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# Effects of High Voltage Pulsed Electric Field on Antioxidant Activity and Extraction of Tea Polysaccharides for Third Grade Ripe Pu'er Tea

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To enhance the antioxidant activity of third grade ripe Pu'er tea, this paper adopts different high-voltage pulsed electric fields (HPEF) to extract the tea polysaccharides and determine their antioxidant activity in each tea sample. The antioxidant activity was measured by four indices, namely the hydroxyl radical (·OH) scavenging effect, the superoxide anion (O2·–) scavenging effect, the DPPH radical scavenging effect, and the total reducibility. Based on the stepwise regression on Matlab, the mathematical model was established to depict the relationship between antioxidant activity, HPEF voltage, and HPEF frequency, and 3D relationship diagrams of the model were drawn to find the best HPEF parameters. It is concluded that the proper HPEF conditions can promote the extraction rate and antioxidant activity of tea polysaccharides in ripe Pu'er tea. The research findings open up a new way to enhance the antioxidant activity of Pu'er tea polysaccharides and shed new light on the extraction and development of natural antioxidants in Pu'er tea.

## 1. Introduction

As the geographical indication (GI) product of China's Yunnan Province, Pu'er tea is immensely popular at home and abroad thanks to its unique health effects (Wang et al., 2015), including but not limited to the prevention of hypertension, hyperlipidaemia and coronary heart disease (Hwang et al., 2003; Hou et al., 2009). The prominent health effects are attributable to the antioxidant activity of bioactive components, namely tea polysaccharides, theanine, caffeine, tea saponins, catechins, tea pigments and minerals. These components have been extracted as raw materials for various pharmaceuticals and healthcare products. In recent years, researchers are competing to enhance the antioxidant activity of Pu'er tea.

One of the most desirable tools for antioxidant enhancement is the high-voltage pulse electric field (HPEF). By this method, the target material is placed between two electrode plates, and applied with continued high-voltage pulse wave. With a short treatment time, low energy consumption, no pollution and limited heat release, the HPEF can preserve the quality of the target material to the greatest extent. Currently, the method has been extensively applied in field like agricultural product processing (Zhong et al., 2007; Liao et al., 2003; Zhong et al., 2004), food sterilization and preservation (Ganeva et al., 2003), natural product extraction (Yin et al., 2005; Eshtiaghi et al., 2002; Loginova et al., 2011), wine aging (Fang Sheng, et al, 2003), assisted extraction and food defrosting (Zhou et al., 2012), to name but a few.

In view of the above, this paper adopts the HPEF to extract tea polysaccharides from ripe Pu'er tea and examines the effect of different HPEFs on the extraction rate and antioxidant activity of the tea polysaccharides. By stepwise regression on Matlab, the antioxidant activity was discussed from such four aspects as hydroxyl radical (·OH) scavenging effect, the superoxide anion ($O_2{\cdot}^{-}$) scavenging effect, the DPPH radical scavenging effect, and the total reducibility. Then, a mathematical model was established to depict the relationship between antioxidant activity, HPEF voltage, and HPEF frequency, and 3D relationship diagrams of the model were drawn to find the optimal HPEF parameters.

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## 2. Materials and Methods

### 2.1 Materials

Raw material: third grade ripe Pu'er tea produced in 2011 from Lincang, Yunnan Province.
Apparatuses: ISO 9001-certified electronic balance (Beijing Sartorius Instrument System Co., Ltd.), DRHH-1 thermostat bath (Shanghai Shuangjie Laboratory Instrument Co., Ltd.), 1,000mL round-bottom flask, condenser pipe, RE-52AA rotary evaporators (Shanghai Yarong Biochemical Instrument Factory), SHZ-D (III) water circulating multi-purpose vacuum pump (Henan Yuhua Instrument Co., Ltd.), funnel, beaker, erlenmeyer flask, watch glass, 722S visible spectrophotometer (Shanghai Jinghua Technology Instruments Co., Ltd.), and test tubes.

