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OPTIMAL EXTRACTION PARAMETERS OF THEABROWNIN FROM SICHUAN DARK TEA

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Abstract

Background: Sichuan Dark Tea is a popular beverage with hypolipidemic and lifting greasy properties in the minority neighborhoods of Sichuan and Tibet regions. The theabrownin, an important pigment of dark tea, has been proven for the role of the hypolipidemic property in Sichuan Dark Tea. The objective of the study investigated the extraction process of theabrownin.

Materials and Methods: Theabrownin was extracted from Sichuan Dark Tea with water and organic solvents. The quadratic regression orthogonally rotational combinational design experiment was performed to obtain the optimal extraction parameters.

Results: The extraction yield of theabrownin was significantly influenced by both water extraction temperature and solid-liquid ratio, and the contribution of these factors on theabrownin yield was as follows: water temperature > solid-liquid ratio > organic solvent temperature. Moreover, the polynomial regression model established could predict the experimental value accurately.

Conclusion: The optimum extraction process of theabrownin from Sichuan Dark Tea was established, which water temperature at 65.69-77.88℃, organic solvent temperature at 13.65-17.48℃ and a solid-liquid ratio of 1:43.58-1:50.75(g/mL).

Key words: theabrownin; extraction; optimization; Sichuan Dark Tea

Abbreviations list: SDT: Sichuan Dark Tea; TB: Theabrownin

Introduction

Sichuan Dark Tea (SDT) is a popular beverage and folk medicine for the ethnic groups living in the regions of Sichuan and Tibet (Li & Li, 2007). It is processed from the rough old leaves of *Camellia sinensis*, and various kinds of microorganisms have played an important role in the production (Xu, 2010; Wen et al., 2015). Recently, more and more attentions have been paid on the physiological functions of dark tea. The hypolipidemic and lifting greasy properties of dark tea have been confirmed (Kuo et al., 2005; Ling et al., 2010; Selena et al., 2010；Fu et al., 2011). As one of the representatives of Chinese dark tea, SDT is also considered as a main source of hypolipidemic active substance (Li & Li, 2007; Bian et al., 2015). Jiang (2007) and Xu (2010) reported that the hypolipidemic property of SDT practically arises from the bioactive component named theabrownin (TB), a kind of material with high molecular weight and complex composition (Wang et al.,2011;Gong et al., 2012;Tan et al.,2012). TB is an oxidation product of catechins, and it contributes to the color of tea liquor and dried tea leaves. Theabrownin was firstly named by Ruan (1983). In black tea liquor, the total content of TB is 4 to 9% (w/w), but in the dark tea liquor this value will be higher (Gong et al., 2012). Nowadays, a lot of studies have suggested that TB perhaps is the main bioactive component resulting in the significant blood lipid-lowering effect of dark tea (Gong et al., 2010). The extraction parameters can significantly affect the quality and quantity of TB. However, few scholars have systematically studied on these parameters so far.

The objectives of this study were: (1) to find out the optimal extraction conditions of TB using a quadratic regression orthogonally rotational combinational design (Xu, 1997); (2) to evaluate the effects of the extraction temperature of water and organic solvent, the liquid-solid ratio and their interactions on the extraction yield of theabrownin.

Materials and Methods

SDT Sample and Regents

Sichuan Dark Tea was purchased from Ya'an tea Co., Ltd. (Ya'an, Sichuan province, People's Republic of China). Sample powder was prepared by pulverizing and passing SDT through a 40-mesh sieve. N-butyl alcohol, chloroform and ethyl acetate were purchased from Kelong Chemical Reagent Co., Ltd. (Chengdu, China) and they were the analytical reagent grade.

Extraction of Theabrownin

The method of quadratic regression orthogonally rotational combinational design was used to find the optimal extraction parameters of TB. The factors and levels of design were showed in table 1.

