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Research Article

The Promotion Effects on Pu'er Tea Aroma of High Voltage Pulsed Electric Field

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Abstract: In order to explore how to improve the aroma of geographical indications products in Yunnan-Pu'er Tea Using Electronic Nose System, the aroma components of Five-star Pu'er ripe tea of Fuyuanchang (2005 vintage) was defected, which treated by different High voltage Pulsed Electric Field (HPEF). And Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) and loadings analysis (Loading) were take on by Winmuster software, then combining response curve peak transuded comparison, the orthogonal test of the results from the experiment was done. The result proved that the content of aroma composition changed prominently after HPEF processing. The three kind of aroma component which include alcohol, organic sulfide and short-chain alkane increased significantly. In the condition of 18 kV/180 Hz/30 min, the three kinds of aroma composition increased the most and the average increase was 12.5%; and under the condition of 12kV/150Hz/45 min, the increase come secondly with the average of 11.1%. In addition, the other promoted condition of HPEF included 18 kV/120 Hz/60 min, 12 kV/120 Hz/60 min and 18 kV/120 Hz/30 min and the first condition was better than the others. The electric field voltage was the essential determinant which effected the content of aroma composition.

Keywords: Aroma composition, electronic nose, High voltage Pulsed Electric Field (HPEF), orthogonal experiment, Pu'er ripe tea

INTRODUCTION

In recent years, the characteristics of tea aroma are focused by many scholars. However, there are many kinds of aromatic substances in tea aroma components and are sensitive to heat, light and oxygen, etc. During the tea processing, some aroma substances are easy to complex chemical reactions, such as oxidation, condensation polymerization, group transfer, etc., which will lead to a change in the content and proportion of aroma components in tea (Zhou *et al*., 2004). For a lot of heat sensitive aroma components, in the traditional processing method including closed, high temperature, long process conditions, some aroma substances more prone to change, influenced the aroma of tea and let tea flavor decreased significantly (Guerin *et al*., 1974). Among many kinds of tea, Yunnan Pu'er tea is taken as a wonderful flower in the tea fields, globally knowned due to its long history, unique quality and remarkable effect (Shao, 2014). Pu'er tea is made up of Yunnan large-leaf variety crude tea produced within a certain range of Yunnan province. According to processing technology categorized as "Sheng" and "Shu" (GB/T 22111, 2008). As its unique flavor and health effects that is gradually known people and well selling in the domestic and foreign markets (Zhou *et al*., 2003; Zhao *et al*., 2005; Zhang *et al*., 2005). There is study show that Pu'er tea's unique aroma is formed by microorganism under wet and hot condition during the pile fermentation process (Ren *et al*., 2011). The component of aroma is closely related the quality of Pu'er tea and it is the most important of its price determinant. It is necessary to find a reasonable and effective way to deal with tea in reducing aging time while increasing the tea aroma composition. Therefore, it is an urgent need for researchers and the market to find a method that can not only keep the composition of tea, but also improve the aroma. In this study, HPEF, a physical method, is used to deal with Pu'er tea, which solve this urgent need. While it increases the aroma of Pu'er tea and also provides a new method that can reduce the aging time of Pu'er tea and shorten the time to meet with consumers.

High-voltage Pulsed Electric Field (HPEF) is a non-heat treatment technology, which is widely used in food industry and is concerned by many scholars in recent years (Guo *et al*., 2001). HPEF is one of the major fields of non-thermal sterilization technology. Because of its advantages, such as low energy consumption, uniform delivery, short time and no pollution, etc., which has been widely used in the food processing industry (Wang *et al*., 2011). Besides sterilization, it is also gradually used in material extraction, wastewater treatment, tobacco

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deinsectization, liquor and wine aging etc (Jeyamkondan *et al*., 1999; Yin *et al*., 2006; Meng *et al*., 2008). However, the effect of HPEF on food composition and some biological macromolecules has been few studied. The many results show that the influence of HPEF on food components such as protein, lipid has been objective existence. Based on a great deal of characteristics of HPEF, the study intends to process samples of Pu'er ripe tea by 9 different conditions (voltage/frequency/time) of High-voltage Pulsed electric Field (HPEF), winmuster software was used to deal with the data of electronic nose detection and the data were analyzed by Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) and Loadings Analysis (Loading), Orthogonal Experimental Analysis (Xu *et al*., 2002; He and Yu, 2005), through the action of HPEF, to increase the contents of aroma components of tea, improve the quality of Pu'er Tea.

EXPERIMENTAL MATERIALS AND METHODS

Samples of Pu'er tea: Samples of Pu'er tea is from the major producing areas of Yunnan, Lincang. It produced in 2005 by Fuyuanchang and marked Five-star ripe tea.

Generating HPEF: The DC High voltage generator is developed by the Institute Of Technology Of Dalian, Static and Special Power Supply Research Center (Wang, 2013). Two groups of capacitors, storage capacitor Ce and pulse capacitor Cp, achieve the charge of pulse capacitor and discharge between reactor plate and ground plate connected to the high voltage interface plate by manipulating two perpendicular spark gap switches RSG1 and RSG2 as one open and one closed, forming steep HPEF between two polar plates (Fig. 1). Major Performance Parameters: Output voltage: 0-60 KV adjustable, pulse width: ≤300 ns, rise time: ≤50 ns, repetition frequency: 0-200 pps adjustable.

Electroporation theory (Elez-Martinez *et al*., 2005; Labreche *et al*., 2005; Jin *et al*., 2009 and Jayaram *et al*., 1992). There will be small holes on the cell membrane with HPEF treatment. The results will lead to selective normal cell membrane appear partially or completely destroyed through the barrier. Base on this theory, we analyzed the whole process of the cell electroporation (Fig. 2), When the applied electric field intensity E reached a certain value and induced membrane potential in the cell membrane has reached the critical value, it is critical membrane potential, called membrane potential of the cell membrane (TMP). At this point, the microporous began to appear on the cell membrane.

When the applied electric field intensity E remains the same, kept the cell membrane pressure balance both inside and outside osmotic, some small molecules (small dots) can still enter the cell through membrane pores, but the cell is still not expansion, after a period of time, pores will be closed on the cell membrane, as shown in Fig. 2. In this way, the cell body not only won't be death because of the cell membrane rupture but also be stronger due to the import of nutrient.

