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IMPACTS OF FLUSHING AND FERMENTATION TIMES ON THE QUALITY OF BLACK TEA

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Flushing and fermentation times are the two main parameters determining the quality of black tea. In this research, the effects of different flushing and fermentation times were studied on the quality of black tea in two clones, 100 and natural Chinese hybrid. Analysis of variance showed there were the significant differences between the clone types, flushing and fermentation times for theaflavin, thearubigin, total color, brightness, tannin and caffeine. Correlation coefficients between all studied traits, except thearubigin and brightness, caffeine and brightness, and total colour and theaflavin/thearubigin ratio, were significant at 1% probability. Regression

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analysis indicated there was a significant linear regression between fermentation time and brightness, tannin, and theaflavin/thearubigin ratio. Also, multiple regression analysis for brightness and total colour indicated that more than 70% of the variation in brightness was explained by theaflavin, caffeine and theaflavin/thearubigin ratio, and more than 68% of the variation in total colour was because of caffeine and theaflavin/thearubigin ratio.

Key words: fermentation and flushing time, quality of black tea, tea clone

INTRODUCTION

Tea is one of the most important strategic crops in Iran. About 32000 ha of farmland in Iran are under tea cultivation. Quality of black tea is the important reason for supplying tea to markets. Factors which influence the quality of black tea more are clone type or genotype, methods of flushing, processing and storing. Tea processing plays a role in determining the final quality of its product. Fermentation is the most important stage among different stages involved in tea processing. During fermentation, polyphenols in the green leaves are converted into stable components including theaflavin, thearubigin and other polymers, through enzymatic and non-enzymatic reactions (OWUOR & MCDOWELL, 1994a). Theaflavin and thearubigin are usually produced more during the fermentation and have a major influence on the quality of black tea. Theaflavin has yellowish-orange colour, when it is dissolved and it plays an important role in giving brightness to black tea. Total concentration of theaflavin in black tea is often 1 to 3%. In contrast, thearubigin has reddish-brown colour and play important role in giving total colour of black tea (MAHANTA & BARUAH, 1992). OWUOR *et al.,* (1994b) and OBANDA *et al*., (2004) showed that the amount of theaflavin and thearubigin formed during fermentation period differs in the clone type. This is because of the genetic variation between clones in producing the prematerial of theaflavin and thearubigin. DEKA & BHATTACHARYYA (1997) studied the quality characteristics of black tea in different flushing times and indicated the flushing time has a significant effect on fermentation period of a clone. OWUOR & ORCHARD (1990) and OBANDA *et al*. (2004) studied the changes in theaflavin and thearubigins during the fermentation period and concluded that increasing the fermentation period decreases theaflavin content and brightness of black tea, whereas thearubigin and total color were increased. In this research, the changes of characteristics related to quality of black tea during different flushing and fermentation periods were studied. The objectives of this study were to obtain and use information on nature of relationships between flushing and fermentation times and the quality of black tea.

MATERIALS AND METHODS

Plant Materials

This study was carried out in the research farm of Tea Research Centre of Iran, Feshalam, Guilan, Iran, in 2007. Two tea clones, 100 (an improved tea clone) and natural Chinese hybrid were studied. Both clone were the same age and had similar shrubs. The structure and shape of tea shrubs were also fitted to research objectives. Leaf flushing was conducted in the standard method (i.e. one bud and one leaf, and/or one bud and two leaves) at the three times, June, August and October.

Tea Processing

After each flushing, all of tea leaves were transferred to Kashef Tea Laboratory at the National Tea Research Centre, Lahijan, Guilan, Iran. To reduce humidity, tea leaves were placed in troughs at 25 to 30 ºC for 14 h until their weight dropped to 70% of initial weight. Then, the withered leaves were chopped out with a CTC machine. After chopping, the leaf particles were passed through a sieve (2 mm mesh) and fermented for 30, 60, 90, 120 and 150 Min. Fermented samples were dried at 105 ºC for 27 Min in a fluid bed drier.

Chemical Experiments

Colorimetry experiments were conducted using spectrophotometry based on MAHANTA & BARURAH (1992) method to determine the percentages of theaflavin (TF), thearubigin (TR), total colour (TC) and brightness (BR). Percentage of Tannin (TN) was measured as described by SMIECHOWSKA & DMOWSKI (2006) and percentage of caffeine (CF) was determined using spectrophotometry based on the LAKIN (1989) method.

