collected scum on the paper was washed with distilled water several times and added to the filtered solution obtained from copper acetate. After the formation and sedimentation of deposits, the solution was filtered and the sediment remained on the filter paper that was washed with distilled water and then burned at 600ºC for 30 minutes. To measure tannin percentage, there is a conversion factor that is 6.305. Tannin percentage was calculated using the following formula:

Tannin percentage = [(weight of sediment after burning ÷ initial sample weight) × conversion factor]

2.4 Measuring Theafelavin, Thearobigin, Flower Color, and Transparency Using Mahanta and Baruah Method (Mahanta and Baruah, 1999)

Nine g dried tea leaf taken from the selected clone was boiled in instilled water and put in a bain-marie for 15 minutes. After being cold, it was filtered using a funnel and cotton. Afterwards, 2 ml of the filtered solution was mixed with 2.5 g oxalic acid to make the volume of the solution 25 ml. One g of sodium sulfate was brought to the volume of 100 ml. Twenty ml of that new solution was added to the extract. Moreover, 20 ml of ethyl acetate was added to the separatory funnel solution. Afterwards, it was shaken for 15 minutes so that phase to would form and keep the upper phase.

An amount of 2 ml of separated solution was put in 25-mm balloons (E2, E3). Two ml of oxalic acid and 6 ml of instilled water was added to E3 balloon, and 8 ml of instilled water was added to E2 balloon. An amount of 4 ml of the separatory funnel phase was added to E1 balloon with a pipette. In the first control balloon (B1), 2 ml of ethyl acetate was brought to the volume of 25 ml using methanol. And in the second control balloon (B2), only 8 ml of instilled water was spilled and brought to the desired volume using methanol. In the third control balloon (B3), 2 ml of instilled was reduced and replaced with 2 ml of ethyl acetate and brought to the desired volume with methanol. Afterwards, the absorption figure was read using spectrophotometer at wavelengths of 380 and 460 nm. Following formulas were utilized to calculate the require percentages.

Teafelavin percentage (TF) = 2.25 E1 (380 nm)
Tearobigin (TR) = [7.06 (2E3(380nm)E1 (380nm))]
Flower color percentage = 12.5E2(460nm)
Transparency percentage = [50E1(460nm)/E2 (460nm)]

Statistical tests used in the study was conducted through Duncan and Sheffe method and results investigated in.

3. RESULTS

The amount of chlorophyll available in 13 clone was calculated. The results showed that the amounts of this pigment were significantly different in different clones; such that the highest amount of chlorophyll a was in clone 548 (treatment 3) and the lowest one was related to clone 100 (treatment 10) (Fig. 1). The highest amount of chlorophyll b was in clone591 (treatment 5) and the lowest one was related to clone100 (treatment10) (Fig. 2).The total amount of chlorophyll (a+b) was also the lowest in clone 100 (Fig. 3) . The amount of carotenoid in clone 100 (treatment 10) was the lowest (Fig. 4). The amount of caffeine in studied clones indicated that the highest amount was related to clone 100 (treatment 10) and the lowest one to clone 581 (treatment 12), which was significantly different (P<0/05) from those of other clones (Fig. 5).

The amount of caffeine and tannin in studied clones indicated that the highest amount were related to clone 100 (treatment 10) and the lowest one to clone 581 (treatment 12), which were significantly different (P<0/05) from those of other clones (Fig. 6).

The amount of Teaflavin in studied colons indicated that the highest amount was related to clone 100 (treatment 10) and the lowest one to clone 581 (treatment 12) which were significantly different (P<0/05) from those of other clones (Fig. 7).

