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FULL-LENGTH RESEARCH ARTICLE

Research on Aging Effect of Unfermented Pu'er Tea by High-Voltage Pulsed Electric Field

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Abstract This essay provides a physical method to improve the "aging" process of Yunnan Pu'er tea, which is pollution-free, energy saving and having no negative effect on the taste or nutrition facts of Pu'er tea. "Aging" is a special storage method in the culture of Yunnan Pu'er tea. After stored for a certain period of time, the aroma and taste of Pu'er unfermented tea would become favorable for drinkers. In addition, as the elevation of people's living standard and the gradual acknowledgment of Pu'er tea's salutary effect, the demand of Pu'er tea increases drastically in recent years. Its characteristic that "getting better" becomes the factor attracting consumer and guiding market price. This essay utilized high-intensity pulsed electric field (HPEF) to process tea samples under different conditions. The results of experiments show that both the taste and aroma of tea samples have improved after the process. This result provides a new method to elevate the quality of Pu'er tea and a theoretical basis for artificial aging of Pu'er tea by HPEF.

Keywords Unfermented Pu'er tea · High-voltage pulsed electric field · Aging effect

Introduction

Pu'er tea is a special kind of fermented tea that is made by raw material produced within certain region in Yunnan Province, China, classified into "Sun-dried Green Tea," "Ripe tea" and "Chen tea" based on different processing technic, the sun-dried green tea is divided into unfermented Pu'er tea, green tea, green brick tea and sun-dried green primary tea [17]. It has a unique fragrance generated during the procedure of aging, and it is also featured by its shiny red, mellow-tasting and long-lasting sweet tea liquor [17]. Pu'er tea is beloved by foreign and domestic tea-drinkers because of its special health efficacy and its "getting better" feature. Differed from the fresh-favored convention of other kinds of tea, Pu'er tea has a unique feature that is "getting better". No matter whether it is fermented or unfermented, the longer it is aged, the better the quality is and the more favorable its aroma would become. Therefore, among all the kinds of tea, Pu'er is the most collectworthy one. Through centuries of productive practice, people have developed a complete process of fermenting, hoarding and aging, especially the highly regarded process of hoarding and aging. Fresh Pu'er tea has incentive, bitter and astringent taste, which needed to be stored for a long period of time for aging to diminish. When it reaches to its best timing of drink, its taste would be transformed to be tender and mellow. As the elevation of people's living standard and the gradual acknowledgment of Pu'er tea's salutary effect, the demand of Pu'er tea surges in recent years, which necessitates the raise of high-quality Pu'er tea production and the decrease in manufacturing cost. Hence, the artificial acceleration of aging process becomes an important field in Pu'er tea study [14, 17, 21].

The HPEF nonthermal procession technique has been universally applied in food industry, which is one of the most potential technologies concerned by numerous scholars in recent years [4]. HPEF is one of the hottest nonthermal sterilization techniques of recent time, which mainly because of its special properties that low energy

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consumption, uniform transmission, speedy processing and non-polluting, which make it universally popular in food industry [15]. In addition to sterilization, it has been more and more widely applied in substance extraction, sewage treatment, tobacco deinsectization and wine aging [7, 10, 12, 20]. However, the existent researches about HPEF application in Pu'er tea aging are still limited. Therefore, in order to provide theoretical basis for artificially accelerated Pu'er tea aging, the experiments in this essay would analyze samples made of Yunnan Mengku large-leaf species, produced in different year by using three groups of HPEF to process unfermented Pu'er tea and systematically study its effect on the quality of tea (extract, tea polyphenols and theine).

Materials and Methods

Pu'er Tea Sample

The seven experiment samples are chosen from the main tea-growing region, Menghai Yunnan, and manufactured by Fuyuanchang in different years. Basic information of samples is specified in Table 1.

Measures of Tea Contents

The Measure of Polyphenols

Reagent ferrisulfas, potassium sodium tartrate, monopotassium phosphate (pure and made domestically).

