Optimization of withering time and fermentation conditions during black tea manufacture using response surface methodology

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ABSTRACT

The central composite design (CCD) technique, a response surface methodology (RSM), was used to study the effect of the withering time and the fermentation conditions of the tea leaves on the black tea quality parameters. A three level, three variable design was adopted. The three independent variables investigated in this experiment, each at three levels, were withering time, the fermentation duration and the fermentation temperature. Responses were represented mathematically by second-order quadratic equation and assessed using polynomial multiple regression model.

The highest levels of the theaflavins (TFs) formation (≥1.25%) was with the withering time at about 19-20 hrs while the fermentation duration was at about 25-30 min (fermentation temperature= 25°C). The gradual increase in the thearubigins (TRs) as the function of the withering time and the fermentation temperature was noticeable. The increase in TR content at >11.5% was minor and related to the narrower ranges of the two parameters studied (x_1 and x_3). The predicted dependence of the TLC evaluation (%) (spectrophotometric measurement) on the fermentation duration (x_2) and on the fermentation temperature (x_3) was statistically significant. The results of this study suggest that the CCD and RSM can be efficiently used in the fermentation process in black tea manufacture.

Keywords: Black tea fermentation, Theaflavins, Thearubins, Total liquor color, Response surface methodology, Experimental design.

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INTRODUCTION

Although all the processing stages contribute greatly to the final quality of black tea but the fermentation step is the most critical stage in view of a number of crucial biochemical events occurring in this process. The endogenous flavanols in the tea leaves undergo various oxidative type of the enzymatic reactions. Catechins as the major component of the polyphenolic fraction of the green tea leaves are the substrates of polyphenol oxidase (PPO) which is the key enzyme in the formation of the black tea components, namely theaflavins (TFs) and thearubigins (TRs). PPO catalyzes the oxidation of hydroxyphenols to their quinone derivatives which thereafter polymerize spontaneously.[1] Contribution of tea leaf peroxidase (POD) to the fermentation process is also known to be considerable.[1,2] Possible role of POD in the oxidative breakdown of TFs and the formation of TRs has been pointed out although, in this coordinated action of PPO and POD enzymes, PPO is shown to be of prime importance in terms of the oxidation of the flavan-3-ol substrates.[2,3] The ratios of these two enzymes in the tea leaves could vary widely and the fate of catalase as the third enzyme in tea leaf is also important in these biochemical events i.e., catalytic action of catalase leads to rapid removal of POD.[3]

Positive health benefits of aqueous extracts of black and green teas have been demonstrated in many studies,i.e., antimutagenic, hypocholesterimic antidiabetic, anti-bacterial, anti-tumor, anti-UV induced oxidative DNA damage and anti-cariogenic properties in a variety of tested animal systems.[4] in fact tea contains antioxidant nutrients and non- nutrient phytochemicals commonly known as flavonoids which have been regarded as safe by US food and drug administration.[4,5]

In addition to physical and genetic properties of tea leaves and the conditions of withering and rolling processes, factors such as temperature and time of the fermentation and availability of O_2 are among known factors which affect these enzymatic reactions. There is strong correlation between quality of the black tea (i.e., total liquid color, TLC) and the quantity of the TF and TR pigments.[6] In fact use of the TF value of the black tea as a measurable parameter for the evaluation of the tea quality has been proposed in the literature. Variety of bio-chemical reactions occurring during the black tea fermentation are critical for evaluating this process and quality of the black tea is quantified by the presence or absence of chemical substances which impart color, brightness, briskness, strength and flavor to the infusion. Beverage black tea made from green tea leaves is the most popular drink in Iran. The annual production of the black tea was about 61000 tons in year 2000 and the cultivating area in Gillan as the major tea manufacturing region in Iran was about 35000 hectare.[7] Usual practice in tea plant cultivation is to use plants having some agronomic advantages such as high yields or plantation of particular clone could lead to good quality tea or the plant resists some adverse environmental conditions or hazards. It is obvious that such agronomic advantages are of economic value. Attempts in most tea plant breeding programmes, are directed toward selecting plants which possess a combination of these described characteristics. A special hybrid of the *Camellia* variety named clone 100 has been developed in the Iran Tea Research Center and introduced to the local tea producers in last two years in view of improving the quality values of the black tea (i.e., high levels of polyphenols) while upon the cultivation, this hybrid expresses less sensitivity to the seasonal temperature changes, typical to the Gillan`s climate. Temperature decrease during winter is the most obvious climatic factors which tea plants experience. Exposure of plants to low temperature causes oxidative stress which is revealed by production of reactive oxygen species (ROS). Levels of these species in plants increase in low temperature and in light and chilling-induced photoinhibition is the fate of the process and the interactions of ROS with the biomolecules cause intense cellular damages.[8] On the basis of the applying rolling stage of the fermentation process during black tea manufacture, the tea production is carried out in either one of the two processes, namely, conventional rolling or unconventional i.e., CTC (cut, tear, curl) method. The rolling stage in the black tea manufacture in Iran, is almost exclusively carried out by conventional process. Despite of the large tea consumption in Iran (the value of the annual consumption is around 11000 tons) the national sale`s market regarding Iranian grown and made tea is at low level and Iran`s tea industry faces very real big problem.