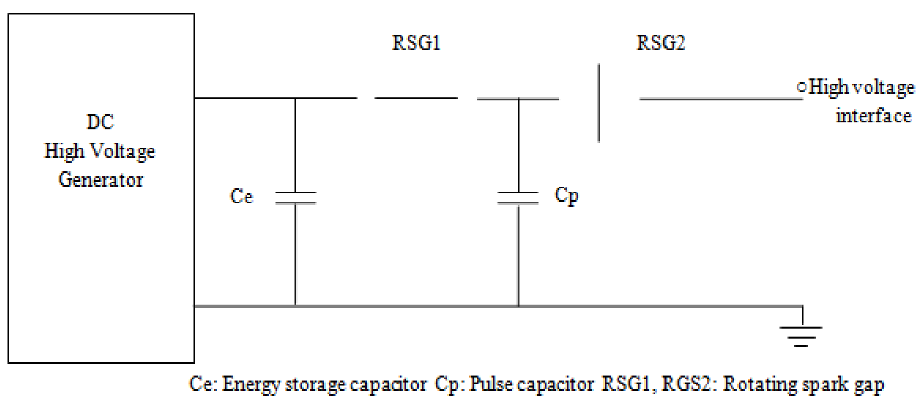

Fig. 1: The generator of High Pulsed Electric Field (HPEF)

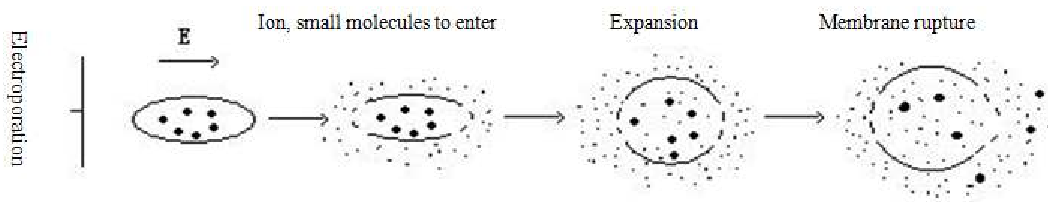

Fig. 2: Reversible breakdown process of cell electroporation

Table 1: Orthogonal test table

Group number		Factors: Electric field voltage (kV) (KV)	Frequency (Hz) (Hz)	Time (min) (Min)	Sample packet number
Levels	1	12	120	30	1-A
	2	12	150	45	1-B
	3	12	180	60	1-C
	4	18	120	45	1-D
	5	18	150	60	1-E
	6	18	180	30	1-F
	7	26	120	60	1-G
	8	26	150	30	1-H
	9	26	180	45	1-I

Table 2: The type and function description of PEN3 electronic nose sensor

Sensor number	Sensor type	Function description	Remarks
1	W1C	Aromatic (Aromatic component)	Toluene,10 mL/m^3
2	W5C	Broadrange (High sensitivity, very sensitive to Nitrogen and Oxygen compounds)	NO_2, 1 mL/m^3
3	W3C	Aromatic (Ammonia, sensitive to aromatic components)	Benzene, Amine, 10 mL/m^3
4	W6S	Hydrogen (Mainly selective for hydrogen)	H_2, 100 mL/m^3
5	W5S	arom-aliph (Alkane, Aromatic component)	Propane, 1 mL/m^3
6	W1S	broad-methane (Sensitive to Methane)	CH_3, 100 mL/m^3
7	W1W	sulphur-organic (Sensitive to Sulfide)	H_2S, 1 mL/m^3
8	W2S	broad-alcohol (Sensitive to Ethanol)	CO, 100 mL/m^3
9	W2W	sulph-chlor (Aromatic component, sensitive to organic sulfide)	H_2S, 1 mL/m^3
10	W3S	methane-aliph (Sensitive to Alkane)	CH_4, 10 mL/m^3

Processing tea sample through HPEF: During the process of HPEF, the parameters affecting the results are pulse voltage, frequency, action time, pulse waveform, processing electrode, temperature, humidity, etc. In this study, the pulse waveform and sample treatment electrodes are well chosen and the main consideration of the 3 variables are: Pulse voltage, pulse frequency, duration of action, 3 levels are: time of action-30 min, 45 min, 60 min; electric field voltage-12 kV, 18 kV, 26 kV; pulse frequency-120, 150, 180 Hz, respectively. Therefore, a three-level OAD with an OA 9(33) matrix was chosen (Table 1).

Electronic nose detection: The electronic nose system is PEN3 produced by AIRSENSE, Germany. It contains 10 different MOX sensors (Table 2), the signal processing module, pattern recognition system and other modules.

Sample preparation: Taking 5 g Pu'er ripe tea samples, placing in fixed container, injecting 100 mL boiling water and quickly covering plastic wrap, detecting them after 10 min. The sensor is easy to cause the detection error by pollute, to ensure the accuracy of the blank sample detection, the electronic nose test was carried out according to the order of the blank sample to the test sample, until the completion of 10 samples.

Electronic nose detection: Using Head Space Adsorption method, detect aroma substances in the tea leaves of the container. Sampling frequency: 1 sec/set; Sensor self cleaning frequency: 120 sec; sensor resetting frequency: 10 sec; sample preparing frequency: 5 sec; sample flow: 400 mL/min; analytical sampling frequency: 70 sec.

RESULTS

Statistical analysis: The aim of study is that the aroma substances were dected, the original data and the blank samples were imported under all experiment condition. The NO.1, 3, 5, 8, 9 and 10 from 10 sensors were selected using determining characteristic gas. The data were analyzed by Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) analysis and difference contribution rate sensor (Loading) analysis. Three kinds of analysis methods have different characters:

- PCA analysis focus on the effect of the main aroma components of tea samples in different experimental conditions;
- LDA analysis focused on classification, as well as the distance analysis between the samples of each group;
- Loading analysis is mainly aimed at the analysis of the contribution rate of different tea aroma components, which can be utilized to determine which kind of gas contributed most to the distinguishment.

RESULTS ANALYSIS

Principal Component Analysis (PCA): PCA analysis results are shown in Fig. 3 and Table 3, points in the same experimental conditions were shown the same