Table 1: Factors and levels of quadratic regression orthogonally rotational combinational design

coding Level	Temperature 1 （X_1）℃	Temperature 2 (X_2)℃	Solid - liquid ratio (X_3)g/mL
-1.6818	50	5	1:10
-1	60	9	1:20
0	75	15	1:35
1	90	21	1:50
1.6818	100	25	1:60

Note: temperature 1 was the water extraction temperature; Temperature 2 was the organic solvent extraction temperature.

The extraction and separation process of TB was as follows: SDT powder was extracted with distilled water 3 times →filtered under vacuum →concentrated under vacuum at 60℃(rotary evaporator RE-3000B, Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China)→extracted with n-butyl alcohol →aqueous solution layer →extracted with chloroform 3 times →aqueous solution layer →extracted with ethyl acetate at the equal volume 3 times →aqueous solution layer →concentrated under vacuum at 60℃→dried to constant weight under vacuumat60℃(Vacuum oven DZF-6050B, Shanghai PUREDU Biochemical Technology Co., Ltd., Shanghai, China)→TB(weighted with Sartorius electronic balanceBSA224S-CW (Sartorius Scientific Instruments (Beijing) Co., Ltd., Beijing, China).

The experiments were performed in triplicate. All the data was analyzed using Minitab 15.0(State College, PA, US).

Results

Establishment and Test of the Quadratic Regression Model

The experimental design and results are shown in Table 2 and the results of variance analyses are presented in Table 3. According to that, a regression model (Eq. (1)) was established by selecting the parameters at a significant level of $p<0.05$. The testing lose effectiveness of fit of the regression was not significant $F_1=3.273$ ($F_{0.05\,(5,\,8)}=3.69$), but the significance test was very significant $F_2=27.730$（$F_{0.01\,(9,13)}=4.19$）, which indicated that the regression model established was suitable. The tested value was consistent with the predicted value perfectly. Additionally, the optimized model (Eq. (2)) was obtained by removing the insignificant factors ($\alpha = 0.1$) after significance test of the regression coefficient.

$$Y=8.55743-0.49772X_1+0.07695X_2+0.89578X_3-0.31554X_1^2-0.26958X_2^2+0.09812X_3^2-0.80750X_1X_2-0.10250X_1X_3-0.03000X_2X_3 \quad (1)$$

$$Y=8.55743-0.49772X_1+0.89578X_3-0.31554X_1^2-0.26958X_2^2-0.80750X_1X_2 \quad (2)$$

Analysis of the Single Factor Effect

The individual effects of water temperature, organic solvent temperature and solid-liquid ratio on the TB extraction yield were investigated individually in the condition that the other factors were at zero level. It was found that TB extraction yield was

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significantly influenced by water extraction temperature and solid-liquid ratio ($p < 0.0001$, Table 3). Fig.1 shows that TB extraction yield increased with the increasing of water extraction temperature and solid-liquid ratio level. However within the testing range, TB extraction yield was less affected by the organic solvent extraction temperature.

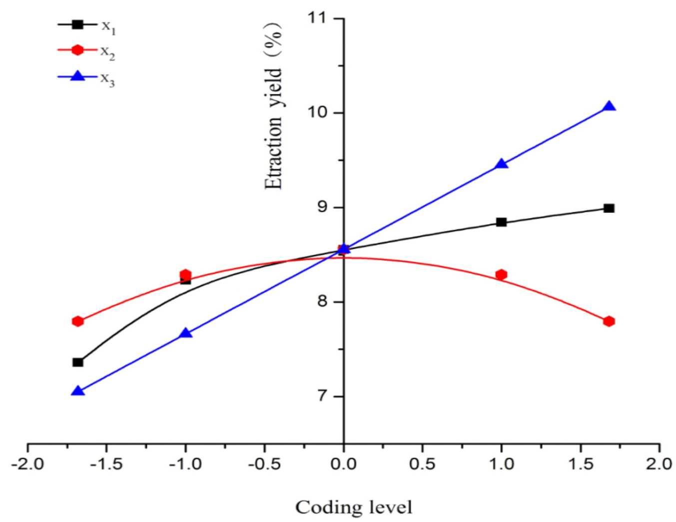

Figure1: Effect of the factors on TB extraction yield

Table 2: Quadratic regression orthogonally rotational combinational design and experimental results