Statistical Analysis

The experiment was carried out in a split-split plot based on randomized complete block design with three replications. The experimental factors were two clones (100 and a Natural Chinese Hybrid) as the main factors, three flushing times (June, August and October, 2006) as the sub factors and five fermentation times (30, 60, 90, 120 and 150 min) as the sub-sub factors. All statistical analyses including ANOVA, Tukey’s test for mean comparisons, correlation coefficients between traits and regression analysis (simple and multivariate) were carried out using the SAS software version 6.12 (SAS, 1989).

RESULTS AND DISCUSSION

Analysis of variance

Data analysis showed that apart from the genetic variations between clones, differences in climatic conditions during the different flushing and fermentation times can also influence the chemical characteristics of black tea such as TF, TR, TC and BR (Table 1). Regarding the significant interactions between clone × flushing time and clone × fermentation time on TF, TR, TC, BR and TN, it seems that each tea clone has a specific fermentation time for each flushing time. On the other hand, the suitable fermentation time of a clone can not be extended to other clones. This is due to genetic diversity between clones in producing the various amounts of chemical components related to quality of black tea.

Results from analysis of variance for quality characteristics in this study support the findings from OWUOR *et al*. (1994), DEKA & BHATTACHARYYA (1997) and OWUOR & OBANDA (2001). Comparison of means indicated there are two- or three-way significant interactions ($P < 0.01$) between clone, flushing time and fermentation time for studied characteristics of black tea (Tables 2 and 3 a,b).

Table 1 Anova of effects of clones, flushing and fermentation times on the quality of black tea.

Source of variation	DF	Mean squares [a]						
		TF	TR	TC	BR	TN	TF/TR	CF
Block	2	0.0022**	0.0006	0.0011	0.0010	0.001	0.0001**	0.0011**
Clone	1	0.098**	0.4976**	1.327**	0.333**	0.1449**	0.00003	0.3858**
Error= Clone x Block	2	0.0000047	0.0016	0.0009	0.0026	0.0010	0.000006	0.0001
Flushing time	2	0.2133**	0.0693**	0.2872**	0.342**	0.1140**	0.0039**	0.1788**
Clone x Flushing time	2	0.1162**	0.7768**	0.0626**	0.6689**	0.1395**	0.0056**	0.0283**
Error= (Block x Flushing time)/Clone	8	0.0004	0.0005	0.0002	0.0016	0.0003	0.00001	0.0002
Fermentation time	4	0.0768**	0.0697 **	0.0378**	0.5009**	0.0211**	0.0049**	0.0033**
Clone x Fermentation time	4	0.0052**	0.0051**	0.0154**	0.0521**	0.0001	0.00007**	0.0001
Flushing time x Fermentation time	8	0.0022	0.0046**	0.0154**	0.0429**	0.0008*	0.00007**	0.0001
Clone x Flushing time x Fermentation time	8	0.0068**	0.0062**	0.0210**	0.0313**	0.0007*	0.00024**	0.000
Error	48	0.0011	0.00069	0.0005	0.0011	0.0003	0.0000092	0.0001

*, ** Significant at 5% and 1% level of probability, respectively.

[a] The symbol of characteristics includes: Theaflavin (TF), Thearubigin (TR), Total color (TC), Brightness (BR), Tannin (TN), Theaflavin to Thearubigin ratio (TF/TR), Caffeine (CF).

Table2.Comparison of means of three way interactions of clone × flushing time × fermentation time for significant characteristics