Experimental design considers simultaneously several factors and attempts to characterize the relationship between the independent variable and one or more dependent or response variables. Response surface methodology (RSM) is an effective tool for optimizing a process in which several factors and their interactions, affect desired response.[9-11] RSM uses an experimental design such as the central composite design (CCD) to fit a mathematical model by least squares technique.[10] Adequacy of the proposed model is then revealed using the diagnostic checking tests provided by analysis of variance (ANOVA). The response surface plots can be employed to study the surfaces and locate the optimum. CCD is a response surface regression which can deal with quadratic terms or equivalently curvature in the response surface. In recent years use of RSM in the evaluation of enzymatic and biological processes has gained importance and is becoming an innovative approach in many research studies and even in industrial operations.[11,12] The objective of the present work was to assess the black tea quality parameters, i.e., TF, TR and TLC of Iranian grown and made tea using a statistical design of experiment. In fact, there are few methods available to detect optimum fermentation time directly or indirectly and very recently an electronic nose was developed to monitor volatile emission pattern during the black tea manufacture.[4] The peaks appeared in the sensor outputs was basis of the detecting the optimum fermentation time in that study. In the present study a CCD and RSM were used to develop mathematical equation providing a quantitative evaluation of the black tea quality parameters as means of optimizing the fermentation process while conventional rolling process as the most common practice in Iran's tea manufacture, has been employed. Withering time, fermentation duration and fermentation temperature were selected as the controllable factors affecting the quality of the black tea. Application of the statistical design to the black tea manufacture provides a systematic procedure for obtaining repeatable results in view of obtaining high quality black tea. This approach might be used as a way of finding the quality of teas in Iranian and international markets.

MATERIALS AND METHODS

Leaf and black tea manufacture

The green tea leaves (the tip and two to three small delicate leaves) were picked. The tea leaves collection was from the tea experimental plantation farm in Gillan (altitude 7 m below sea level). The collected tea leaves were spread out on wire trays (600 g) and withered by passing cool air at about 25°C, for pre-determined time. Withered tea leaves were processed using conventional method. Humidity of air in the site of the tea leaves collection and during withering process was 60-80% and 75-80%, respectively. During fermentation process, humidity was controlled at 90%. The macerated leaves were then spread out in trays and placed in fermentation cabinet for a period of pre-determined time of oxidative fermentation while the fermentation temperatures were set at those levels used in this work. The fermented tea leaves were dried in a mini-fluid bed drier at 105°C for 26 min, the dried black teas after reaching its temperature to the room temperature, packed in plastic bags and stored until further analysis. For each 15 experiments according to the CCD arrangement and as it is shown in Table 2.9 g dried black teas were packed. The dried black teas were subjected to chemical analysis. The quality parameters studies were theaflavin, thearubigin, highly polymerized substances and total liquor color. The solvent extraction of tea was carried out according to procedure described elsewhere.[1] For each experiment, 600 fresh green leaves was used (duplicate). The dried black teas were subjected to chemical analysis without sorting. A tea infusion was made with 200 ml of boiling water into a tared flask having 4 g of the tea. The flask was held on the water bath for 10 min and the infusion filtered using whatman #1 filterpaper and appropriate volume was taken for the chemical analysis.