### 2.2 HPEF device

The HPEF device consists of a DMC-200 high-voltage pulsed power supply (Dalian Dingtong Technology Development Co., Ltd.) and a processing device.
The main performance parameters of the high-voltage pulsed power supply are as follows:
Output voltage: 0~60kV;
Input voltage: AC 220V±10%;
Duty ratio of output pulse: 0~70%;
Output pulse frequency: 80Hz~2,000Hz;
Output power: 2,000W.

### 2.3 Experimental Method

(1) Setting of HPEF parameters
The main HPEF parameters include voltage, frequency and duration. Based on the previous findings, the voltage was set to 11 levels in 12kV~22kV, the frequency to 80Hz, 99Hz, 121Hz, 139Hz and 162Hz, and the duration to 55min. The specific parameters are listed in Table 1 below.

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

| Group | Voltage (kV) | Frequency (Hz) | Group | Voltage (kV) | Frequency (Hz) | Group | Voltage (kV) | Frequency (Hz) |
|---|---|---|---|---|---|---|---|---|
| 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 | | | |

(2) Extraction and measurement of tea polysaccharides
The alcohol precipitation method was employed to extract tea polysaccharides. First, the tea was placed in rotary evaporators to remove most of its moisture. Then, the Sevage solution (trichloromethane: n-butyl alcohol = 4:1) was added to the tea, and the mixture was oscillated for 20~30min. After that, absolute ethyl alcohol was added by twice the volume percent of the supernatant. Next, the mixture was filtered and let stand for 24h for drying. In this way, the author managed to extract the tea polysaccharides.
The content of tea polysaccharides was determined by phenol-sulfuric acid spectrophotometry, and the calibration curve was drawn as follows.

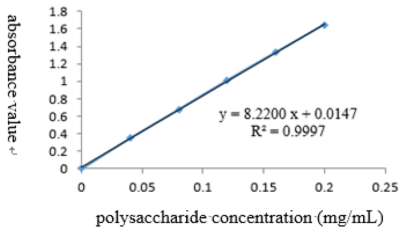


*Figure 1: Polysaccharides standard curve*

(3) Determination of antioxidant activity of tea polysaccharides

As mentioned above, the antioxidant activity of tea polysaccharides was measured by four indices: the ·OH scavenging effect, the $O_2\cdot^-$ scavenging effect, the DPPH radical scavenging effect, and the total reducibility.

The scavenging effects can be obtained by the following formula:

scavenging rate (%) = $[1-((A_1-A_2))/A_0]\times100$

The ·OH scavenging effect was determined by sodium salicylate complexometry (Xu et al., 2006). The specific steps are as follows.

First, mix 2mL 20mg/mL tea polysaccharides solution with 2mL 9mmol/L $FeSO_4$ and 2mL 9mmol/L $H_2O_2$, and let the mixture react at room temperature for 20min. Then, add 2mL 9mmol/L sodium salicylate solution, and let the mixture react at room temperature for another 20min. After that, measure the absorbance $A_1$ of tea polysaccharides in different groups at λ=510nm. Next, replace sodium salicylate solution with 2mL distilled water, and repeat the steps above to obtain the absorbance $A_2$. In addition, substitute the tea polysaccharides solution with 2mL distilled water, and repeat the steps above to obtain the absorbance $A_0$.

The $O_2\cdot$– scavenging effect was determined by pyrogallol autoxidation (Guo et al., 2007). The specific steps are as follows.

First, take 4mg/mL tea polysaccharides solution as the base solution, and prepare tea polysaccharides solutions with the concentration of 0.05mg/mL, 0.1mg/mL, 0.2mg/mL, 0.3mg/mL, 0.4mg/mL, 0.5mg/mL, and 0.6mg/mL, respectively. Then, take 0.2mL tea polysaccharides solution of each concentration, add it with 5.7mL 50mmol/L Tris-HCl buffers (pH=8.2), and let the mixture react in 25°C water for 20min. After that, add 0.1mL 6mmo1/L pyrogallol solution and let the mixture react for 5min. Finally, measure the absorbance $A_1$ at λ=360nm. Next, replace pyrogallol solution with distilled water of the same volume, and repeat the steps above to obtain the absorbance $A_2$. In addition, substitute the tea polysaccharides solution with 2mL distilled water, and repeat the steps above to obtain the absorbance $A_0$.

