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Effects of High Voltage Pulsed Electric Field on Antioxidant Activity of Tea Polyphenols for Yunnan Pu'er Tea

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In order to improve the antioxidant activity of Pu'er tea, the authors use different high voltage pulsed electric field (HPEF) to deal with the third grade Pu'er ripe tea, extract the tea polyphenols and determine antioxidant activity of tea polyphenols in each tea samples by four indexes, namely the ability of scavenging hydroxyl radical (·OH), the ability of scavenging superoxide anion (O2·–), the ability of scavenging radical DPPH and total reducibility. Then antioxidant activity of tea polyphenols is analyzed by stepwise regression analysis on Matlab, establish the mathematical model of its antioxidant activity and voltage, frequency. The results show that HPEF could decrease the content of tea polyphenols in Pu'er ripe tea, and promote it to translate into Theabrownines (TBs) and so on. The suitable conditions of HPEF can improve the antioxidant activity of Pu'er tea to a certain extent. This study provides a new physical method for improving the antioxidant activity of Pu'er tea polyphenols, and provides technical support for the extraction and development of natural antioxidants in Pu'er tea.

1. Introduction

Pu'er Tea, as a geographical indication product in Yunnan Province, has been beloved by domestic and international tea lovers because of its unique quality and many health benefits (Wang et al., 2015; GB/T 30766-2014). There are many health functions of Pu'er tea like the lipid-lowering diet, prevention and treatment of coronary heart disease, falling hypertension, anti-aging and so on(Hwang et al., 2003; Hou et al., 2009; Kuo et al., 2005; Lee et al., 2013). Among of these functions, the most important mechanism is its antioxidant effect.

High voltage pulsed electric field (HPEF) is the process of treating high voltage pulse waves intermittently between two parallel electrode plates. It has been widely applied in agricultural products and food processing industry for its many advantages such as short processing time, low energy consumption, no pollution and so on (Yin et al., 2007; Zhong et al., 2007; Liao et al., 2003). At present, domestic and foreign scholars have achieved certain results in food sterilization and preservation (Zhang et al., 2012), extraction of natural products (Ganeva et al., 2003; Yin et al., 2007; Yin et al., 2005; Eshtiaghi et al., 2002; Loginova et al., 2011), wine aging (Fang et al., 2003), extraction (López et al., 2008) and other fields mainly using the physical means HPEF. This paper deals with the extraction of tea polyphenols from tea samples by HPEF, and studies the effect of HPEF on the extraction rate and antioxidant activity of Pu'er tea polyphenols. Using Matlab to analyze the antioxidant activity of tea polyphenols by stepwise regression analysis, the author establishes the mathematical model between antioxidant activity and HPEF voltage and frequency, and draws the three-dimensional relationship graph to find the best HPEF treatment parameters.

2. Materials and methods

2.1 Experimental materials and equipment

Experimental materials: three-grade Pu'er ripe tea produced in 2011 in Lincang.

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Experimental instruments: ISO 9001 type electronic balance (Beijing Sartorius Co. Ltd.), reflux extraction device (1000ml round bottom flask, an condensing tube, an iron stand, alcohol lamp and asbestos net), extraction device (a 250ml separatory funnel, an iron stand, 150ml beakers and 400ml beakers); drying device (a tripod, an alcohol lamp, 250ml beaker, surface vessel and asbestos net); 722S spectrophotometer (Shanghai Jinghua Science and Technology Co. Ltd.), some beaker tubes and some 50mL beakers.

2.2 High voltage pulsed electric field device

The HPEF process device consists of two parts: high voltage pulse power supply and processing device. The core part of the device is a high voltage pulse power supply. The main performance parameters of the high voltage pulse power supply are as follows:
Output voltage: 0~60kV; Input voltage: AC 220V ± 10%Output pulse duty ratio: 0~70%; Output pulse frequency: 80Hz~2000Hz;Output power: 2000W.

2.3 Experimental method

High voltage pulsed electric field process Pu'er ripe tea
Voltage, frequency and time are the main parameters affecting the high voltage pulsed electric field. According to the preliminary research results of the project team, the setting time is 55min, the frequency is 80Hz, 99Hz, 121Hz, 139Hz and 162Hz, the voltage is 12kV-22kV, and their parameters are shown in Table 1.