Experimental design and data analysis

As shown in Table 1, a CCD in the form of 2^3 full factorial designs was used. The first eight treatment combinations form a 2^3 factorial design. The next six treatment combinations are referred to the axial runs, because they lie on the axes defined by the design variables. The last treatment combination represents the center run and this arrangement of CCD as shown in Table 1 is in such a way that allows the development of the appropriate empirical equations (i.e., the second-order polynomial multiple regression equation).[9-11] The model for predicting the quality of the black tea (response variable) was expressed as:

$$\hat{y}_i = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} x_i x_j \quad (1)$$

Where $\hat{y}_i$ represents the response variable; β_0 is the intercept coefficient; β_i, β_{ii} and β_{ij} are the regression coefficients; n is the number of variables studied; and x_i and x_j represent the regressor variables. The Design Expert version 7 software was used for the data analysis.

RESULTS AND DISCUSSION

Central composite design and fitted regression models as related to the black tea quality assessment

In the present work, the relationship between three criteria of the tea quality evaluation namely TF, TR and TLC and three controllable factors namely withering time (WT), fermentation duration (FD) and fermentation temperature (FT) was studied. A CCD arrangement shown in Table 1 allows the development of mathematical equations where each response variable y is assessed as a function of withering time (x_1), fermentation duration (x_2) and temperature of fermentation (x_3) according to the following equation:

$$\hat{y} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_{11}^2 + \beta_{22} x_{22}^2 + \beta_{33} x_{33}^3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (2)$$

Where the regression coefficients are the intercept (β_0), linear ($\beta_1, \beta_2, \beta_3$), interaction ($\beta_{12}, \beta_{13}, \beta_{23}, \beta_{123}$) and quadratic ($\beta_{11}, \beta_{22}, \beta_{33}$).

The results obtained were then analyzed by ANOVA to assess the goodness of fit. Only terms found statistically significant were included in the model. Therefore a new ANOVA was performed for the reduced mathematical model. The result of ANOVA (statistical parameter) is shown on Table 2. The models for TF and TR formation and TLC assessment were significant by the F test at the 5% confidence level (Prob>F<0.05). Table 3 also shows those regression coefficients remained in the models while the statistically non-significant terms have been dropped. The relative contribution of each of the controllable variables to each of the three response variables studied in the present work was directly measured by the respective coefficient in the reduced (i.e., fitted) model. The following fitted regression models were used to quantitatively investigate the effects of withering time, fermentation duration and temperature of fermentation on the characterization of the black tea quality considering the clone 100 and use of the conventional rolling stage for the black tea manufacture:

TF formation: $y_{TF} = 1.22 + 0.15x_1 + 0.051x_2 - 0.053x_1x_2 - 0.28x_2^2$

TR formation: $y_{TR} = 9.71 + 1.51x_1 + 0.60x_2 + 0.59x_3 + 0.57x_1x_3 - 1.62x_1^2 - 0.92x_2^2 + 1.48x_3^2$

TLC evaluation: $y_{TLC} = 2.60 + 0.29x_1 + 0.51x_2 + 0.20x_3 + 0.17x_1x_3 - 0.43x_2^2 + 0.49x_3^2$

Statistical parameters obtained from the ANOVA for the reduced model of the black tea quality assessment are given in Table 2. Since R^2 always decreases when a regressor variable is dropped from a regression model, in statistical modeling the adjusted R^2 which takes the number of regressor variables into account, is usually selected.[13] The R^2 coefficient gives the proportion of the total variation in the response variable explained or accounted for by the predictors (xs) included in the model. In the present study, the adjusted R^2 were 0.96 and 0.98 for TF and TR formation, respectively. The adjusted R^2 for the TLC evaluation was 0.89. The R^2 coefficient in this study ensured a satisfactory adjustment of the quadratic model to the experimental data and more than 85% of the variations in the obtained data can be explained by the suggested mathematical equations. High R^2 values do not necessarily mean that formation of TF and TR during the black tea manufacture proceed through the appearance of the same individual intermediates and are strongly correlated. As it is given in Table 2, the coefficient of variance (CV) for the TF and TR formation and TLC evaluation has been found to be 3.84, 3.10 and 7.35, respectively. The CV as the ratio of the standard error of estimate to the mean value of the observed response (as a percentage) is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if its CV is not greater than 10%. The adequate precision value, is a measure of the 'signal to noise' and was found to be in the range of 16 to 29 which indicates an adequate signal (see Table 2). A ratio >4 is desirable. The predicted models can be used to navigate the space defined by the CCD. The experimental results obtained are given also in Table 1 while the predicted values for the dependent variables were included in the same table. This would allow an evaluation of the reliability of the empirical models. Moreover, in order to gain a better understanding of the results, the predicted models are presented in Figs. 1, 2 and 3 as the three-dimensional response surface plots.