The DPPH radical scavenging effect was determined by the method of Zhang et al. (2007) The specific steps are as follows.

First, prepare 0.2mmol/L DPPH radical anhydrous ethanol solution in a brown bottle at 0~4 °C. Then, mix 2mL 20mg/mL tea polysaccharides solution with 2mL 0.2mmol/L DPPH radical anhydrous ethanol solution in a test tube, and let the mixture stand for 0.5h. After that, measure the absorbance $A_1$ at λ=517nm. Next, replace the DPPH radical anhydrous ethanol solution with anhydrous ethanol solution of the same concentration, and repeat the steps above to obtain the absorbance $A_2$. In addition, substitute the tea polysaccharides solution with 2mL 20mg/mL anhydrous ethanol solution, and repeat the steps above to obtain the absorbance $A_0$.

The total reducibility of tea polysaccharides was determined by the method of Zou et al. (2014) The specific steps are as follows.

First, take 4mg/mL tea polysaccharides solution as the base solution, and prepare 20mL tea polysaccharides solutions with the concentration of 0mg/mL, 1mg/mL, 2mg/mL, 3mg/mL, 4mg/mL and 5mg/mL, respectively. Then, take 1mL tea polysaccharides solution of each concentration, add it with 1mL 2mol/L phosphate buffer (pH=6.6) and 1mL potassium ferricyanide solution (ω=1%), and let the mixture stand for 20min. After that, add 0.25mL ferric chloride (ω=0.1%) and 2mL distilled water, and let the mixture stand for 20min. Finally, measure the absorbance A at λ=360nm. The value of A is positively correlated with the total reducibility.

## 3. Results and Analysis

### 3.1 Effect of HPEF on the extraction of tea polysaccharides

For the tea samples in the blank group and those under different HPEFs, the content of tea polysaccharides was calculated according to the standard curve equation: y=8.2200x+0.0147 ($R_2$=0.9997). The results are listed in Table 2.

*Table 2: The content of tea polysaccharides in its crude extraction*

| Group | Content of tea polysaccharides (mg/mL) | Group | Content of tea polysaccharides (mg/mL) | Group | Content of tea polysaccharides (mg/mL) |
|---|---|---|---|---|---|
| CK | 0.1590 | 19 | 0.1638 | 38 | 0.1659 |
| 1 | 0.1661 | 20 | 0.1649 | 39 | 0.1633 |
| 2 | 0.1672 | 21 | 0.1595 | 40 | 0.1629 |
| 3 | 0.1631 | 22 | 0.1632 | 41 | 0.1628 |
| 4 | 0.1644 | 23 | 0.1605 | 42 | 0.1637 |
| 5 | 0.1595 | 24 | 0.1646 | 43 | 0.1633 |
| 6 | 0.1644 | 25 | 0.1650 | 44 | 0.1646 |
| 7 | 0.1632 | 26 | 0.1641 | 45 | 0.1668 |
| 8 | 0.1560 | 27 | 0.1661 | 46 | 0.1649 |
| 9 | 0.1656 | 28 | 0.1606 | 47 | 0.1635 |
| 10 | 0.1596 | 29 | 0.1611 | 48 | 0.1640 |
| 11 | 0.1588 | 30 | 0.1660 | 49 | 0.1629 |
| 12 | 0.1654 | 31 | 0.1643 | 50 | 0.1627 |
| 13 | 0.1640 | 32 | 0.1629 | 51 | 0.1657 |
| 14 | 0.1616 | 33 | 0.1660 | 52 | 0.1649 |
| 15 | 0.1637 | 34 | 0.1645 | 53 | 0.1640 |
| 16 | 0.1628 | 35 | 0.1637 | 54 | 0.1598 |
| 17 | 0.1632 | 36 | 0.1634 | 55 | 0.1531 |
| 18 | 0.1645 | 37 | 0.1635 | | |

It is clear that the tea samples under HPEF had a higher content of tea polysaccharides than those in the blank group. For instance, the content in group 2 (13kV/80Hz) reached the peak value of 0.1672mg/mL, 5.09% higher than that of the blank group. This means proper HPEF conditions can increase the content of tea polysaccharides in its crude extraction.