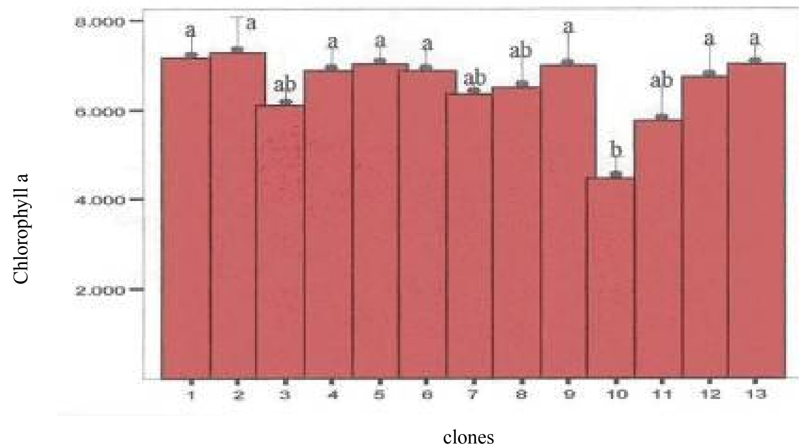


Fig. 1. Changes of Chlorophyll a in tea different clones

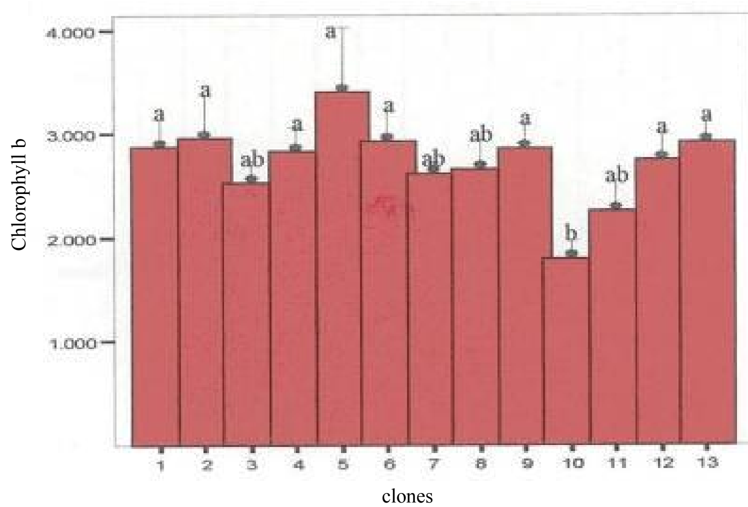


Fig. 2. Changes of Chlorophyll b in tea different clones

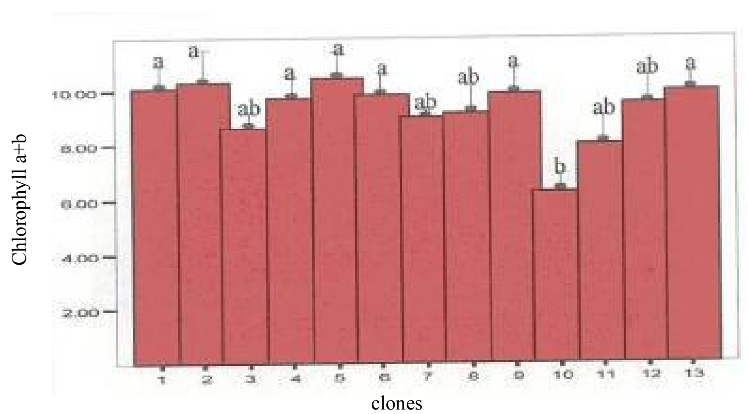


Fig. 3. Changes of Chlorophyll (a +b) in tea different clones

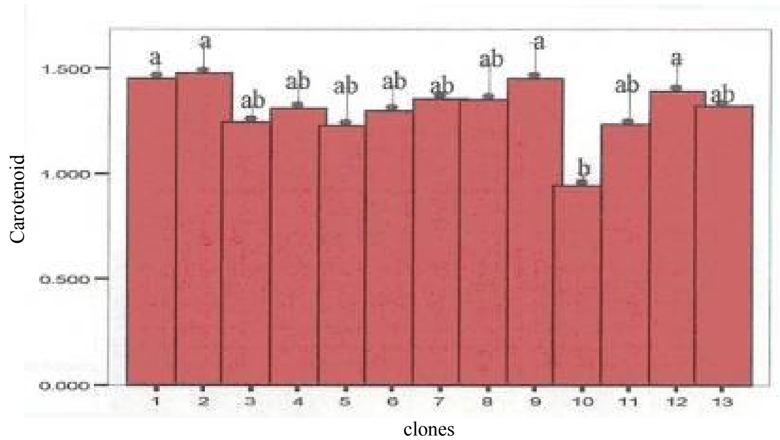


Fig. 4. Changes of Carotenoid pigments in tea different clones

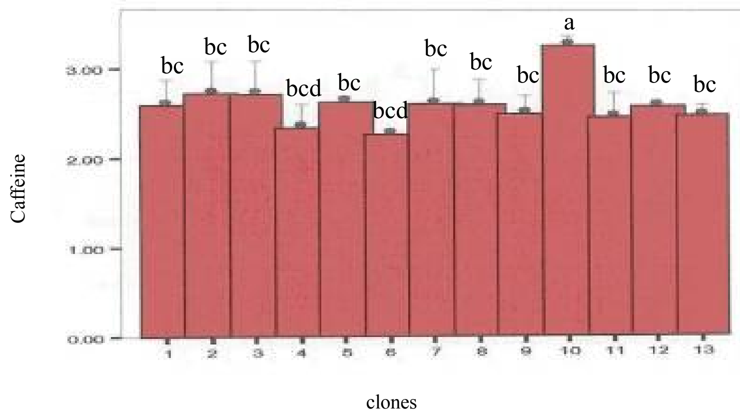


Fig. 5. Changes of Caffeine in tea different clones

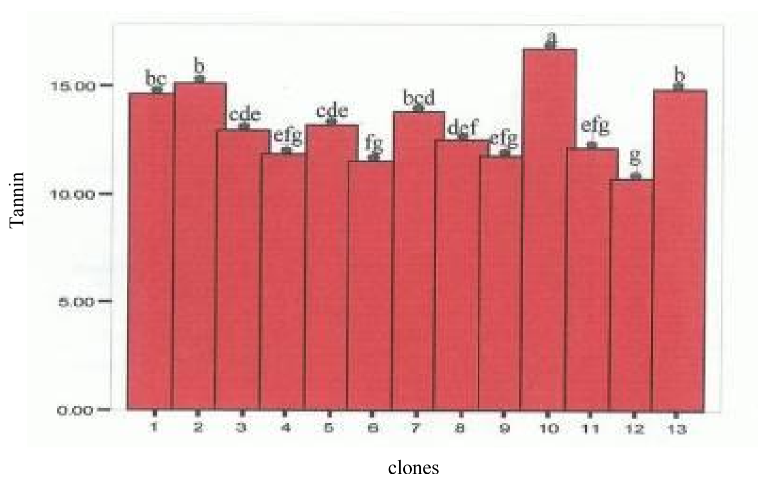


Fig. 6. Changes of Tannin in tea different clones

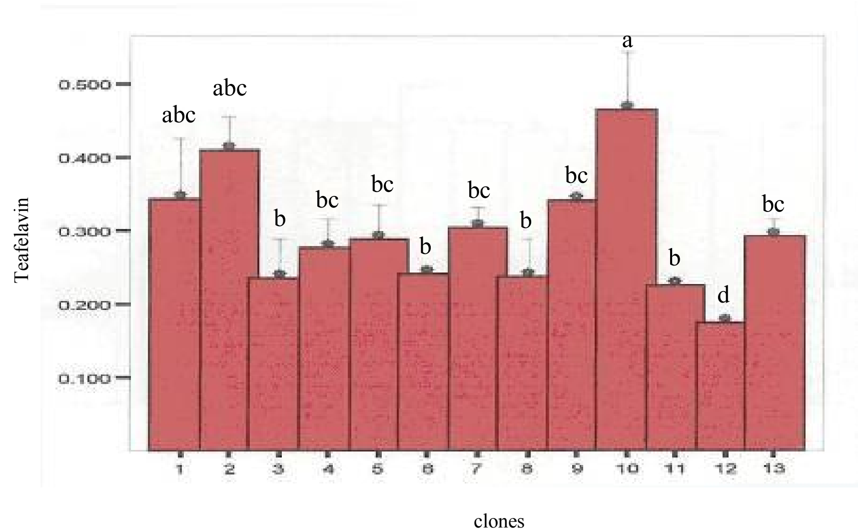


Fig. 7. Changes of Theaflavin in tea different clones

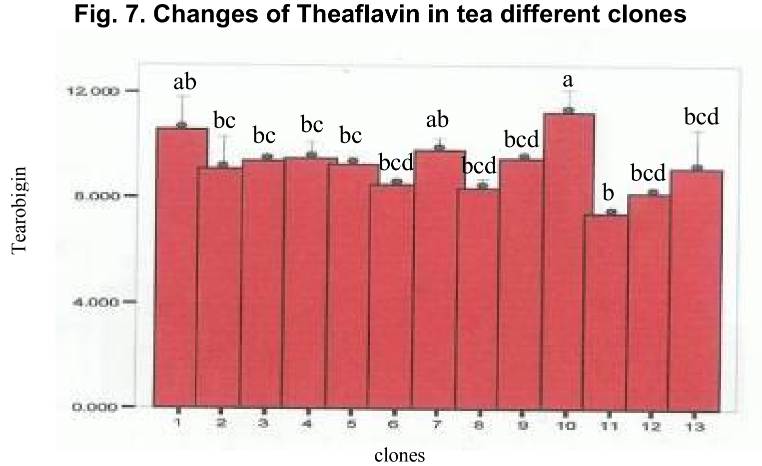


Fig. 8. Changes of Thearobigin in tea different clones

The amount of Tearobigin in studied clone indicated that the highest amount was related to clone100 (treatment10) and the lowest one to clone 416 (treatment11), which were significantly different (P<0/05) from those of other clone (Fig. 8).

The amount of pigmentes such as chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenides in studied were spring higher than summer which wasn't significantly different(P<0/05) from those of other clones (Fig. 9).

The amount of qualities such as caffeine, tannin, Theafelavin (TF), Thearobigin (TR) in studied were spring higher than summer which wasn t significantly different (P<0/05) (Fig. 10). Quantity factors such as transparency, function, and total color in studied were summer higher than spring which wasn't significantly different (Fig. 11). Mean of function in summer was higher than spring which wasn't significantly different (P<0/05) (Fig. 12).

The results of correlation between the properties indicated that there is a negative correlation between the amount of pigments and quality factors such as Teafelavin (TF), caffeine, and tannin. It can be concluded that the less the amount of the pigments (chlorophyll and carotenoid), the higher the quality of dried tea will be. However, there was a significant positive (P<0/05) correlation between chlorophyll a and chlorophyll b at 1% probability.

Level that is as chlorophyll increases, carotenoid will increase. It can be stated that since carotenoids affect tea flavor, they are considered as an important factor in tea quality.