Response surface plotting and optimization of the black tea quality assessment

For the graphical interpretation of the interactions between the controllable variables (i.e., joint effect(s) of two or more factors), the use of three-dimensional plots of the regression model is highly recommended.[10,11] Variables giving quadratic and interaction terms with the largest absolute coefficients in the fitted models, were chosen for the axes of the response surface plots to account for the curvature of the surfaces. The 3-D plots obtained from RSM analysis are shown in Figs. 1, 2 and 3. Two independent variables namely withering time and fermentation duration were selected for TFs and TLC while the third remaining variable i.e., temperature of fermentation had the value which led to the assessment of the black tea quality parameters for TRs formation, the selected variables for the axes were x_1 and x_3. Fig. 1 shows while in one axis (the axis was identified by the withering time, x_1) there is a linear increase in theaflavin formation, in the other axis (the axis was indicated by the fermentation duration, x_2) there is increase in TFs only up to a certain extent which decreases thereafter. This indicates that a critical duration of fermentation is involved up to which TF formation is increased and it is not so, after that critical time of fermentation. TFs as the black tea quality criterion (major contribution to the color of the liquor) decreases during course of the fermentation.[14] Orange-colored pigments of black tea, TFs, are oxidized products of PPO, the enzyme which catalyzes condensation of gallocatechin (GC) and a simple catechin. The occurrence of this biochemical reaction results in formation of a flavanol with benzotropolone ring system.[15] There are four main TFs and the amount of each individual TF varied with the clones.[5] Therefore the TF compositional variations due to country of origin could also be related to climatic, genetic variations while fermentation duration always plays a key role in the process.[15] It is not know whether the pattern of the TFs distribution is genetically controlled and is stable to the different environment/growing conditions.[16] The degree of wither is also important on the activity of polyphenol oxidase. Lowest level of the TF formed (<0.8%), was with x_1 at about 10-12 hrs and fermentation duration (FD $\leq$ 30) min. the TF levels ranged between 0.9-1% when withering time increased to 15-18 hrs with FD at about 70 min. The highest levels of the TF formation ($\geq$1.25%) was at x_1=19-20 hrs when FD=90-100 min. The statistical analysis of the results obtained by the work reported elsewhere using different clones, showed that the longer the wither time, the lower was the TF levels. In that study whithering condition was under hot and dry atmosphere (36°C dry bulb and 18°C wet bulb) while the tea leaves were macerated using CTC method. Importance of the experimental design is to plan the experiment(s) in advance and treat data according to a pre-determined logical order. Experimental design has not been used in the works of other researchers assessing the black tea quality parameters.[6] The relationship between total TFs and the individual theaflavins have been studied for Kenyan black tea.[17] Not necessarily levels of all of the individual TFs were correlated well with total TFs. Based on the results, the authors concluded that the higher levels of total TFs are likely to have high contents of the theaflavin monogallates.

The decreasing trend of the enzyme activity during withering and also mechanical injury of the fresh tea leaves, is a serious problem while the need to control the moisture content of the green leaves has been emphasized.[17,18] The increase of the enzyme activity on rolling stage is found to be due to the occurrence of the biochemical events in the tea leaves such as the release of PPO because of the gradual dissolution of the chloroplast membrane. The increased level of the enzyme activity in the beginning of the fermentation is therefore, quite possible while the activity decreases as the fermentation progresses and the colored tea pigments are formed when a combination of orthodox+ rotorvane+ orthodox systems were used to find optimum fermentation time for Turkish black tea while the TF value was considered as the sole criterion of the tea quality.[19] With two leaves and a bud, the TF content was at the highest level while decrease in TF levels, due to the additional leaves, was said to be due to probably lower levels of polyphenols and PPO activity although the activities were not measured in that work.