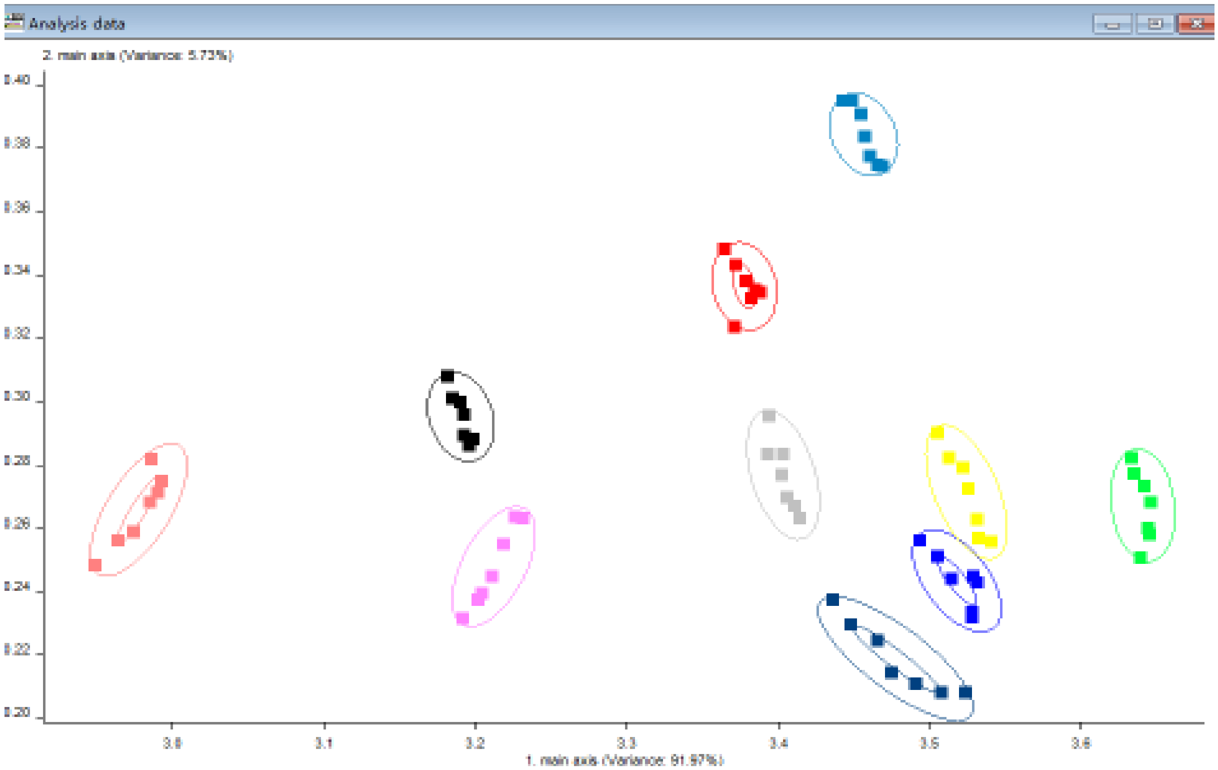

Fig. 3: Principal component analysis of tea samples treated with different experimental conditions

Table 3: PCA differentiation analysis and principal component contribution rate

PCA-Analysis
Normalization: PCA:
Matrix: Correlation-M.
Algorithm: PCA
Variance: : 97.697%
Main axis 91.965%
Main axis 5.7318%
Pattern File :
Discrimination power:

	1-A	1-B	1-C	1-D	1-E	1-F	1-G	1-H	1-I	11-A
1-A		0.979	0.979	0.956	0.909	0.822	0.975	0.997	0.993	0.996
1-B	0.979		0.997	0.997	0.974	0.961	0.990	0.999	0.998	0.999
1-C	0.979	0.997		0.953	0.983	0.955	0.992	0.996	0.984	0.998
1-D	0.956	0.997	0.953		0.979	0.852	0.992	0.997	0.987	0.997
1-E	0.909	0.974	0.983	0.979		0.943	0.957	0.998	0.994	0.997
1-F	0.822	0.961	0.955	0.852	0.943		0.982	0.992	0.973	0.982
1-G	0.975	0.990	0.992	0.992	0.957	0.982		0.997	0.992	0.999
1-H	0.997	0.999	0.996	0.997	0.998	0.992	0.997		0.987	0.990
1-I	0.993	0.998	0.984	0.987	0.994	0.973	0.992	0.987		0.993
11-A	0.996	0.999	0.998	0.997	0.997	0.982	0.999	0.990	0.993	

color, grouping and color matching are shown in Table 3. The Blank sample number is 11-A, marked with black color. The PCA analysis showed that the first component's contribution rate to distinction is 91.965%; the second component's contribution rate to distinction is 5.7318%. The sum of contribution rates of these two major components is 97.697%, so it's sufficient to say that the two major components represent the sample's prime information characteristic. Table 3 showed that there was significant difference in the aroma components of tea samples treated by HPEF and without any treatment, the discrimination degree reached 0.96, while the maximum value is 1, which displayed that HPEF had an obvious effect on the aroma of Pu'er ripe tea. PCA analysis can be very good to distinguish between the processing of different experimental conditions.

Linear Discriminant Analysis (LDA): LDA analysis results are shown in Fig. 4, which show that there is a clear difference between tea samples in different HPEF conditions, the processing samples in different HPEF conditions can be better to distinguish by linear discriminant method, which explained that volatile components were changed, have some differences and could be detected by electronic nose between groups of

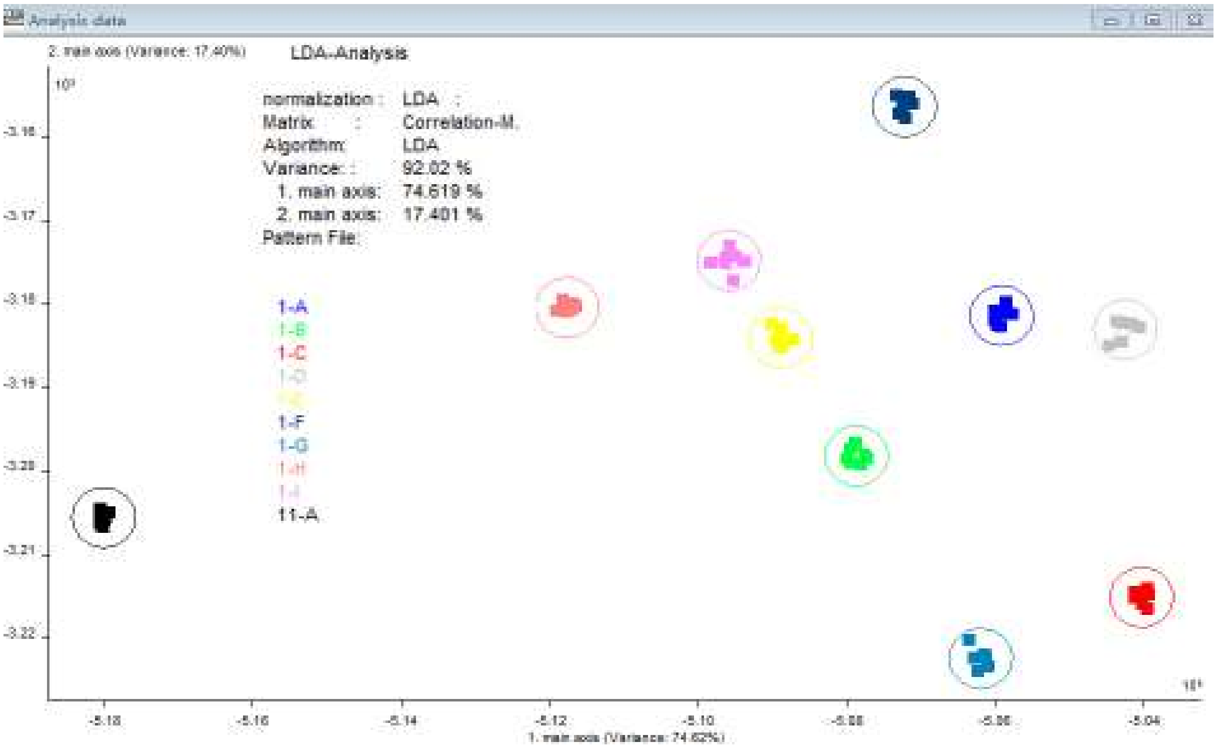

Fig. 4: Linear discriminant analysis of tea samples treated with different experimental conditions