Instruments electronic balance (FA1204B, Shanghai Precision Instruments Co.), ultraviolet spectrophotometer (T6 New Age, Beijing Purkinje General Instrument Co., Ltd.), thermostatic water bath (HWS-24 electric-heated thermostatic water bath, Shanghai Yiheng Scientific Instrument Co.) and Small plant sample mill (DFT-100 Portable).

Testing method measure 0.2 g of ground tea sample to make mother liquor, using pipette, respectively, remove gallic acid liquid, water (as control), testing liquid for each 1.0 mL into test tube, adding 5.0 mL Folin–Ciocalteu for each test tube and shaking, reacting 3–8 min, adding 4.0 mL 7.5 % Na_2co_3 liquor. Watering and constant volume to scale, shaking. Place 60 min at room temperature. Using 10-mm cuvette, the absorbance (A) was measured by spectrophotometer at the wavelength of 765 nm [4].

Formula of calculating the content of polyphenols:

$$\text{Polyphenols } (\%) = \frac{A \times V \times d}{\text{SLOPE}_{\text{std}} \times m \times 10^6 \times m_1} \times 100$$

A the absorbance of sample test liquid; V the volume of sample extract, 10 mL; d dilution factor; *SLOPEstd* slope of standard curve of gallic acid; m dry matter content of the sample (%); and m_1 the quality of sample, unit: g.

The Measure of Tea Extracts

The measure of tea extract is in accordance with People's Republic of China National Standard GB/T 8305-2002 "the measurement of tea extract" [2].

The Measure of Theine

After ground by plant crusher, the tea samples are filtered by 80-mesh sieve. Weigh the appropriate amount of pulverized sample, placing it in a flask of tea, operating according to GB/T 8312-2002 to measure the content of theine [3].

The Generation of HPEF

The devices adopted are the DC high-voltage generator developed by Dalian University of Technology, Static Electricity and Special Power Research Institute, coupled with energy storage capacitor Ce and pulse capacitor capacitance Cp. Through the alternate on–off of two perpendicular spark gap switch, RSG1 and RSG2, the charging of capacitor and its discharging between the reactor plate connected to the high-voltage interface and the ground plate are achieved, and the steep high pulsed

Table 1 Tea samples

Number	Production name	Type	Grade	Production date	Place
1	Fuyuanchang Five-star	Unfermented Pu'er tea	1–3	2005	Lincang
2	Fuyuanchang Five-star	Unfermented Pu'er tea	1–3	2006	Lincang
3	Fuyuanchang Five-star	Unfermented Pu'er tea	1–3	2007	Lincang
4	Fuyuanchang Five-star	Unfermented Pu'er tea	1–3	2008	Lincang
5	Fuyuanchang Five-star	Unfermented Pu'er tea	1–3	2009	Lincang
6	Fuyuanchang Five-star	Unfermented Pu'er tea	1–3	2010	Lincang
7	Fuyuanchang Five-star	Unfermented Pu'er tea	1–3	2011	Lincang

electric field is formed between the two plates, as shown in Fig. 1.

The main performance parameters are: Output voltage: 0–60 kV adjustable, pulse width: ≤300 ns, frontier rise time: ≤50 ns and repetition frequency: 0–200 pps adjustable.

Electroporation theory [6, 13]—under HPEF effect—pores will appear on cell membrane, which will result in partial or complete destruction of normal selective barriers of cell membrane. Based on this theory, we can analyze the whole process of cell electroporation according to Fig. 2. When the size of the applied electric field strength E reaches a certain value, the induced membrane potential added on the cell membrane reaches a critical value, which is critical membrane potential, also known as transmembrane potential (TMP). At this point, microporous begins to appear on cell membrane.

When the applied electric field strength E remains unchanged and osmotic balance inside and outside the cell membrane is ensured, some small molecules (represented by small dot) can still enter the cells through the membrane pores, but the cells will not expand. After a period of time, the micropores on cell membrane will close again, as shown in Figs. 1, 2. Thus, instead of dying because of membrane rupture, cells would become hardier due to import of nutrients [18].