The response surface plot of thearubigins formation as a function of withering time and fermentation temperature is shown in Fig. 2. The plot shows fluctuating nature, levels of the TR formation at about 7-8% corresponded to the withering time of less than 12 hrs while the temperature of the fermentation was in about the investigated range (20°-30°C) and when the withering time increases to 15 hrs, the TR content increases to about 9.5% considering the same range for x_3 variable. Highest amount of TR as a small fraction, was about ≥11.47% and occurred when withering interval was about 16-20 hrs and the temperature range for the fermentation was about 28°-30°C (see Fig. 2). The TR content for the Keyan black tea ranged from (%) 4.8 to 19 and the authors[4] found that rise in the levels of the total TR within 10-20 hrs wither was less than that found within 20-35 hrs withering time. In the present study the gradual increase in the TR levels at 10-14 hrs of withering time and at the range of fermentation temperature investigated (20°-30°C) was noticeable (see Fig. 2). While the rise in TR content at ≥11.47% was minor and related to the narrower range(s) of the withering interval (16-20 hrs) and the fermentation temperature (28°-30°C). Statistical analysis of the results reported by other researcher showed that the thearubigins reduced black tea brightness scores and as the authors pointed out the results of those spectrophotometric measurements were in agreement with the brightness results obtained through sensory evaluation study.[6] Works of the other researchers and other show that in the production of bright black teas, cultivars need to be selected with potential of leading to low TR levels.[6,17] The use of TF formation as a way of predicting optimum fermentation conditions has been found not to be satisfactory for some black teas since presence of other chemical components in black are also important in the tea quality assessment.[15] The levels of TFs and TRs, on a dry weight basis, although are different and ranged from 0.3-2% and 10-20%, respectively, but both of these phenolic pigments together contribute to the tea brew characteristics such as color, strength and body.[5] Extensive work of the other researchers[20,21] on effect of tea variety on flavonoid content of blak tea with emphasize on TFs and TRs contents, showed that Assam and Kenya (both Indian-hybrid teas) had higher levels of TFs as compared to that of other black teas. CTC method of rolling was used for the above named varieties whereas for China and Darjeling varieties, with conventional method of rolling, the teas had lowest TFs. In the present study values of the TFs and TRs in the form of ratio are given in Table 3. The lowest level of the TF/TR ratio was for 20 hrs as the withering time, 150 min as the fermentation duration and 30°C as the fermentation temperature, although the ratio was >0.1 in all the experiments. The TF/TR ratios for those black teas produced by the conventional method of rolling were low at about 0.035 to 0.037 (Ceylon, China, Darjeeling) while the ratios for the Assam and Kenya black teas produced by using CTC method of rolling were high at about 0.08.[21] In the present work the ratio of TF/TR was more comparable with the ratio obtained for the teas processed by CTC while the conventional type of the process has been used.

Fig. 3 shows the predicted dependence of the TLC spectrophotometric evaluation (%) on the fermentation duration and on the fermentation temperature when the withering time was kept constant at 15 hrs. The response surface plot for the TLC is shown in Fig. 3 it shows that the highest level of the TLC measurement at about 2.76%, was corresponded to the fermentation duration between 100-140 min while the withering time was at about 15-20 hrs. Statistical analysis of the results obtained on the Kenyan black tea showed that TF and TR contents accounted for 90% of the variations of the TLC measured spectrophotometrically, although the coefficients of TF and TR were positive and negative, respectively.[6] The authors pointed out that in order to study the contribution of the TR to TLC evaluation, it is important to fractionate the TR and two fractions of the it namely, TRSI and TRSII have been found to play key role(s) in assessing TLC. Moreover, TF and TRSI and TRSII together, could give a better prediction of liquor brightness than TF and total TR alone.[6]

The concept of using the composition of green tea leaves in predicting the black tea quality has been extended and possible role of the ratio of gallated to non-gallated flavan-3-ols in the quality of Kenyan plain black tea has been studies.[17,20] The flavan-3-ols are the major flavonoid class in tea.[21,22] High amounts of theaflavin digallate equivalent, low levels of TRs and the scores of the brightness led to increased levels of the sensory evaluation. Based on the results obtained, the authors suggested that amount of some fractions of flavan-3-ol present in the green tea leaf can be used to predict the black tea quality.[22] For clone 100 used in the present work, more systematic approaches are needed not only to study the flavonoid content of the green tea leaf but also the pattern of the flavan 3-ols distribution. The need for establishing a database for monitoring quality of the Iranian grown and made black tea is thus, emphasized.