Run	Experimental scheme			Y	$\hat{Y}$
	X1	X2	X3		
1	1	1	1	7.53	7.61
2	1	1	-1	6.51	6.08
3	1	-1	1	9.53	9.13
4	1	-1	-1	7.45	7.48
5	-1	1	1	10.67	10.42
6	-1	1	-1	8.3	8.48
7	-1	-1	1	8.5	8.71
8	-1	-1	-1	6.95	6.66
9	-1.6818	0	0	8.52	8.50
10	1.6818	0	0	6.5	6.83
11	0	-1.6818	0	7.5	7.67
12	0	1.6818	0	7.78	7.92
13	0	0	-1.6818	7.13	7.33
14	0	0	1.6818	10.23	10.34
15	0	0	0	8.73	8.56
16	0	0	0	8.42	8.56
17	0	0	0	8.13	8.56
18	0	0	0	8.74	8.56
19	0	0	0	8.67	8.56
20	0	0	0	8.66	8.56
21	0	0	0	8.54	8.56
22	0	0	0	8.37	8.56
23	0	0	0	8.81	8.56

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X1、X2、X3 in the table were coded for temperature 1(℃), temperature 2(℃) and solid-liquid ratio（g/mL）respectively; Y was theabrownin extraction yield measured (%)； $\hat{Y}$ was theabrownin extraction yield predicted(%).

Table 3: Variance analysis of experimental results

Parameter	Sum of squares	*df*	Mean square	F-value	*P*-value
X1	3.3831	1	3.3831	37.33541	0.00004
X2	0.0809	1	0.0809	0.89245	0.36204
X3	10.9586	1	10.9586	120.93823	0.00000
$X1^2$	1.5704	1	1.5704	17.33051	0.00111
$X2^2$	1.1420	1	1.1420	12.60357	0.00356
$X3^2$	0.1658	1	0.1658	1.82949	0.19925
X1 X2	5.2165	1	5.2165	57.56835	0.00000
X1 X3	0.0840	1	0.0840	0.92757	0.35307
X2 X3	0.0072	1	0.0072	0.07946	0.78247
Model	22.6142	9	2.5127	F2=27.730	0.00001
Residual	1.1780	13	0.0906		
Lack of fit	0.7912	5	0.1582	F1=3.273	0.03940
Pure error	0.3868	8	0.0484		
Total	23.7921	22			

Analysis of the Interaction between the Factors

The interaction between the temperature of water and organic solvent extraction had a significant effect on TB extraction yield ((p= 0.0000, Table 3). Fig. 2 shows that TB extraction yield increased with water temperature and organic solvent temperature in the condition of the solid-liquid ratio of 1:35(g/mL). If the temperatures were at a low level, they positively and significantly influenced the TB extraction yield; but at a high level, they negatively affected the extraction yield.

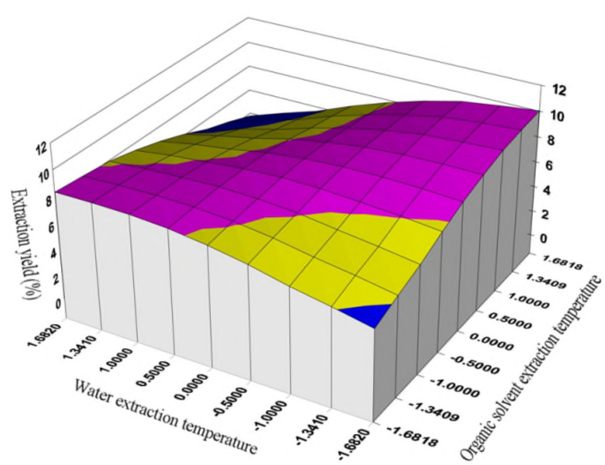

Figure 2: Interaction of the temperature of water extraction and organic solvent extraction on TB extraction yield in the condition of solid - liquid ratio of 1:35 (g/mL)