Processing Tea Samples with HPEF

Main Parameters of HPEF effect are electric field strength (voltage), frequency and duration of action, processing the above-mentioned seven kinds of unfermented Pu'er tea on the basis of orthogonal test. According to the laboratory's experience, 3 different groups of HPEF are chosen with different electric field intensity, different frequencies and different time (Tables 2, 3) to process the tea. Firstly, the devices are appropriately adjusted. After the warm-up, tea samples are placed between the electric field plates and the device is turned on. A, B and C, three groups of conditions, are adjusted, respectively, to complete the experiment, after which the frequency and voltage are set to minimum. Then, the device is turned off and the equipment is discharged. The tealeaves are removed and sealed after the discharge.

Results and Discussion

Sensory Evaluation of Tea

Three national tea tasters are invited to do sensory evaluations to tea samples with respect to liquor, aroma, taste and leaves. The color of unfermented Pu'er tea is yellow based. The liquor changed most is the 2005 raw tea, whose color is orange-red. After the HPEF process, the aroma became closer to Chenxiang compared to the original tea sample; the color turned into orange, the pungent taste significantly decreased and tended to be mellow. From the sensory analysis, the conclusion is that, after a long period of storage, the sensory quality and the degree of aging of both fermented and unfermented Pu'er tea will be significantly changed. The effects of HPEF process are close to the natural aging results in sensory qualities (Tables 4, 5).

Detection and Comparison of Tea Inclusions

Detection and Comparison of Tea Inclusions Produced in Different Years

1. Comparison of tea polyphenols content in Pu'er tea produced in different years

The results of polyphenols content in seven kinds of unfermented Pu'er tea samples are shown in Table 6. The same brand of tea, if stored for different times, tea polyphenols content is different: With the extension of storage time of tea, tea polyphenols content shows a clear downward trend; if processed with different techniques, tea

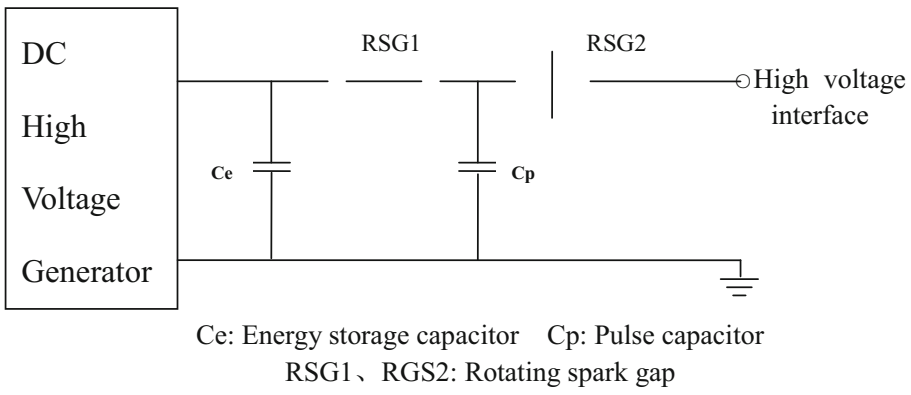

Fig. 1 Generator of high-intensity pulsed electric field

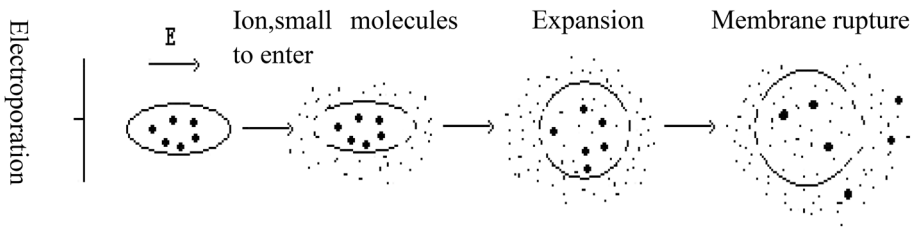

Fig. 2 Reversible breakdown process of cell electroporation

Table 2 Paramenters of HPEF

Serial number	Voltage (KV)	Frequency (Hz)	Time (min)
A	10	60	45
B	14	120	60
C	20	150	30