CONCLUSIONS

The effect of some processing conditions during the black tea manufacture, on the assessment of the quality parameters of the teas was studied using central composite design experiment. The RSM was used aiming to extend our knowledge of the optimization process for black tea manufacture, and the results obtained in this work show that the statistical design of experiment can be an interesting approach and a useful tool not only in the optimizing the tea fermentation process but also in explaining qualitatively and quantitatively the fermentation behavior of the tea leaves of the clone 100 used in this work while the conventional rolling stage has been employed. This approach might be useful in providing an acceptable quality of teas in Iranian and international markets.

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Table 1. Arrangement of the CCD for the three independent variables used in the present study along with the theoretically predicted values for the three response variables.

Experiment no.	Variable levels/Coded values Withering time (x_1)	Fermentation duration (x_2)	Fermentation temperature(x_3)	Responses(%) y_{TF}	y_{TR}	y_{TLC}
1	-1	-1	-1	0.69(0.69)	6.57(6.63)	1.63(1.80)
2	+1	-1	-1	1.10(1.08)	8.25(8.47)	2.11(2.08)
3	-1	+1	-1	0.90(0.85)	7.51(7.72)	2.59(2.84)
4	+1	+1	-1	1.09(1.11)	9.69(9.46)	3.06(2.96)
5	-1	-1	+1	0.69(0.70)	6.67(6.72)	1.96(2.06)
6	+1	-1	+1	1.10(1.10)	10.62(10.26)	2.44(2.58)
7	-1	+1	+1	0.90(0.95)	7.61(7.70)	2.92(2.75)
8	+1	+1	+1	1.09(1.06)	12.06(12.27)	3.39(3.99)
9	-1	0	0	1.07(1.05)	6.52(6.35)	2.30(2.04)
10	+1	0	0	1.37(1.38)	9.58(9.78)	2.78(2.80)
11	0	-1	0	0.89(0.90)	8.14(8.32)	1.60(1.76)
12	0	+1	0	1.00(1.01)	9.34(9.21)	2.56(2.81)
13	0	0	-1	1.22(1.30)	10.36(10.54)	2.81(3.05)
14	0	0	+1	1.22(1.19)	11.60(11.79)	3.14(3.36)
15	0	0	0	1.22(1.19)	9.57(9.79)	2.54(2.71)

The actual values are also given in the parenthesis. The actual values for the three independent variables were x_1: 10, 15, 20 hrs; x_2: 30, 90, 150 min; and x_3:20, 25, 30ºC theses were based on the variables levels which coded as: -1, 0 and +1.

Table 2. Statistical parameters obtained form ANOVA for the fitted models.

Statistical parameter	y_{TF}	y_{TR}	y_{TLC}
R^2	0.9709	0.9882	0.9423
R^2 adjusted	0.9593	0.9763	0.8990
$F_{exp}{}^{*}$	83.53	83.46	21.77
Prob>F	0.0001	0.0001	0.0002
Std. Dev	0.040	0.28	0.19
CV	3.84	3.10	7.35
PRESS	0.035	2.65	1.11
Adequate precision	29.641	26.549	16.362

*F_{exp} defined as the ratio of the mean square of the model to mean square of the error.

Table 3. TF/TR ratio for some of the black teas.

TF/TR ratio*									
Withering time (hrs)			Fermentation duration (min)			Fermentation temperature (°C)			
10	15	20	30	90	150	20	25	30	ref
0.1214	0.1159	0.1124	0.1101	0.1304	0.1104	0.1164	0.1206	0.1043	Present study

* The TF/TR ratio for several popular tea are: 0.0789 for Assam (CTC); 0.0822 for Kenya(CTC); 0.0373 for Ceylon(conventional); 0.0362 for China (conventional); 0.0342 for Darjeeling (conventional).[21,22]