Characteristics [a]	Clone	Flushing time	Fermentation time (Min) [b]				
			30	60	90	120	150
TF	100	June	1.13^{abc}	1.12^{abc}	0.98^{bcde}	0.85^{defghi}	0.70^{ghij}
		Aug.	1.16^{abc}	1.15^{abc}	1.01^{bcde}	0.85^{efghi}	1.00^{bcde}
		Oct.	1.07^{bcd}	1.05^{bcd}	0.81^{efghi}	0.85^{defghi}	0.73^{fghij}
	Hybrid	June	0.65^{ijk}	0.63^{jk}	0.63^{jk}	0.64^{jk}	0.48^{k}
		Aug.	1.21^{ab}	1.36^{a}	1.21^{ab}	1.05^{bcd}	0.87^{defgh}
		Oct.	0.89^{def}	0.92^{cdef}	0.86^{defg}	0.67^{hij}	0.68^{hij}
TR	100	June	10.40^{d}	10.79^{cde}	10.89^{cde}	10.69^{cdef}	10.55^{defg}
		Aug.	7.84^{mn}	9.4^{hijk}	10.04^{efg}	9.88^{fghij}	10.39^{defg}
		Oct.	11.29^{b}	12.33^{a}	12.45^{a}	12.60^{a}	12.15^{ab}
	Hybrid	June	7.78^{mn}	8.76^{kl}	8.99^{jk}	9.18^{ijk}	9.69^{ghij}
		Aug.	9.94^{efg}	11.96^{ab}	12.13^{ab}	12.34^{a}	11.63^{abc}
		Oct.	6.30^{p}	7.03^{no}	7.13^{no}	8.05^{lm}	8.15^{lm}
TC	100	June	2.39^{fg}	2.67^{ef}	2.71^{e}	2.37^{gh}	2.41^{fg}
		Aug.	2.66^{ef}	3.45^{ab}	3.31^{abc}	3.06^{cd}	3.58^{a}
		Oct.	3.05^{cd}	3.45^{ab}	3.20^{bc}	3.19^{bc}	3.04^{cd}
	Hybrid	June	1.59^{lm}	1.71^{kl}	1.88^{jk}	2.04^{ij}	2.08^{ij}
		Aug.	2.81^{de}	2.40^{fg}	2.27^{ghij}	2.11^{i}	3.13^{c}
		Oct.	1.44^{m}	2.10^{i}	2.13^{hi}	2.05^{ij}	2.12^{i}
BR	100	June	40.42^{c}	36.18^{def}	31.44^{hij}	28.23^{jklm}	25.25^{mno}
		Aug.	35.04^{ef}	32.40^{ghi}	28.56^{jkl}	24.82^{no}	22.94^{op}
		Oct.	29.55^{ij}	26.03^{lm}	21.49^{p}	22.74^{op}	21.27^{pq}
	Hybrid	June	33.16^{fg}	27.32^{kl}	27.11^{kl}	22.95^{op}	18.91^{q}
		Aug.	37.08^{d}	53.25^{a}	47.71^{ab}	44.65^{bc}	24.56^{no}
		Oct.	38.43^{d}	33.56^{fgh}	33.27^{fgh}	27.19^{klmn}	25.97^{lmn}
TF/TR	100	June	0.112^{b}	0.104^{cd}	0.090^{de}	0.074^{ef}	0.067^{fg}
		Aug.	0.130^{a}	0.130^{a}	0.107^{bc}	0.086^{de}	0.086^{de}
		Oct.	0.094^{d}	0.084^{de}	0.065^{fg}	0.064^{fg}	0.060^{g}
	Hybrid	June	0.080^{ef}	0.071^{fg}	0.069^{fg}	0.059^{g}	0.055^{g}
		Aug.	0.120^{a}	0.114^{bc}	0.099^{cd}	0.087^{de}	0.075^{ef}
		Oct.	0.130^{a}	0.133^{a}	0.121^{ab}	0.075^{ef}	0.083^{ef}

[a] The symbol of characteristics are the same as in Table 1.

[b] Treatments with a same letter in each characteristic are not significant at 1% level of probability.

Table 3. Comparison of means a clone × flushing time interactions for tannin (TN) and caffeine (CF) B. flushing time × fermentation time interactions for tannin (TN).

A. clone × flushing time

Characteristics	Clone	Flushing time	Mean [a]
TN	100	June	12.4573[c]
		Aug.	12.412[c]
		Oct.	13.9387[a]
	Hybrid	June	10.5307[e]
		Aug.	13.492[b]
		Oct.	11.68.7[d]
CF	100	June	2.9[c]
		Aug.	3.314[a]
		Oct.	3.09[b]
	Hybrid	June	2.336[e]
		Aug.	3.06[b]
		Oct.	2.43067[d]

[a] Treatments with a same letter in each characteristic are not significant at 1% level of probability.

B. flushing time × fermentation time

Characteristic	Flushing time	Fermentation time (Min) [a]				
		30	60	90	120	150
TN	June	11.9867[def]	11.7683[ef]	11.5233[f]	11.3667[f]	10.825[g]
	Aug.	13.6317[a]	13.2867[ab]	12.7633[bc]	12.4667[cd]	12.6117[bc]
	Oct.	13.495[a]	13.08[ab]	12.8717[bc]	12.33[cde]	12.2717[cde]

[a] Treatments with a same letter in each characteristic are not significant at 1% level of probability.