Table 1: HPEF voltage and frequency settings (Time=55min)

NO.	Voltage(kV)	(Hz) Frequency	NO.	Voltage(kV)	(Hz) Frequency	NO.	Voltage(kV)	(Hz) Frequency
1	12	80	20	20	99	39	17	139
2	13	80	21	21	99	40	18	139
3	14	80	22	22	99	41	19	139
4	15	80	23	12	121	42	20	139
5	16	80	24	13	121	43	21	139
6	17	80	25	14	121	44	22	139
7	18	80	25	15	121	45	12	162
8	19	80	27	16	121	46	13	162
9	20	80	28	17	121	47	14	162
10	21	80	29	18	121	48	15	162
11	22	80	30	19	121	49	16	162
12	12	99	31	20	121	50	17	162
13	13	99	32	21	121	51	18	162
14	14	99	33	22	121	52	19	162
15	15	99	34	12	139	53	20	162
16	16	99	35	13	139	54	21	162
17	17	99	36	14	139	55	22	162
18	18	99	37	15	139			
19	19	99	38	16	139			

Extraction and measurement of tea polyphenols
Extract the tea polyphenol standard solution from 1.0mL, 2.0mL, 3.0mL and 4.0mL into the four 450mL flask, and add distilled water to filter and dilute to 10mL, then add 10mL ferrous tartrate solution, finally add phosphate buffer (pH=7.5) to the scale, mixing into the cuvette with blank reagent as a reference. The absorbance value (A) is measured at λ=540nm, and then draw a standard curve as shown in Figure 1.

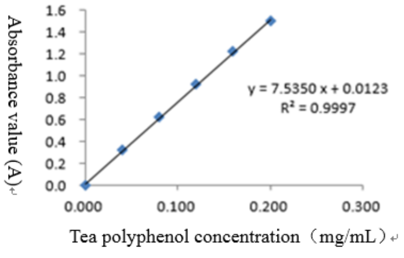

Figure 1: Polyphenol standard curve

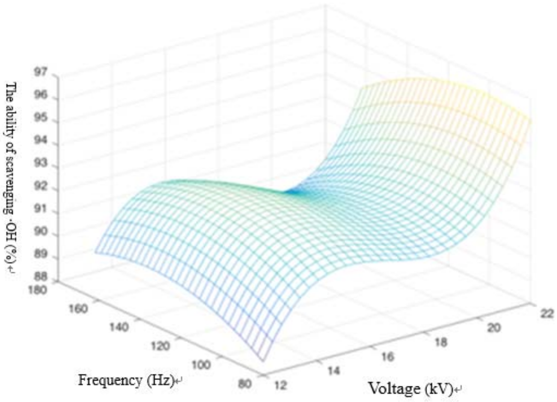

Figure 2: Scavenging·OH for polyphenols

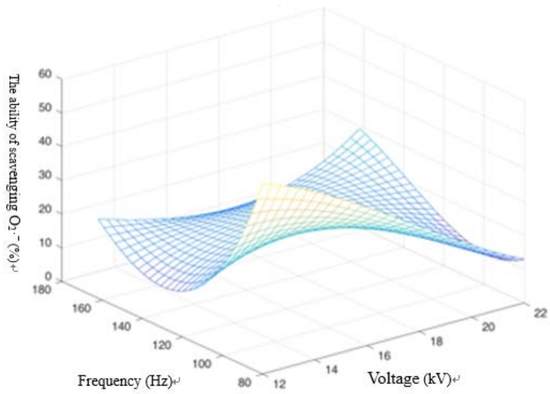

Figure 3: Scavenging O2·– for tea tea polyphenols

Determination of antioxidant activity of tea polyphenols

The determination of antioxidant activity of tea polyphenols contains four indexes: hydroxyl radical (·OH) scavenging ability, superoxide anion (O2·–) scavenging ability, DPPH radical scavenging ability and total reducibility.

Among of these, the calculation formula of the scavenging rate is: The scavenging rate(%)=[1-(A_1-A_2)/A_0]×100

Determination of the scavenging ability of ·OH: Sodium salicylate (XU Jianguo, et al, 2006) was used to determine the scavenging ability of tea polyphenols on ·OH.

Determination of the scavenging ability of O2·–: utilize the pyrogallol autoxidation method (Guo et al., 2007) to determine scavenging effects of tea polyphenols on O2·– determination of scavenging capacity of DPPH radical: According to the method from Zhang et al. (2007), and others, we can determine the scavenging effect of tea polyphenols on DPPH radical

Determination of total reducibility of tea polyphenols: According to the method from Zou et al. (2014) and others, we determine the total reducibility.