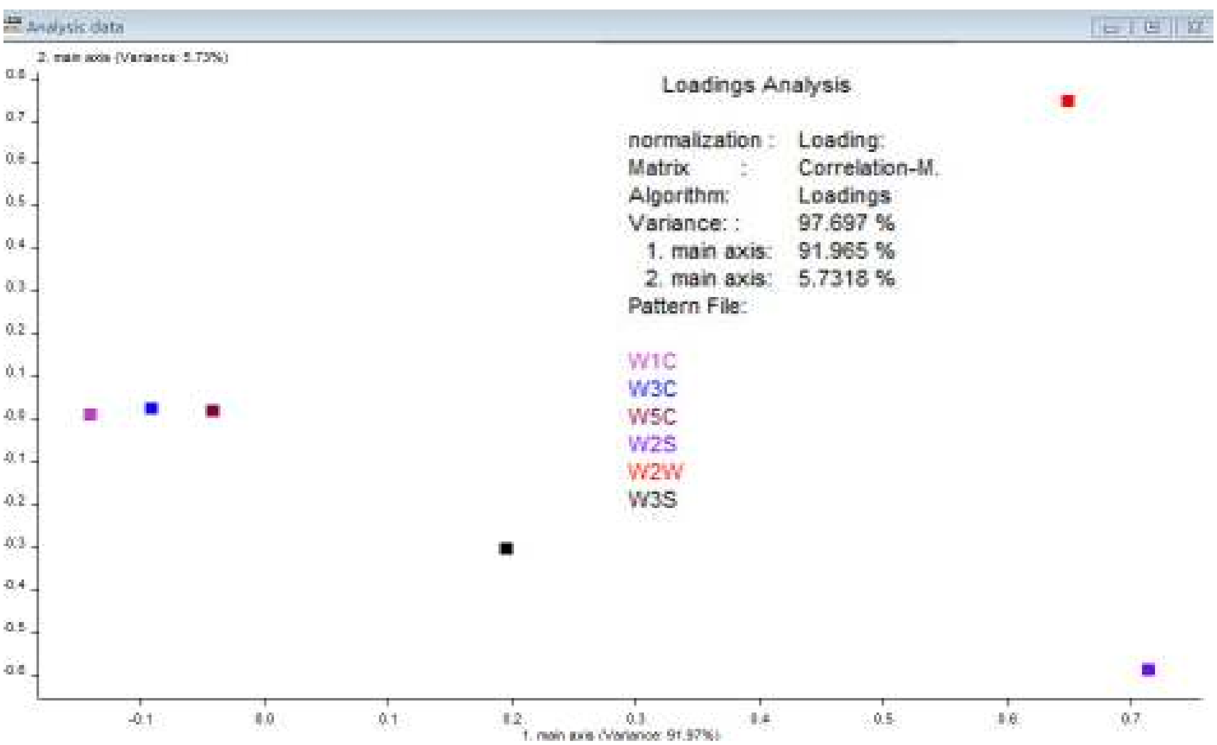

Fig. 5: Loading analysis of tea samples treated with different experimental conditions

tea samples. Especially the blank sample (black) and processing sample have a greater distance, difference is more obvious, separation effect is better after the HPEF treatment.

Difference contribution rate sensor analysis (loading): Loading analysis results are shown in Fig. 5, 6 sensors were selected for Loading analysis and found that No. 8 sensor has the highest contribution to the first