Table 3 Tea sensory evaluation of Pu'er raw tea in different years

No.	Date (Year)	Soup color	Aroma	Taste	Ye Di	Score
1	2005	Reddish orange, bright	Aged fragrant(sweet)	Pure and mild	Tan	96
2	2006	Reddish orange	Aged fragrant(sweet))	Mellow	Tan	92
3	2007	Reddish orange, bright	Aged fragrant(sweet)	Mellow	Tawny	90
4	2008	Thick yellow	Aged fragrant(sweet)	Mellow	Tawny	87
5	2009	Thick yellow	Aged fragrant(sweet)	Mellow	Kelly	84
6	2010	Thick yellow	Slight-aged fragrant(sweet)	Mellow	Kelly	82
7	2011	Thick yellow	Slight-aged fragrant(sweet)	Mellow (bitter)	Kelly	80

Table 4 Comparison of tea polyphenols content

No.	Name	Type	Date (Year)	Grade	Tea polyphenols content (%)
1	Fuyuanchang	Unfermented Pu'er tea	2005	1–3	13.14
2	Fuyuanchang	Unfermented Pu'er tea	2006	1–3	19.06
3	Fuyuanchang	Unfermented Pu'er tea	2007	1–3	24.02
4	Fuyuanchang	Unfermented Pu'er tea	2008	1–3	28.57
5	Fuyuanchang	Unfermented Pu'er tea	2009	1–3	31.83
6	Fuyuanchang	Unfermented Pu'er tea	2010	1–3	35.11
7	Fuyuanchang	Unfermented Pu'er tea	2011	1–3	38.74

polyphenols content is also different: The tea phenol content in unfermented Pu'er tea is significantly higher than that in fermented Pu'er tea.

2. Comparison of tea extract from unfermented Pu'er tea produced in different years

The results show that the same brand of tea, if stored for different times, tea extract content is different. With the extension of storage time of tea, tea extract content shows a clear downward trend.

3. Comparison of theine from unfermented Pu'er tea produced in different years

The experimental results show that (Table 6), the same brand of tea, if stored for different times, tea extract content is different: With the extension of storage time tea, tea theine content was measured and the results show that with the passage of storage time, theine showed a downward trend, which indicates that the storage time is an important factor affecting the theine content.

Table 5 Comparison of tea extracts contents

No.	Name	Type	Date (Year)	Grade	Tea extracts contents (%)
1	Fuyuanchang	Unfermented Pu'er tea	2005	1–3	23.14
2	Fuyuanchang	Unfermented Pu'er tea	2006	1–3	26.06
3	Fuyuanchang	Unfermented Pu'er tea	2007	1–3	28.57
4	Fuyuanchang	Unfermented Pu'er tea	2008	1–3	31.83
5	Fuyuanchang	Unfermented Pu'er tea	2009	1–3	35.11
6	Fuyuanchang	Unfermented Pu'er tea	2010	1–3	38.74
7	Fuyuanchang	Unfermented Pu'er tea	2011	1–3	42.66

Table 6 Comparison of the theine content

No.	Name	Type	Date (Year)	Grade	The theine content (%)
1	Fuyuanchang	Unfermented Pu'er tea	2005	1–3	3.369
2	Fuyuanchang	Unfermented Pu'er tea	2006	1–3	3.903
3	Fuyuanchang	Unfermented Pu'er tea	2007	1–3	4.012
4	Fuyuanchang	Unfermented Pu'er tea	2008	1–3	4.325
5	Fuyuanchang	Unfermented Pu'er tea	2009	1–3	4.62
6	Fuyuanchang	Unfermented Pu'er tea	2010	1–3	4.895
7	Fuyuanchang	Unfermented Pu'er tea	2011	1–3	5.011

Detection and Comparison of Tea Inclusions After HPEF Process

1. Effect of HPEF on unfermented Pu'er tea polyphenols

Tea polyphenol is a general designation of multiple phenolic compounds contained in tea, which is colorless and bitter, with strong convergence. Its main component is catechin, accounting for 60 to 80 % of total polyphenols, which is prone to be non-enzymatic auto-oxidized during storage, forming fresh taste-related theaflavins (TFs), concentration of tea taste-related thearubigins (TRs) and the dark resulting tea theabrownins (TBs) and other browning substance [8, 22].