### 3.2 Effect of HPEF on the ·OH scavenging effect of tea polysaccharides

The best-fit binary cubic equation was established through multiple nonlinear fittings of the relationship between the scavenging effect (Y), HPEF voltage ($X_1$) and frequency ($X_2$). The regression model is as follows:

$$Y=94.232+0.55945X_1+0.061745X_2-0.035053X_1^2-0.00049171X_2^2+0.00027475X_1X_2+0.00067568X_1^3+0.000001202X_2^3$$

Since the P-value (5.34e-08) is less than 0.01 and the intercept/P-value of each coefficient is less than 0.5, the model is applicable to the prediction of the ·OH scavenging effect of tea polysaccharides in tea samples under HPEF. The 3D diagram was drawn below according to the model.

As shown in Figure 2, when the HPEF voltage fell in 12~18kV, the ·OH scavenging effect of tea polysaccharides grew stronger and then became weaker with the increase in frequency; when the HPEF voltage was higher than 18kV, the scavenging effect was improved on the whole. The best scavenging effect appeared at $X_1$=22kV and $X_2$=162Hz.

### 3.3 Effect of HPEF on the O2·– scavenging effect of tea polysaccharides

The best-fit binary cubic equation was established through multiple nonlinear fittings of the relationship between the scavenging effect (Y), HPEF voltage ($X_1$) and frequency ($X_2$). The regression model is as follows:

$$Y=-35.784+2.2099X_1+2.1266X_2-0.0073939X_2^2-0.028182X_1X_2$$

Since the P-value (3.75e-08) is less than 0.01 and the intercept/P-value of each coefficient is less than 0.05, the model is applicable to the prediction of the $O_2·^-$ scavenging effect of tea polysaccharides in tea samples under HPEF. The 3D diagram was drawn below according to the model.

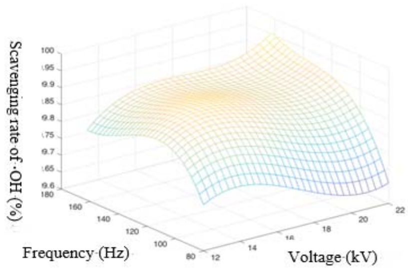


*Figure 2: Three-dimensional diagram of the rate of scavenging tea polysaccharides for ·OH*

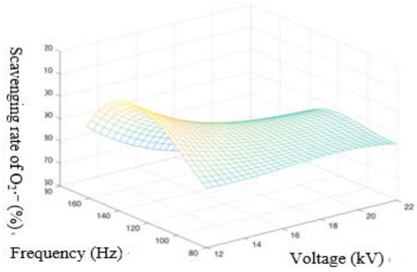


*Figure 3: Three-dimensional diagram of the rate of scavenging tea polysaccharides for O2·–*

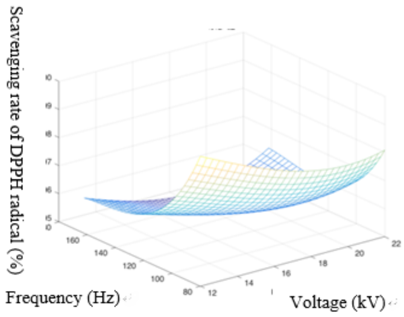


*Figure 4: Three-dimensional diagram of the rate of scavenging tea polysaccharides for DPPH*

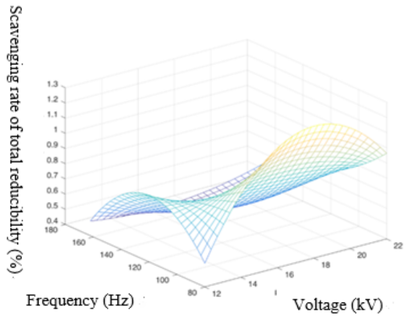


*Figure 5: Three-dimensional diagram of total reducibility of tea polysaccharides*

As shown in Figure 3, when the HPEF voltage ranged from 12kV to 18kV, the $O_2\cdot^-$ scavenging effect of tea polysaccharides grew stronger and then became weaker with the increase in frequency; when the HPEF frequency was lower than 100Hz, the scavenging effect remained basically stable with the change in voltage; when the frequency was higher than 100Hz, the scavenging effect decreased with the increase in voltage. The best scavenging effect appeared at $X_1$<14kV and $X_2 \in$ (120, 140) Hz.