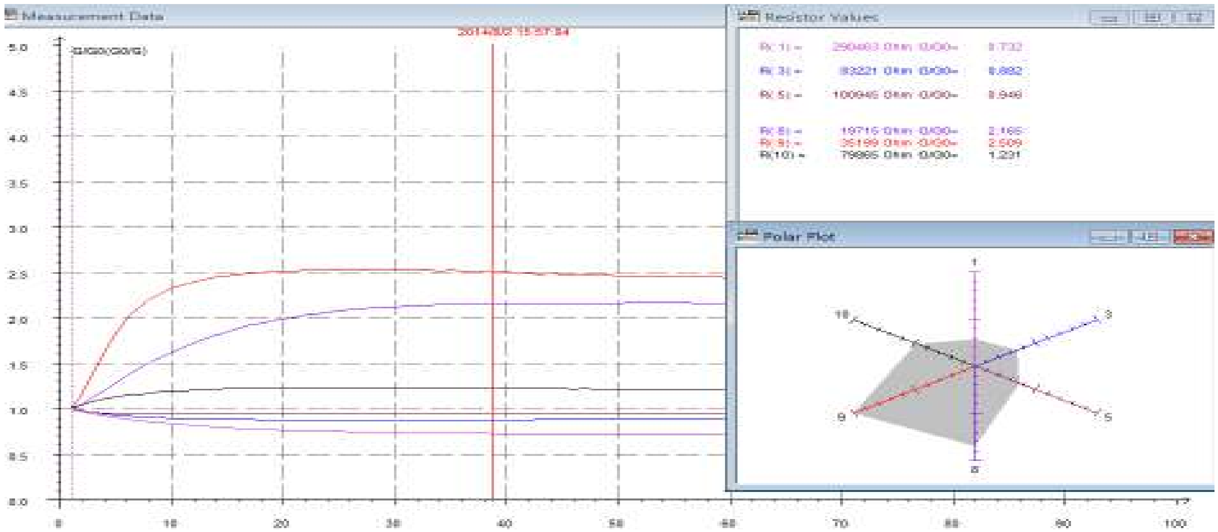

Fig. 6: The sensor characteristic response curve and radar chart of blank sample

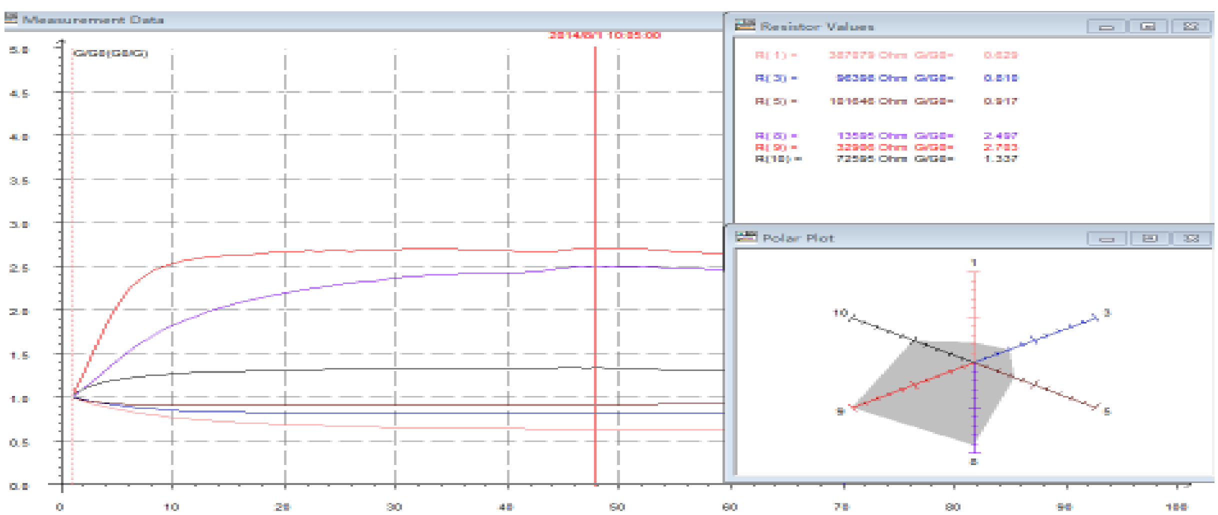

Fig. 7: The sensor characteristic response curve and radar chart of group 1-A

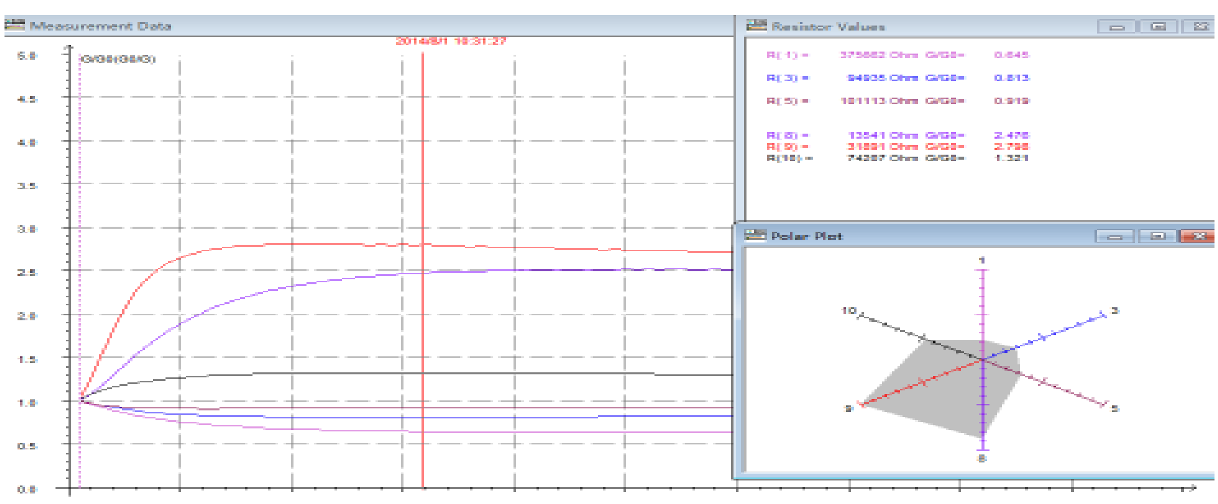

Fig. 8: The sensor characteristic response curve and radar chart of group 1-B

major aroma component, followed NO. 9 and 10 sensor. No. 9 sensor contribution has the highest contribution to second kinds of main aroma components.

Analysis of sensor characteristic response curve: Sensor characteristic response curve is in the data collection process, the conductivity sensor to obtain the ratio trends over time. At about T = 40s each sensor response curve gradually stabilized and select No. 1, 3, 5, 8, 9, 10 sensor to make response curve analysis. Blank and sensor characteristics of each experimental group and radar response curves shown in Fig. 6 to 15,