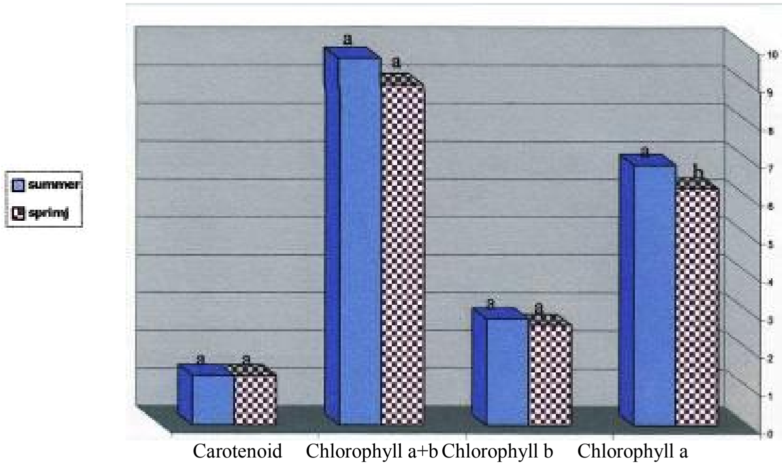


Fig. 9. Mean of pigments in two times of spring and summer

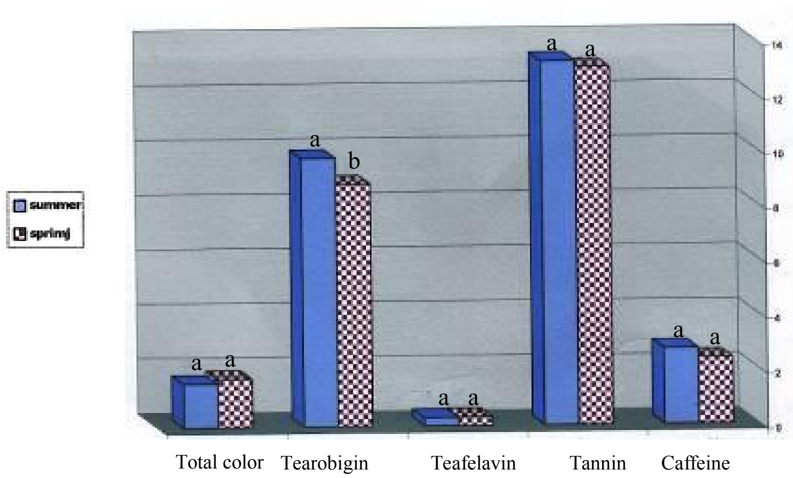


Fig. 10. Mean of qualities in two times of spring and summer

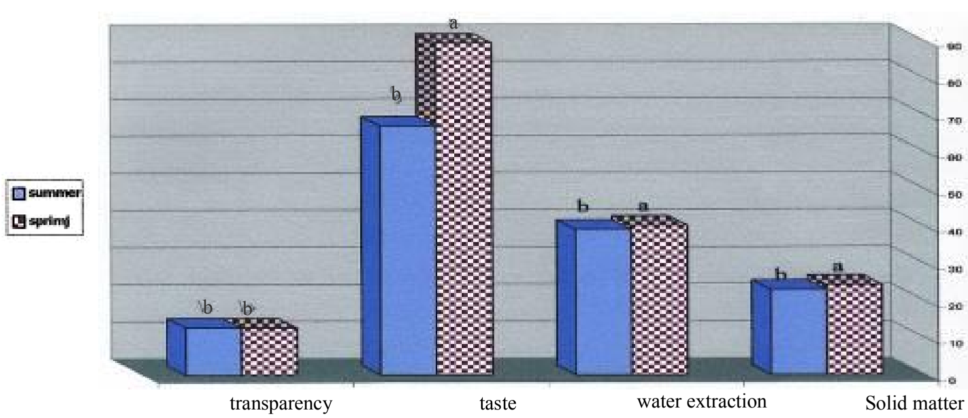
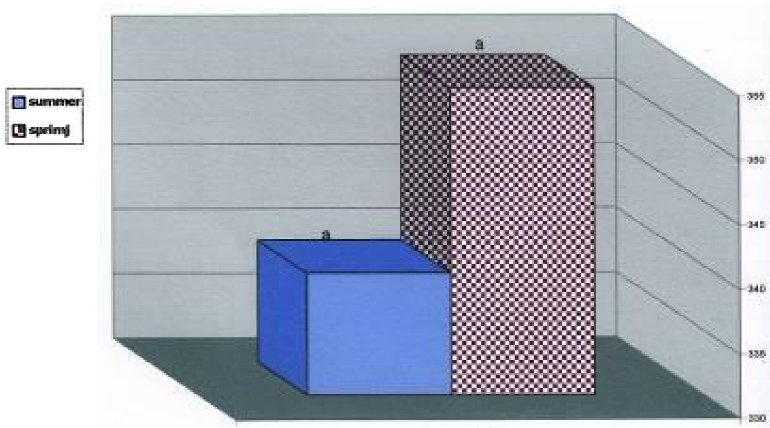


Fig. 11. Mean of quantities in two times of spring and summer



Function

Fig. 12. Mean of function in two times of spring and summer

4. DISCUSSION

The present study is the first investigation conducted on these 13 selective clones of Guilan and Mazandaran in order to choose the best clone with better quality tea. The present study was conducted in order to investigate the effects of chlorophylls and carotenoids on determining the quality and quantity of dry tea.

According to the results of the study, the amount of chlorophyll pigment was different in the 13 selective clones. Clone 100 was proved to have the lowest amount of chlorophyll a, chlorophyll band chlorophyll (a+b), which was related to the effect of environmental factors on pigments.

Liyanage et al. [12] concluded that chlorophyll plays a significant role in tea blackness that is one of the most important factors in commercial evaluation of tea.