Correlation Coefficients

Correlation coefficients between all studied traits, except for TR and BR, CF and BR, and TC and TF/TR ratio, were significant at 1% probability (Table 4). The correlation coefficients between TC and BR and TR and TF/TR were negative and statistically significant ($P < 0.01$). The negative correlation between TC and BR is important and show that increase of total color (TC) is associated with the decrease of brightness (BR). OWUOR & ORCHARD (1990), OWUOR (1992), OWUOR & OBANDA (2001) and OBANDA *et al*. (2004) reported the same results. Results from correlations between characteristics related to quality of black tea indicated that due to the complicated relationships between them, the quality of black tea can not be assessed on the basis of a limited number of characteristics.

Simple Regression Analysis

Regression analysis between fermentation time as the independent variable (X) and each of the studied characteristics as the dependent variable (Y) showed that there are significant linear relationships between fermentation time and theaflavin (TF), brightness (BR), tannin (TN) and TF/TR ratio (Figure. 1). Regression equation between TF and fermentation time was fitted as:

$$TF = 0.751 - 0.0013X \qquad R^2 = 0.8943 \qquad [Eq. 1]$$

With respect to Eq. 1, more than 89% of the variation in TF was explained by fermentation time. So, increasing fermentation time from 30 Min to 150 Min resulted to a significant decrease in theaflavin content (Figure. 1). Decreasing theaflavin content can also be due to a decrease in the production of chemical pre- materials and polyphenol oxidase activity. Also, the conversion of theaflavin into thearubigin and/or the combining ability of theaflavin with other components such as amino acids may be other reasons for decreasing theaflavin content during the fermentation process.

Regression equation between BR and fermentation time (X) was evaluated as:

$$BR = 3.729 – 0.0034X \qquad R^2 = 0.9342 \qquad [Eq. 2]$$

On the basis of Eq. 2, BR decreases by the increase in fermentation time, and about 93% of the variation in BR is explained by fermentation time (Figure. 1). Increasing fermentation time is associated with producing the complex components with high molecular weights, such as prontocyanidines and TR. Therefore, increasing these components may explain decrease in BR during the fermentation process. Similar results have also been reported by OWUOR & ORCHARD (1990) and OWUOR & OBANDA (2001), who showed decrease in TF and BR during fermentation process.

Regression equation between TN and fermentation time (X) was fitted as Eq. 3:

$$TN = 2.651 – 0.0007X \qquad R^2 = 0.9792 \qquad [Eq. 3]$$

More than 97% of the variation in TN was explained by fermentation time, so increasing one Min of fermentation time in the range of 30 to 150 Min decreased TN of black tea about 0.0007% (Figure. 1). The main reason for this is to consume tannins (polyphenols) as the pre- material for to create TF, TR, prontocyanidines, bis-flavanols and theaflavic acids. As result of combination tannins with other components such as caffeine may also be another reason for decreasing tannin during fermentation period.

The regression equation between TF/TR ratio and fermentation time (X) is shown in Eq. 4:

$$TF/TR = 0.115 - 0.0004X \qquad R^2 = 0.9297 \qquad \text{[Eq. 4]}$$

With regard to Eq. 4, more than 92% of the variation in TF/TR ratio was explained by fermentation time, so increasing fermentation time resulted to decrease in TF/TR ratio (Figure. 1). This may be due to decreasing TF. Also, increasing TR due to its production from tannins and/or change of other components such as TF and prontocyanidines may be other reasons for decreasing TF/TR ratio.

Results from simple regression analyses between fermentation time and characteristics related to quality of black tea indicated that fermentation time significantly influences the content and changes of these characteristics. These characteristics depend on plant genetic potentials to produce chemical pre-materials; however fermentation period can have a great effect on expressing this genetic capability.