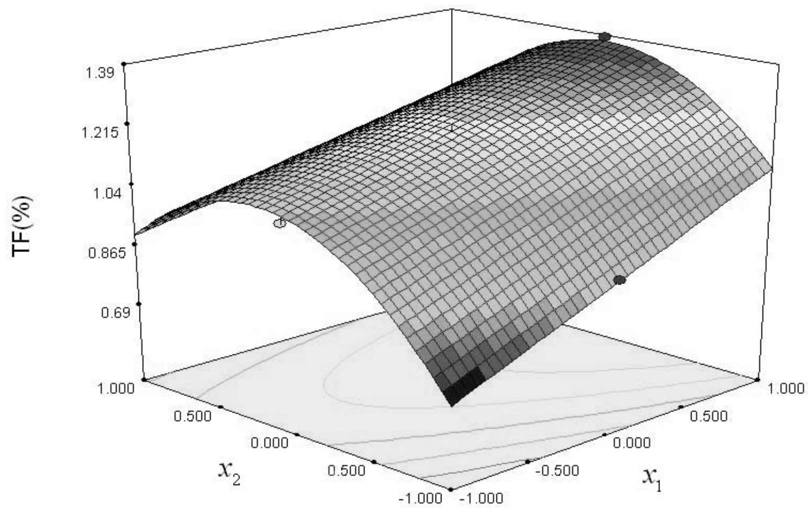

Fig.1. second- order response surface plot in TF formation. Dependence of y_{TF} on the withering time (x_1) and fermentation duration (x_2) is shown (fermentation temperature, x_3=0).

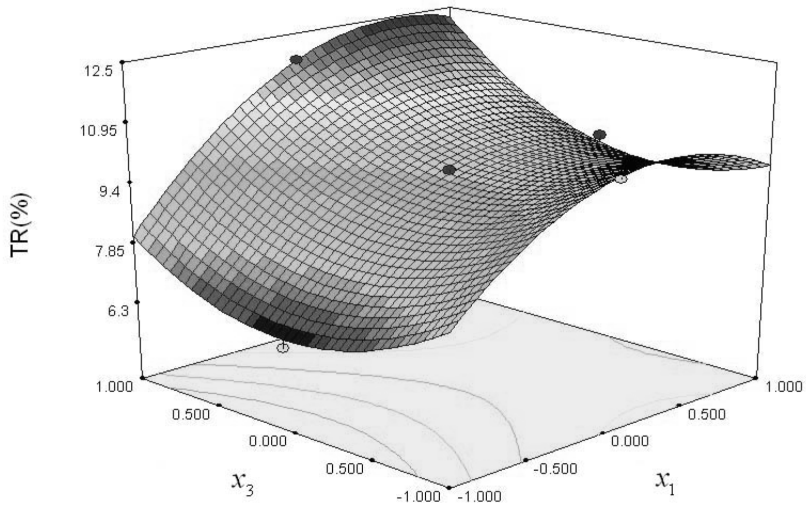

Fig.2. second- order response surface plot in TR formation. Dependence of y_{TR} on the withering time (x_1) and fermentation temperature (x_3) is shown (fermentation duration, x_2=0).

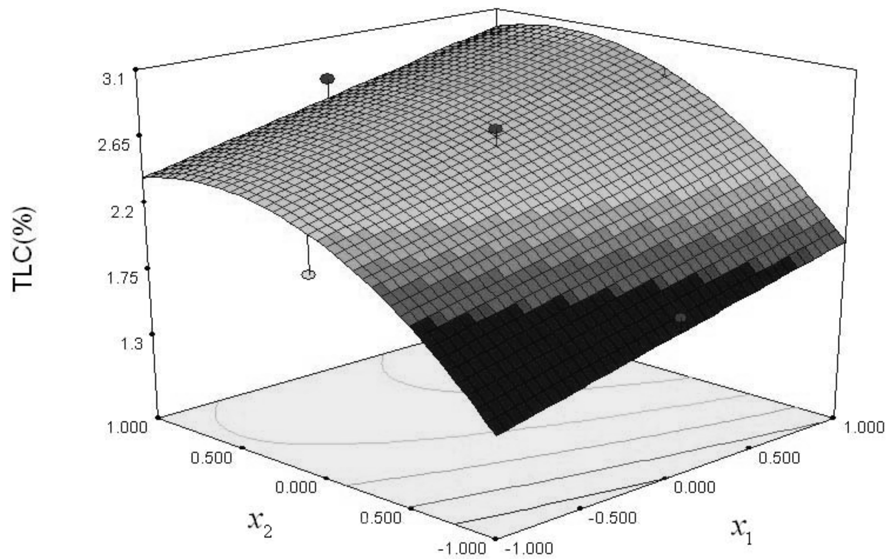

Fig.3. second- order response surface plot in TLC evaluation. Dependence of y_{TLC} on the withering time (x_1) and fermentation duration (x_2) is shown (fermentation temperature, x_3=0).