After undergoing a lot of changes during fermentation process, chlorophyll turns into pheophytin (black colour) and pheophorbide (brown color). Tea bushes with light colour yield lower quality tea. Ravichandran et al. [13]. reported that tea bushes with light color indicate lower quality tea. Generally, tea leaf blackness is related to its high quality although any leaf with any colour can have high quality. Bera et al. [14] stated that leaves with more chlorophyll produce lower quality tea. However, chlorophyll in tea making processes like fermentation and drying plays a significant role in tea blackness that is one of the most important factors in commercial evaluation of tea. The amount of chlorophyll can change due to factors like leaf type, leaf age, rainfall increase, presence of shade, and different seasons. Based on the investigations conducted on the relation of chlorophyll and carotenoid with antioxidant activities in tea, it can be stated that when medical and health properties are concerned and not tea quality, clones with more pigments are more important because they have more antioxidant activities. In natural conditions, there are a lot of factors that affect tea quality. From among these factors are climate conditions, tea clone, harvest time, cultivation method, and damaging factors. Polyphenols, amino acids, caffeine, tannin, Theaflavin, and Thearobigin enhance tea flavor [15].

Carotenoids are among pigments that significantly affect qualitative and quantitative properties of tea. Carotenoid has a positive correlation with chlorophyll but a negative one with Thearobigin that cause transparency and color in tea. The more the amount of chlorophyll, the more the amount of carotenoid will be. In the present study, from among 13 studied colons, clone 100 had the lowest amount of carotenoid and the highest amounts of Theaflavin and Thearobigin [16,17].

Taylor et al. [18] concluded that β carotene had the highest negative correlation with quality of black tea. The more the amount of carotenoid decreases, the better the flavor of the tea will be. It is likely that during tea production process, changed volatile and non-volatile components are created from β carotene which has a negative relation with quality of black tea [19].

Theaflavins are responsible for the taste, brightness and contributes to the colour of black teas. The thearubigins are responsible for thickness and colour of both the liquors and infusion. These chemical attributes, including black tea brightness and colour are referred to as the plain tea quality parameters. Indeed theaflavins have become a critical parameter in estimating the quality of black teas [20].

The two most important chemical groups that decide the liquor characteristics of black CTC (cut, torn and curled) tea are Theaflavins (TF) and Thearubigins (TR). Hence, a quick estimation of concentration of these compounds can significantly contribute to the evaluation process for the quality of finished tea in an objective manner [21].

There was a significant relation between Theaflavin and black tea quality. Therefore, in most cases Theaflavin of black tea is considered as useful and measurable criterion of tea quality [22]. In their studies, Gud et al. [23] concluded that high amount of Thearobigin can also improve tea quality and is the reason for absence of chlorophyll carotenoid and flavonol glycosides.

The time of cultivate of leaves tea influence on quality and quantity factors such as Theaflavin, Thearobigin, caffeine, tannin, transparency, function, and total color. Climate, tea clones, different times affect on function,quality and quantity [24]. Spring tea is desired in relation to summer tea because of in summer increase fibrous tissue and reduced quality and quantity factors in leaves tea. Geoghraphical area effective in both quality and quatity of aromatic materials similar theaflavin, thearobigin and bitter substances such as tannin, caffeine also transparency, function, a solid matter nd total colour [25]. Obanda et al. [26] in reviews on clone 100 and hybrid types observed that qualitative properties include water extract, caffeine, Theaflavin total colour and brightness in clone 100 better than the hybrid types. According to studies conducted by Rufigari Hagighat et al. [27] clone 100,than for mean of function, and quality is higher than other clones .Therefore clone 100 known hopeful clone that the results agrees with it .

Theaflavins are responsible for the taste, brightness and contributes to the colour of black teas. The thearubigins are responsible for thickness and colour of both the liquors and infusion. These chemical attributes, including black tea brightness and colour are referred to as the plain tea quality parameters. Indeed theaflavins have become a critical parameter in estimating the quality of black teas [28].

5. CONCLUSION

In the present study, from among 13 studied selective clones, clone 100 had the highest amounts of caffeine, tannin, Theaflavin, and Thearobigin and the lowest amounts of chlorophyll and carotenoid, which caused this colon to be selected as the better one among 13 studied clones in terms of tea quality. It was concluded that there was a significant relation between black tea quality and high amounts of Theaflavin, Thearobigin, tannin, and caffeine. Moreover, chlorophyll and carotenoid have negative effect on quality of black tea, which is in line with the results obtained for clone 100.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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