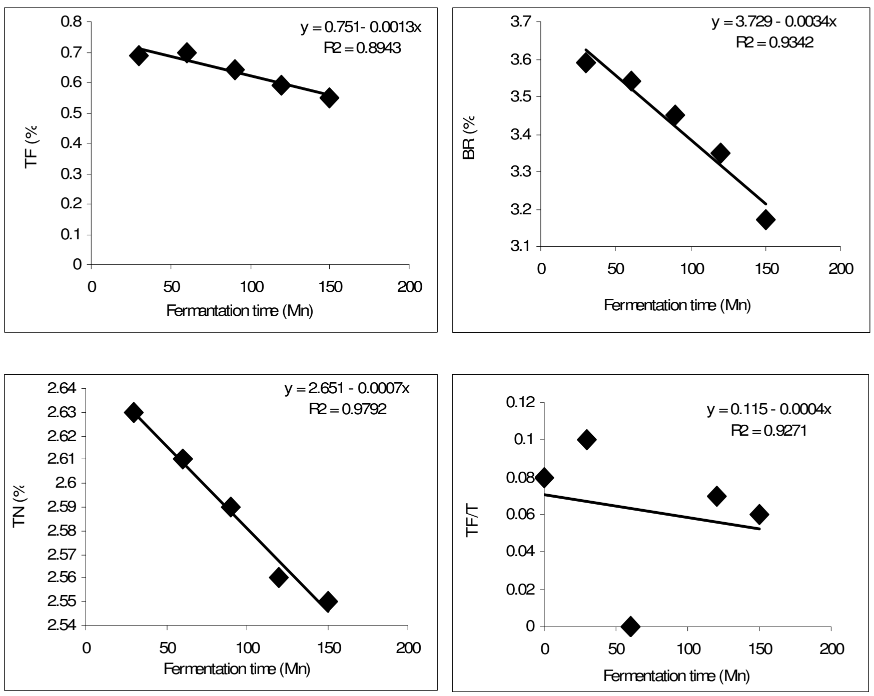

Figure. 1. Linear regression between fermentation time as the independent variable (X) and theaflavin (TF), tannin (TN), brightness (BR) and TF/TR ratio as the dependent variables (Y).

Table 4.Simple correlation coefficients between characteristics related to quality of black tea.

Characteristics [a]	TF	TR	TC	BR	TN	TF/TR
TR	0.304**					
TC	0.424**	0.621**				
BR	0.689**	-0.091	-0.281**			
TN	0.748**	0.555**	0.604**	0.331**		
TF/TR	0.687**	-0.407**	-0.005	0.707**	0.326**	
CF	0.729**	0.588**	0.795**	0.188	0.760**	0.281**

** Significant at 1% level of probability.

[a] The symbol of characteristics are the same as in Table 1.

Multivariate Regression Analysis

To study the effect of studied characteristics on BR and TC, multivariate regression analyses were separately conducted between BR and TC as dependent variable (Y) and the other traits as independent variables. The best equation was fitted using stepwise method.

Multivariate regression analysis for BR as dependent variable and TF, TR, TN, CF and TF/TR ratio as independent variables indicated that TF, TF/TR and CF explain more than 70% of the total variation in BR (Table 5a). The best regression model was as Eq. 5:

$$BR = 2.01605TF + 2.23904TF/TR - 1.45224CF + 3.8981 \quad R^2 = 0.704 \qquad [Eq. 5]$$

Eq. 5 shows that an increase of 2% in TF and TF/TR ratio and a decrease of 1% in CF will increase BR about 1%. Regarding high correlation coefficients between TF and BR (0.689, $P < 0.01$) and TF/TR ratio and BR (0.707, $P < 0.01$), this result was expected. Also, decreasing BR as a result of caffeine components such as TR which results in the production of tea cream (POWELL *et al.* 1992), can explain the negative relationship between BR and CF content in Eq. 5. It is notable that TF/TR ratio plays an important role in producing BR. Therefore, it can be concluded that not only TF and TR, but also the ratio of these two characteristics (i.e. TF/TR) are effective characteristics determining quality of black tea.

Results from multivariate regression analysis for total color (TC) as dependent variable and TF, TR, TN, CF and TF/TR ratio as independent variables showed that only CF and TF/TR ratio have significant effect on TC (Table 5b), and the following equation was achieved:

$$TC = 1.5228CF - 1.9246TF/TR - 0.6293 \qquad R^2 = 0.6894 \qquad [Eq. 6]$$

Based on this equation, about 69% of the variation in TC was explained by CF and TF/TR ratio. As shown in Eq. 6, TC of black tea increases by decreasing TF/TR ratio and CF content. These relationships can be explained by the high correlation between CF and TC (0.795, $P < 0.01$) and the role of CF in producing tea cream (POWELL *et al*., 1992) as well as decreasing TF/TR ratio by increasing TR, regarding the high correlation between TR and TC (0.621, $P < 0.01$). As it is noted for BR, TF/TR ratio was effective in producing final TC of black tea. Thus, the importance of this characteristic for determining the quality of black tea and as a characteristic which determines the optimum fermentation time is clearly obvious.