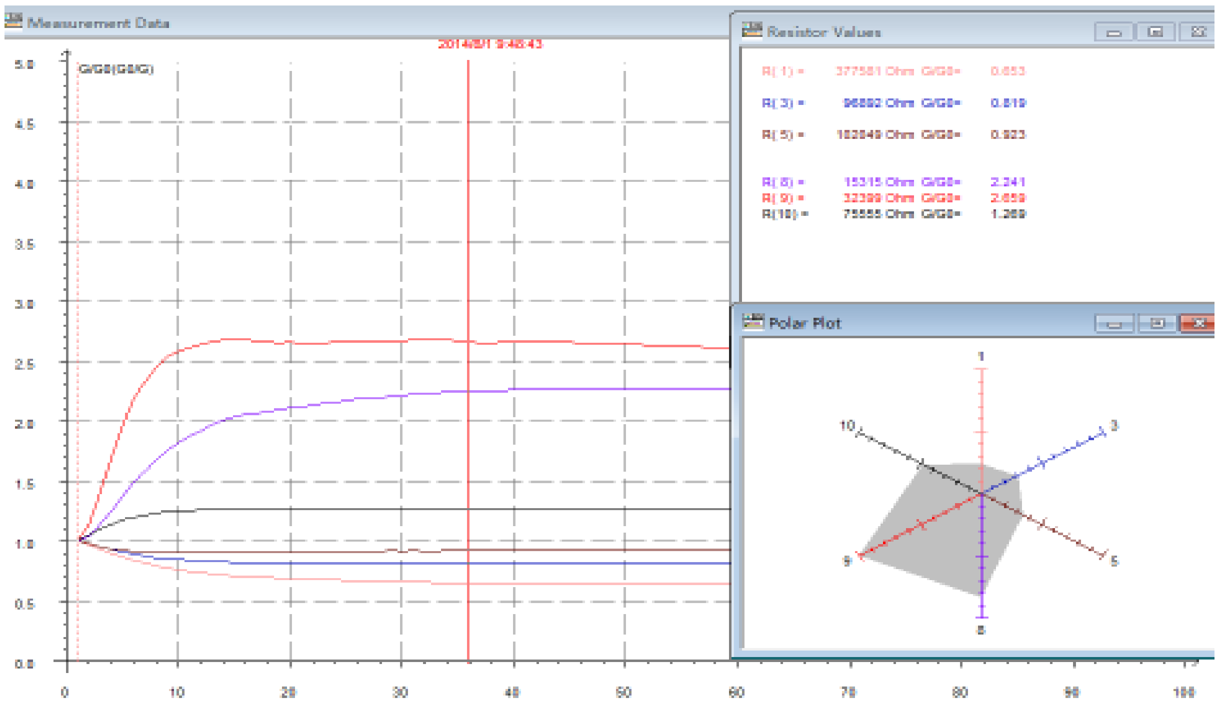

Fig. 9: The sensor characteristic response curve and radar chart of group 1-C

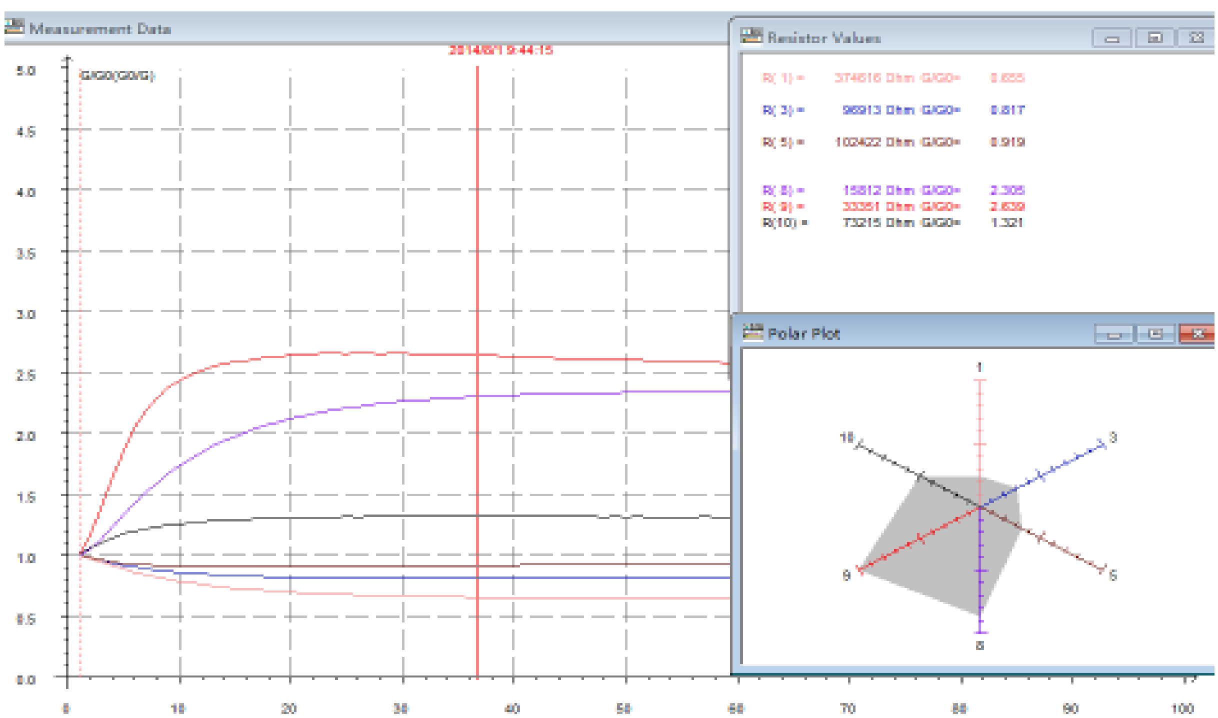

Fig. 10: The sensor characteristic response curve and radar chart of group 1-D

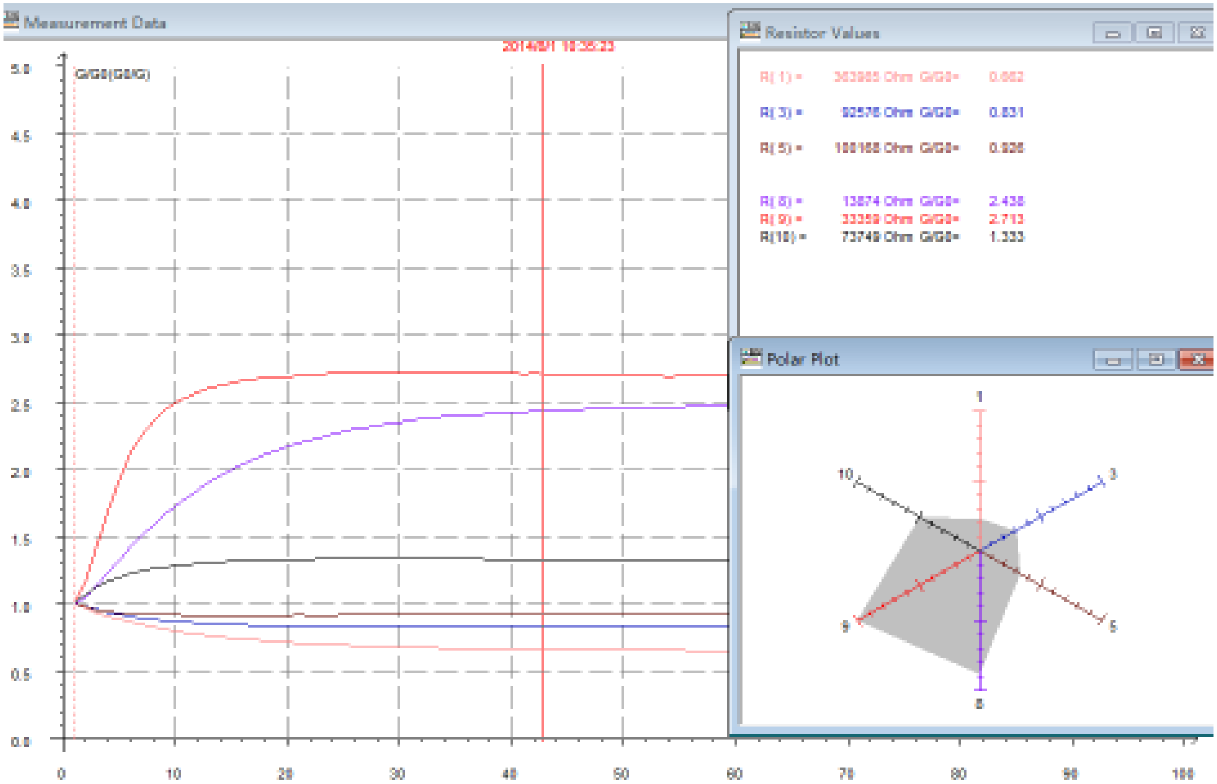

Fig. 11: The sensor characteristic response curve and radar chart of group 1-E

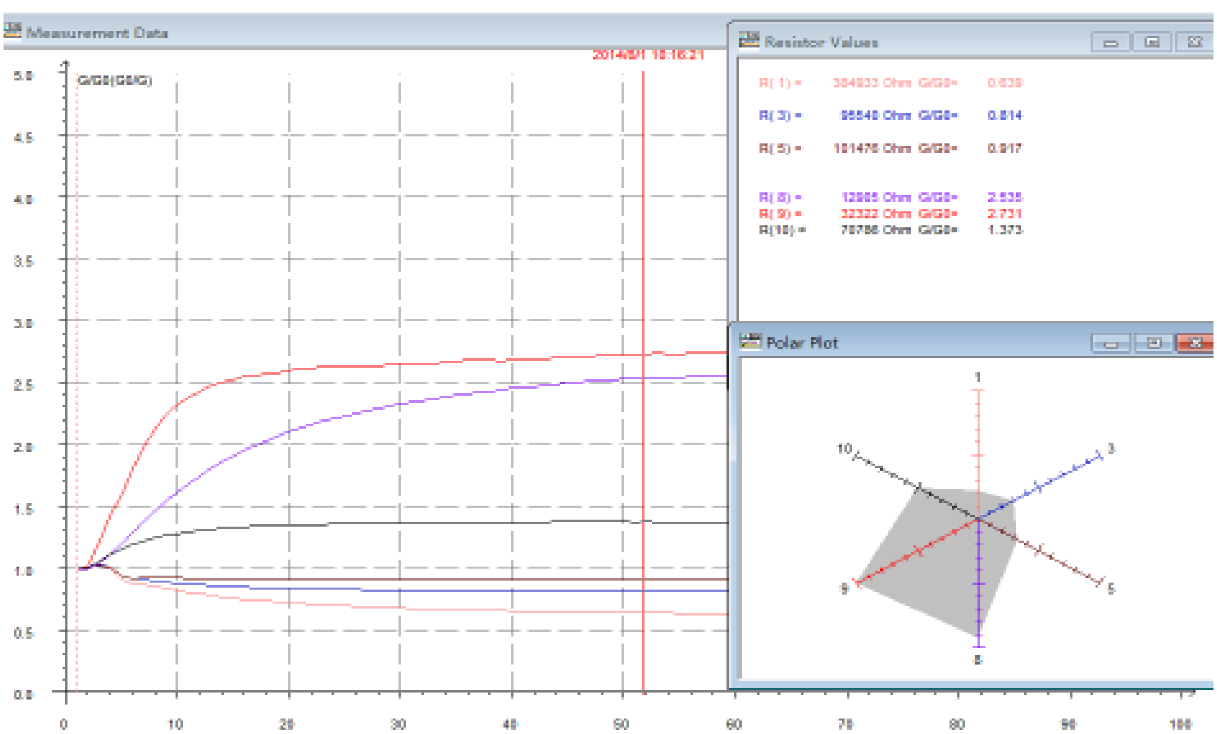

Fig. 12: The sensor characteristic response curve and radar chart of group 1-F

R (1), R (3), R (5), R (8), R (9), R (10) represent the NO.1, 3, 5, 8, 9, 10 sensor, its value is the ratio of the measured conductivity sensor G/G0.

According to the sensor response curve, after a smooth response curve, ratio of electronic nose sensors conductivity of a sample in each group (G/G0) are