Figure 3 shows that after different conditions of HPEF process, tea polyphenol content tends to decrease wherein the sample processed by HPEF B (14 kV/120HZ/60 min) has a significant reduction in polyphenol content. According to Yongguang Yin and others' studies [15, 20], HPEF process could increase dry red wine extract content, improve the color stability of wine and reduce its alcohol content, which is basically the same trend as naturally aged wine. After HPEF process, the sensory quality of tea is enhanced, the main chemical components of tea polyphenols content tends to decrease, which is consistent to the naturally aging tendency.

Studies have shown with the extension of storage time, tea polyphenols content tend to decrease [9]. Figure 3 shows that short-term HPEF treatment can also cause the reduction in tea polyphenols content. During storage, if the

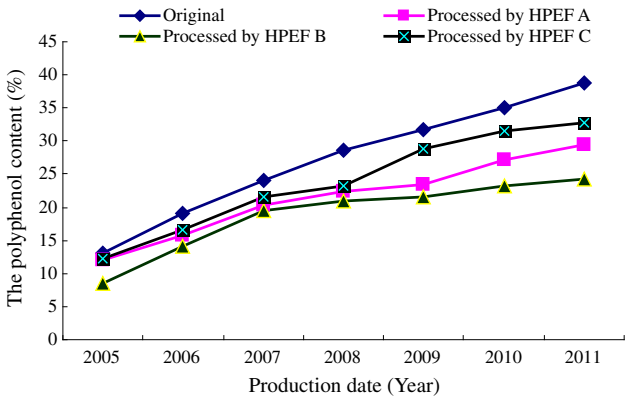

Fig. 3 Effect of HPEF on the polyphenols content in tea

retention of polyphenols is excessive, particularly astringent catechins, tea would taste bitter. By contrast, if the retention of polyphenols is too little, tea convergence would be weakened, and the flavor would be insipid. Therefore, during storage, the retention of water-soluble polyphenols should be moderate. The retention rate of tea polyphenols is different if treated by different high-pressure treatments. The retention rate depends on sensory evaluation results.

2. Effect of HPEF on the extract content unfermented Pu'er tea

As shown in Fig. 4, with the extension of storage time of tea, tea extract content shows a clear downward trend. Tea