Table 5.Multivariate linear regression for A. brightness (BR) and B. total color (TC) as dependent variable and TF, TR, TN, CF and TF/TR ratio as independent variables.

A. brightness (BR)

Regression model [a]	Degree of freedom	Mean squares	Regression coefficients				
			Adjusted R^2	Intercept	TF/TR	TF	CF
TF/TR residual	1 88	2.6311** 0.0298	0.50	2.7472**	7.8404**		
TF/TR-TF residual	2 87	1.5230** 0.0254	0.579	2.49**	4.8966**	0.8021**	
TF/TR-TF-CF residual	3 86	1.2347** 0.0181	0.704	3.8981**	2.2390*	2.0160**	-1.4522**

$^{*, **}$ Significant at 5% and 1% level of probability, respectively.

[a] The symbol of characteristics are the same as in Table 1.

B. total color (TC)

Regression model [a]	Degree of freedom	Mean squares	Regression coefficients				
			Adjusted R^2	Intercept	TF/TR	TF	CF
CF residual	1 88	1.6227** 0.0107	0.6325	-0.6305**	-	1.3997**	
CF-TF/TR residual	2 87	0.8843** 0.0091	0.6894	-0.6293**	-	1.5228**	-1.9246**

$^{*, **}$ Significant at 5% and 1% level of probability, respectively.

[a] The symbol of characteristics are the same as in Table 1.

CONCLUSION

Clone type and flushing time are the most essential factors in to produce black tea, so the quality of black tea can be controlled with changing the fermentation time corresponding to the clone type and flushing time. Thus, it is necessary to determine the suitable fermentation time for each clone in each flushing time.

Results from correlations between characteristics related to quality of black tea indicated that due to the complicated relationships between them, the quality of black tea can not be assessed on the basis of a limited number of characteristics. In other words, since each of these characteristics contribute to this quality, it is necessary to consider all characteristics to achieve the best quality of black tea. Regulation of fermentation period can be controlled by plant genetic potential at a large extent and achieve maximum quality of black tea. Multivariate regression analyses for BR and TC (as the dependent variables) and theaflavin, thearubigin, tannin, caffeine and TF/TR ratio (as the independent variables) indicated the changes of characteristics determining quality of black tea do not have similar trends. Therefore, to determine the optimum quality of black tea, all characteristics need to be studied. In other words, when one of the characteristics is at highest level, the other characteristics may be at lower levels. Thus, to produce high quality of black tea, it is necessary to all characteristics to be at optimum levels.

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UTICAJ VREMENA DOBIJANJA CRVENE BOJE I FERMENTACIJE NA KVALITET CRNOG ČAJA

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I z v o d

Vreme dobijanja crvene boje i fermentacijesu dva glavna parametra koji određuju kvalitet crnog čaja. Vršena su ispitivanja različitog vremena crvenjenja i vremena fermentacije kod dva klona i prirodnih kineskih hibrida.na kvalitet crnog čaja.
Analiza variance je pokazala da postoje značajne razlike među tipovima klona, crvenjenja i vremena fermentacije za čajni flavin, čajni rubigin, ukupnu boju, bistrinu, tannin i kafein. Koeficijenti korelacije svih ispitivanih osobina, izuzev čajnog birubigina i bistrine, kafeina i bistrine, ukupne boje i odnosa čajnog flavina i čajnog rubiginina su značajni na nivou verovatnoće 1 %. Regresiona analiza ukazuje da postoji linearna regresija između vremena fermentacije I bistrinee, tanina, čajnog flavina I odnosa čajnog flavina i čajnog rubigina. Multipla analiza regresije za bistrinu I ukupnu boju ukazuje takođe da se više od 70 % variranja bistrine objašnjava čajnim flavinom, kafeinom I odnosom čajnog flavina I čajnog rubigina, a više od 68 % variranja ukupn boj je posledica uzrokovana kafinom I odnosom čajnog flavina I čajnog rubigina.

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