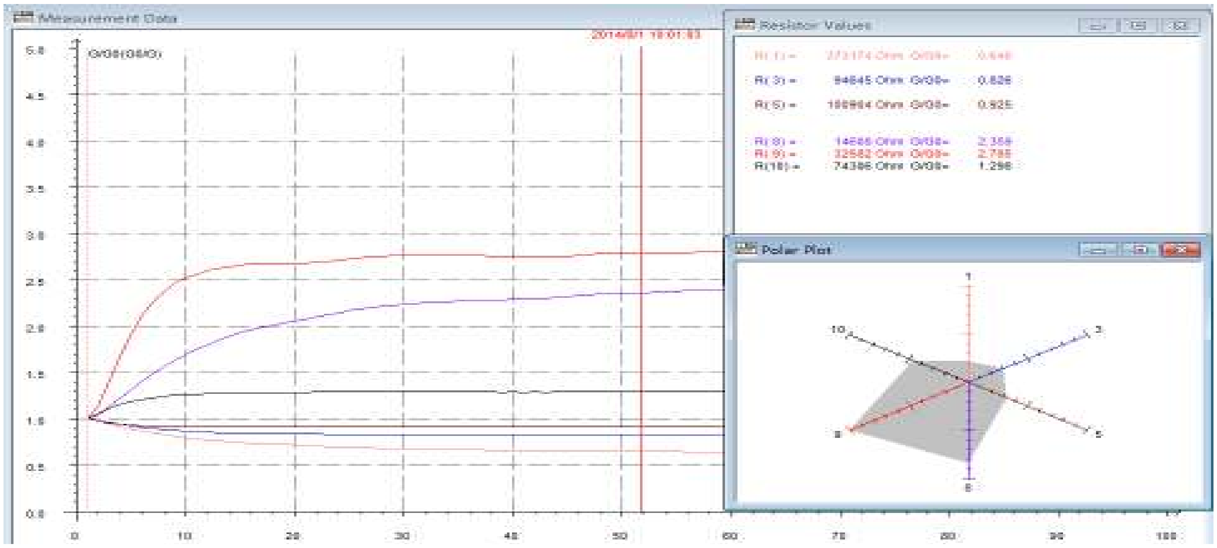

Fig. 13: The sensor characteristic response curve and radar chart of group 1-G

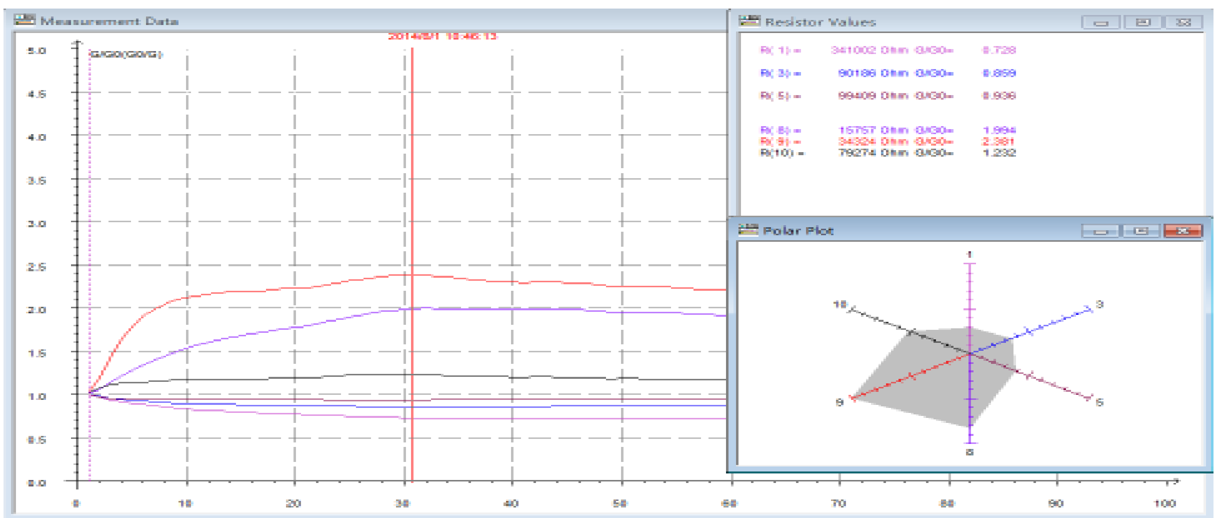

Fig. 14: The sensor characteristic response curve and radar chart of group 1-H

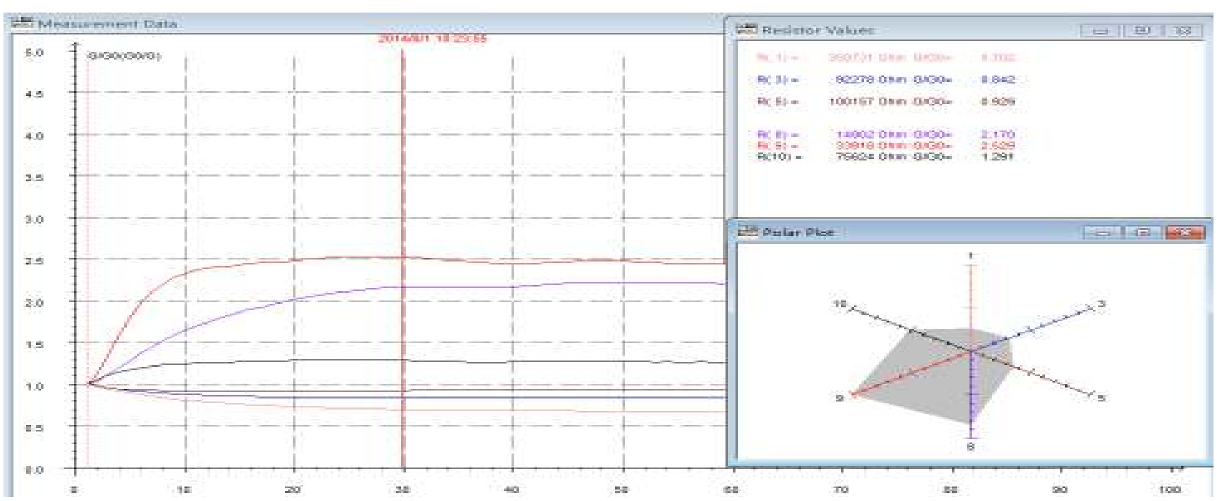

Fig. 15: The sensor characteristic response curve and radar chart of group 1-I

Table 4: The conductivity ratio G/G0

Sample group	1	3	5	8	9	10	Field conditions
Blank samples	0.732	0.882	0.946	2.165	2.509	1.231	None
1-A	0.629	0.810	0.917	2.497	2.703	1.337	12 kV/120 Hz/30 min
1-B	0.645	0.813	0.919	2.476	2.789	1.321	12 kV/150 Hz/45 min
1-C	0.653	0.819	0.923	2.241	2.659	1.269	12 kV/180 Hz/60 min
1-D	0.655	0.817	0.919	2.305	2.639	1.321	18 kV/120 Hz/45 min
1-E	0.662	0.831	0.926	2.438	2.713	1.333	18 kV/150 Hz/60 min
1-F	0.639	0.814	0.917	2.535	2.731	1.373	18 kV/180 Hz/30 min
1-G	0.649	0.826	0.925	2.359	2.785	1.298	26 kV/120 Hz/60 min
1-H	0.728	0.859	0.936	1.994	2.381	1.232	26 kV/150 Hz/30 min
1-I	0.702	0.842	0.929	2.170	2.529	1.291	26 kV/180 Hz/45 min

Table 5: The contrast of increase conductivity ratio (G/G0)

Groups	No.8 (%)	No.9 (%)	No.10 (%)	Mean (%)
1-A	15.3	7.7	8.6	10.5
1-B	14.4	11.2	7.3	11
1-C	3.5	6.0	3	4.2
1-D	6.5	5.2	7.3	6.3
1-E	12.	8.1	8.3	9.7
1-F	17.1	8.8	11.5	12.5
1-G	9.0	11	5.4	8.5
1-H	-7.8	-5.1	0.1	-4.3
1-I	0.5	0.8	4.9	2.1

Table 6: No.8 sensor visual analysis chart

Column	1	2	3	4	
Factors	Voltage	Frequency	Time	None	Result
Test 1	1	1	1	1	15.300
Test 2	1	2	2	2	14.400
Test 3	1	3	3	3	3.500
Test 4	2	1	2	3	6.500
Test 5	2	2	3	1	12.600
Test6	2	3	1	2	17.100
Test7	3	1	3	2	9.000
Test8	3	2	1	3	-7.800
Test9	3	3	2	1	0.500
Mean 1	11.067	10.267	8.200	9.467	
Mean 2	12.067	6.400	7.133	13.500	
Mean3	0.567	7.033	8.367	0.733	
Range	11.500	3.867	1.234	12.767	

extracted as shown in Table 4. As it can be seen from Table 4, for Pu'er ripe tea processed by HPEF, alcohol, organic sulfides, short chain hydrocarbons aromatic component content are detected increased in varying degrees by No. 8, 9, 10 sensor.