3. Results and analysis

3.1 Effect of HPEF on Extraction of tea polyphenols

Table 2: The content of tea polyphenols in its crude extraction

NO.	Tea polyphenols content(mg/mL)	NO.	Tea polyphenols content(mg/mL)	NO.	Tea polyphenols content(mg/mL)
CK	0.0118	19	0.0087	38	0.0105
1	0.0077	20	0.0136	39	0.0104
2	0.0082	21	0.0112	40	0.0100
3	0.0090	22	0.0067	41	0.0107
4	0.0089	23	0.0091	42	0.0094
5	0.0099	24	0.0093	43	0.0089
6	0.0091	25	0.0102	44	0.0087
7	0.0099	26	0.0110	45	0.0110
8	0.0096	27	0.0100	46	0.0106
9	0.0077	28	0.0089	47	0.0098
10	0.0094	29	0.0106	48	0.0100
11	0.0085	30	0.0094	49	0.0089
12	0.0098	31	0.0093	50	0.0085
13	0.0091	32	0.0104	51	0.0101
14	0.0106	33	0.0098	52	0.0091
15	0.0114	34	0.0106	53	0.0083
16	0.0098	35	0.0114	54	0.0077
17	0.0102	36	0.0112	55	0.0088
18	0.0075	37	0.0108		

According to the standard curve equation of polyphenols y=7.5350x+0.0123 (R2=0.9997), to calculate the content of tea polyphenols in blank group and treated by different HPEF groups in crude extraction of tea, its contents are shown in Table 2.

From the table, most of crude extraction after treated by HPEF, its content of tea polyphenols in tea samples is lower than the blank group (CK). Among that, the content of tea polyphenols of the twenty-second group (22kV/99Hz) is the lowest, which is 0.0067mg/mL, and the content of tea polyphenols is 43.22% lower than that in the blank group.

3.2 The effect of HPEF on tea polyphenols to scavenge·OH

The regression model is as follows:

$$Y=-107.31+34.214X_1+0.21157X_2-0.00413X_1X_2-2.0427X_1^2-0.000597X_2^2+0.040711X_1^3$$

The optimal regression model P = 2.56e-08 (less than 0.001), this model is significant, in the model, the P values of the intercept and the coefficients are <0.05.The model can be used to forecast and analyze the tea polyphenols' scavenging ability on ·OH after the HPEF treatment in tea samples. Draw a three-dimensional graph according to the model as Figure 2.

When the HPEF voltage is 12-18kV, the removal ability of tea polyphenols on ·OH strengthens with the increase of frequency, then weaken; When the HPEF voltage is greater than 18kV, the total removal rate increases with the increase of frequency. When the HPEF condition is about 22kV/162Hz, the tea polyphenols has the best scavenging effect on ·OH.

3.3 The effect of HPEF on tea polyphenols to scavenge O2·–

The regression model is as follows:

$$Y=1703.9-169.71X_1-30.035X_2+3.1584X_1X_2+4.3041X_1^2+0.12554X_2^2-0.08333X_1^2X_2-0.01357X_1X_2^2+0.000367X_1^2X_2^2$$

The optimal regression model P = 3.75e-08 (less than 0.001), this model is significant, in the model, the P values of the intercept and the coefficients are <0.05. The model can be used to forecast and analyze the tea polyphenols' scavenging ability on O2·– after the HPEF treatment in tea samples. Draw a three-dimensional graph according to the model as Figure 3.

When the HPEF voltage is constant, the removal ability of tea polyphenols on O2·– strengthens with the increase of frequency, then weakens. When the voltage is 22kV, the tea polyphenols has the best scavenging effect on O2·–.

3.4 The effect of HPEF on tea polyphenols to scavenge radical DPPH

Stepwise regression analysis is performed the ability of tea polyphenols to radical DPPH after HPEF treatment , and use two third-order polynomial equations to have multivariate nonlinear fitting, on the scavenging capacity (Y) and voltage of HPEF (X1)and the frequency (X2), establish equation, and then get the optimal fitting equation. The regression model is as follows:

$$Y=838.81-6.0972X_1-19.195X_2+0.18985X_1^2+0.16874X_2^2-0.000478\ X_2^3$$

The optimal regression model P =6.52e-12 (less than 0.001), this model is significant, in the model, the P values of the intercept and the coefficients are <0.05. The model can be used to forecast and analyze the tea polyphenols' scavenging ability on radical DPPH after the HPEF treatment in tea samples. Draw a three-dimensional graph according to the model as Figure 4.