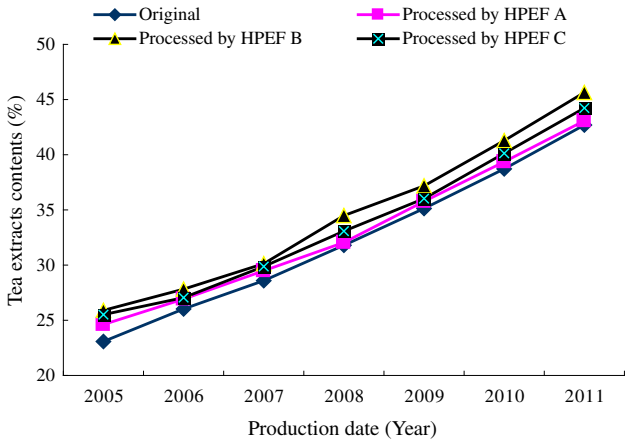

Fig. 4 Effect of HPEF on the extract content in tea

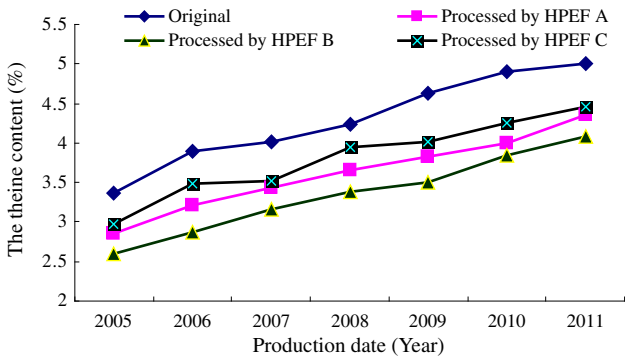

Fig. 5 Effect of HPEF on the theine content in tea

extracts content increases after HPEF process: Tea samples treated by HPEF B (14 kV/120 Hz/60 min) had largest increase in tea extract content. The results provide a new method to improve the tea extract content of antiqued Pu'er tea. They also provide a theoretical basis for the future development of Pu'er tea deep processing and functional food.

3. Effect of HPEF on the tea theine

Theine is a kind of alkaloids, bitter and odorless, white or slightly yellow powder or satin-like needle-shaped crystals, an important tea taste substances. Its hydrogen bond association complex with theaflavin has a fresh and mellow taste, whose content is often considered an important factor of tea quality.

According Guofan He's research, during tea processing, theine increases as the pile process, after which processed tea is higher than the original tea in the theine content [6]. Hence, the theine content of unfermented Pu'er tea samples is lower than that of fermented tea samples. Shuhong Zhou and others' studies have shown that during storage, radiation treatment to tea could decrease its theine content [22].

The experimental results show that(Fig. 5) after processed by different conditions of HPEF, tea theine content of unfermented Pu'er is lower than that of control sample, which indicates that HPEF treatment can reduce theine content. Compared to the control sample, the HPEF B (14 kV/120 Hz/60 min) significantly reduced theine content of tea samples processed. The reduction in theine content, the key quality indicators of tea, after HPEF process, is consistent to natural aging downward tendency. The results of this study not only provides a new way to enhance the quality of tea, but also provides a theoretical basis for further discussion of the use of HPEF on tea aging.

Conclusions

The longer Yunnan unfermented Pu'er tea is stored, the brighter and oranger the liquor, the higher the aroma. During the storage, the infused leaves gradually change from green to yellow-green to yellow-red and become more flexible. In short, the sensory quality of tea changes as time goes by, the taste becomes better and better, which is called "aging" in Yunnan Pu'er tea culture. Pu'er tea's "getting better" feature has become an important factor attracting consumer and guiding market price, so how to use physical methods to artificially accelerate Pu'er tea's aging is a hot research topic in tea industry.

After HPEF process, the aroma tends to be Chen tea aroma and the taste tends to be mellow compared to the original control group. From the sensory analysis, the sensory quality of tea processed by HPEF is close to natural result of aging. Data and charts present a consistent reduction in tea polyphenols and theine on HPEF-processed Pu'er tea with naturally aged Pu'er tea. Due to the significant reduction in polyphenols, the taste of Pu'er tea becomes mellow, spicy and convergence are alleviated, and salutary effectiveness has significantly enhanced [11, 23].

Theine is one of the causing factors of bitter tea taste. The reduction in theine makes tea's taste mild and mellow, more acceptable for the majority of the crowd. In this study, the HPEF treatment also increased its tea extract content, which further improves its taste while artificially aging it.

HPEF has accelerated effect on aging process. Using this technology can greatly shorten the aging time of unfermented Pu'er tea and improve the quality of health and safety, which provides a new way to enhance the quality of Pu'er tea. It also provides a theoretical basis for artificially aging process by HPEF and a possibility for further industrial processing.

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Compliance with Ethical Standards

Conflict of interest We have nothing to disclose.

Author's Contributions Chen Ting was mainly responsible for the experimental principle and design. Peng Wen was mainly responsible for the data analysis. Zhao Yan was mainly responsible for analysis of the contents of tea. Liu Yijia was mainly responsible for the article's translation. Wang Baijuan was mainly responsible for the overall structure of the article.

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