### 3.4 Effect of HPEF on the DPPH scavenging effect of tea polysaccharides

The best-fit binary cubic equation was established through multiple nonlinear fittings of the relationship between the scavenging effect (Y), HPEF voltage ($X_1$) and frequency ($X_2$). The regression model is as follows:
$Y=109.72-0.083284X_1-0.173017X_2+0.00059166X_2^2$
Since the P-value (4.54e-08) is less than 0.001 and the intercept/P-value of each coefficient is less than 0.05, the model is applicable to the prediction of the DPPH radical scavenging effect of tea polysaccharides in tea samples under HPEF. The 3D diagram was drawn below according to the model.
As shown in Figure 4, when the voltage was regular, the DPPH radical scavenging effect of tea polysaccharides generally decreased with the increase in frequency; when the frequency was irregular, the scavenging effect was basically immune to voltage increase. Hence, frequency is the main influencing factor of the DPPH radical scavenging effect. The best scavenging effect appeared at $X_1$<14kV and $X_2$≈80Hz.

### 3.5 Effect of HPEF on the total reducibility of tea polysaccharides

The best-fit binary cubic equation was established through multiple nonlinear fittings of the relationship between the scavenging effect (Y), HPEF voltage ($X_1$) and frequency ($X_2$). The regression model is as follows:
$Y=-44.954+5.3492X_1+0.70703X_2-0.14596X_1^{2}0-0.0026079X_2^2-$
$0.081965X_1X_2+0.0022262X_1^2X_2+0.00029876X_1X_2^2-0.000008071X_1^2X_2^2$
Since the P-value (9.22e-08) is less than 0.001 and the intercept/P-value of each coefficient is less than 0.05, the model is applicable to the prediction of the reducibility of tea polysaccharides in tea samples under HPEF. The 3D diagram was drawn below according to the model.
As shown in Figure 5, when λ=360nm and $X_1$>14kV, the total reducibility exhibited a decline trend with the increase in frequency; when $X_1$<14kV, the total reducibility first increased and then decreased with the frequency growth; the strongest reducibility appeared at $X_1$>18kV and $X_2$≈80Hz.

## 4. Conclusion

In this paper, the HPEF was adopted to extract the polysaccharides from third grade ripe Pu'er tea. Then, the content of tea polysaccharides in different groups of tea samples were compared one by one. The comparison shows that proper HPEF conditions can increase the extraction rate, i.e. the content of tea polysaccharides in its crude extraction. After that, the scavenging effects and total reducibility of tea polysaccharides were analysed by stepwise regression on Matlab. Based on the P-value, the regression model was proved applicable to the prediction of the relationship between the antioxidant activity of tea polysaccharides, HPEF voltage and HPEF frequency. Then, the 3D diagrams were drawn according to the model.
Since the P-value is always below 0.01, the optimal regression model is significant enough for prediction of the relationship between antioxidant activity of tea polysaccharides, HPEF voltage and frequency. The model analysis reveals that the HPEF can improve the antioxidant activity of tea polysaccharides in ripe Pu'er tea to a certain extent. It is also concluded that the best ·OH scavenging effect appeared at $X_1$=22kV and $X_2$=162Hz; the best $O_2\cdot^-$ scavenging effect appeared at $X_1$<14kV and $X_2 \in$ (120, 140) Hz; the best DPPH radical

scavenging effect appeared at $X_1$<14kV and $X_2$≈80Hz; the strongest reducibility appeared at $X_1$>18kV and $X_2$≈80Hz.
To sum up, the proper HPEF conditions can promote the extraction rate and antioxidant activity of tea polysaccharides in ripe Pu'er tea. The research findings open up a new way to enhance the antioxidant activity of Pu'er tea polysaccharides and shed new light on the extraction and development of natural antioxidants in Pu'er tea.

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