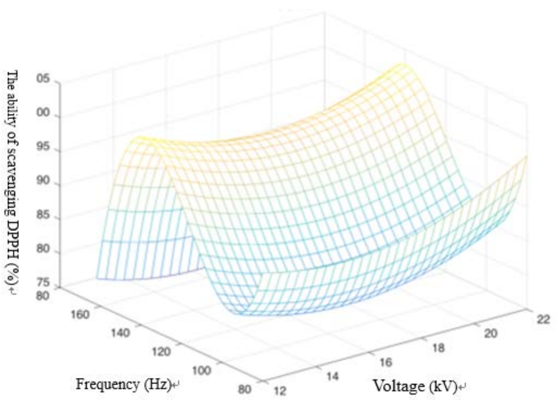

Figure 4: Scavenging DPPH for tea polyphenols

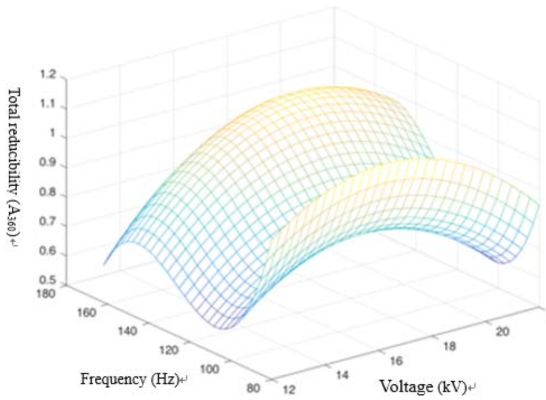

Figure 5: Total reducibility of tea polyphenols

When the HPEF voltage is constant, the removal ability of tea polyphenols on radical DPPH weakens with the increase of frequency, then strengthens and weakens. When the frequency is about 140Hz, the tea polyphenols has the best scavenging effect on DPPH.

3.5 The effect of HPEF on total reducibility of tea polyphenols

The optimal regression model P =2.43e-12 (less than 0.001), this model is significant, in the model, the P values of the intercept and the coefficients are <0.05. The model can be used to forecast and analyze the tea polyphenols' total reducibility after the HPEF treatment in tea samples. Draw a three-dimensional graph according to the model as Figure 5.
When the wavelength is 360nm and the HPEF frequency is constant，the total reducibility of tea polyphenols strengthens with the increase of frequency, then weakens. When the voltage is 16-20kV and the frequency is about 80Hz or 160Hz, HPEF can improve the total reducibility of tea polyphenols.

4. Conclusions

By using high voltage pulsed electric field to deal with the Pu'er Tea and extract the tea polysaccharides from tea samples, the contents of tea polyphenols in tea samples are compared, and the results show that the appropriate HPEF could decrease the content of tea polyphenols. Secondly, scavenging effects of tea polyphenols and its total reducibility are analyzed by stepwise regression analysis on Matlab, then establish the corresponding optimal regression model and draw the three-dimensional relationship diagram. The results show that the significance test probability of the optimal regression model P<0.01, that is the model is distinct. We can see that through the analysis of the corresponding regression model: HPEF can improve the antioxidant activity of Pu'er tea polyphenols to a certain extent, and 1) When the voltage is 22kV, the tea polyphenols have the best scavenging effect on ·OH; 2) When the frequency is less than 100Hz and the voltage is less than 16kV, tea polyphenols has the greatest scavenging ability for O2·–; 3)The main factor affecting the scavenging ability on DPPH radical is frequency; when the frequency is about 140Hz, the scavenging ability of tea polyphenols on DPPH radical is the highest; 4) When the voltage is 16kV~20kV and the frequency is about 80Hz and 160Hz, the total reducibility of tea polyphenols is the strongest.
Therefore, the suitable high voltage pulsed electric field can improve the antioxidant activity of Pu'er tea polyphenols. This study provides a new physical method for improving the antioxidant activity of Pu'er tea polyphenols, and provides a new technical support for the extraction and development of natural antioxidants, and the deep processing and the development of functional food of Pu'er tea.

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