The Importance of the Factors

The importance of the factors was analyzed with the method of contribution rate: The mean square ratio of the significant test of regression coefficient was set as $F_{(j)}$,$F_{(ij)}$ and $F_{(jj)}$, Setting $\delta = \begin{cases} 0 & if \quad F \le 1 \\ 1 - \dfrac{1}{F} & if \quad F \ge 1 \end{cases}$

The contribution rate of factors could be established in accordance with Xu(1997) as:

$$\Delta_j = \delta_j + \frac{1}{2}\sum_{\substack{i=1 \\ i \ne j}}^{m} \delta_{ij} + \delta_{jj} \qquad (j = 1, 2, \cdots, m)$$

According to the mean square ratio F presented in Table 3, the contribution rate of water extraction temperature, organic solvent extraction temperature and solid-liquid ratio on TB extraction yield were Δ_1=2.406829, Δ_2= 1.411972, Δ_3=1.445131. It was evident that the influence of the factors on the extraction yield was: water temperature ＞ solid-liquid ratio＞ organic solvent temperature.

Optimization and Validation of the Extraction Factors

The optimal parameters were obtained using the frequency analysis method. The interval of [-1.6818, 1.6818] was divided into 5 parts, length frequency was set as 0.8409, the programs contained 5^3=125 combinations, in which, the TB extraction yield of 53 groups were over 8.27％. The result of frequency analysis of Eq. (1) is presented inTable4.

In the 95% confidence interval, the program that extraction yield over 8.27％was water extraction temperature of 65.69-77.88℃, organic solvent extraction temperature of 13.65-17.48℃ and solid-liquid ratio of 1:43.58-1:50.75(g/mL). The mean value of the parameters (X_1=-0.316, X_2=0.095, X_3=0.816, which mean water temperature at 70.29℃, organic solvent temperature at 15.57℃ and a solid-liquid ratio of 1:47.16(g/mL) were used to verify the optimal result. Consequently, the extraction yield measured was 9.62％, higher than 8.27％. The value is much closer to the theory value of 9.54％. It was evident that the regression model established had good fitness.

Table 4: The probability distribution of X_i in the combined application

Level of factors	X1		X2		X3	
	times	frequency	times	frequency	times	frequency
-1.6818	13	0.2453	8	0.1509	1	0.0189
-1.0000	13	0.2453	11	0.2075	4	0.0755
0.0000	13	0.2453	12	0.2264	12	0.2264
1.0000	8	0.1509	11	0.2075	17	0.3208
1.6818	6	0.1132	11	0.2075	19	0.3585
$\overline{X}$	-0.3160		0.0950		0.8160	
$S_{\overline{X}}$	0.1570		0.1640		0.1230	
95%confidence interval	（-0.625，-0.008）		（-0.226，0.416）		（0.576，1.057）	
Extraction parameter	（65.69，77.88）		（13.65，17.48）		（43.58，50.75）	

Discussion

In this study, TB extraction yield increased with the extraction temperature of water and solid-liquid ratio. The results are consistence with those found in Pu-er Tea (Fang et al., 2012). However, it is considered that the high temperature may result in the inactivation of theabrownin (Wang et al., 2014). In this work, the optimum water extraction temperature obtained was 65.69-77.88℃, which is much lower than the temperature employed in TB extraction of Liubao Tea and Pu-er Tea (Yi et al., 2010; He et al., 2012; Fang et al., 2012). Additionally, our results showed that the optimum organic solvent temperature was 13.65-17.48℃. The extraction yield of TB reached the max when the organic solvent temperature was 15℃. Therefore, it is suggested that low temperature of organic solvent extraction within a certain range can improve the yield of TB.

Acknowledgements

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