For No. 8, 9, 10 sensor HPEF has promoted three corresponding aroma components of nine experiments tea samples and blank sensor measured conductivity ratio G/G0 increase comparing results shown in Table 5. Seen from Table 5: at experimental conditions of 1-F (18 kV/180 Hz/30 min), an increase of No.8 sensor corresponding alcohol aroma ingredients is 17.1%, the increase of No.10 sensor corresponding to a short-chain hydrocarbons fragrance ingredients is 11.5%, both of which increase is the maximum growth rate of all experimental conditions. The increase of No.9 sensor corresponding to aromatic components is 8.8%. The average increase in three aroma components was 12.5%, the biggest average increase. The No.9 sensor's corresponding to a maximum increase of aroma components is at experimental conditions of group1-B (12 kV/150 Hz/45 min), an increase of 11.5%.

Orthogonal test analysis: Visual orthogonal experiment analysis of the data was obtained in Table 5.

After HPEF treatment, visual analysis chart No. 8, 9, 10 sensor corresponding aroma components as well as the average increase of three aroma components shown in Table 6 to 9. The size of range response the size of affect of the factor to the test results.

As it can be seen from Table 6, No. 8 sensor detects alcohol aroma components, in voltage, frequency, time of three factors influence on the aroma components in descending order of electric field voltage, frequency, time. As the results, we can get the experimental conditions within range, HPEF promote optimal conditions alcohol aroma component is 18 kV/120 Hz/60 min. Similarly available HPEF promote organic sulfides, short chain hydrocarbons optimal conditions aroma components were 12 kV/120 Hz/60 min, 18 kV/120 Hz/30 min. The optimal conditions of promoting three types of aroma components are 18 kV/120 Hz/60 min.

Table 7: No.9 sensor visual analysis chart

Column	1	2	3	4	
Factors	Voltage	Frequency	Time	None	Result
Test 1	1	1	1	1	7.700
Test 2	1	2	2	2	11.200
Test 3	1	3	3	3	6.000
Test 4	2	1	2	3	5.200
Test 5	2	2	3	1	8.100
Test 6	2	3	1	2	8.800
Test 7	3	1	3	2	11.000
Test 8	3	2	1	3	-5.100
Test 9	3	3	2	1	0.800
Mean 1	8.300	7.967	3.800	5.533	
Mean 2	7.367	4.733	5.733	10.333	
Mean 3	2.233	5.200	8.367	2.033	
Range	6.067	3.234	4.567	8.300	

Table 8: No.10 sensor visual analysis chart

Column	1	2	3	4	
Factors	Voltage	Frequency	Time	None	Result
Test 1	1	1	1	1	8.600
Test 2	1	2	2	2	7.300
Test 3	1	3	3	3	3.000
Test 4	2	1	3	3	7.300
Test 5	2	2	1	1	8.300
Test6	2	3	2	2	11.500
Test7	3	1	2	2	5.400
Test8	3	2	3	3	0.100
Test9	3	3	1	1	4.900
Mean 1	6.300	7.100	6.733	7.267	
Mean 2	9.033	5.233	6.500	8.067	
Mean 3	3.467	6.467	5.567	3.467	
Range	5.566	1.867	1.166	4.600	

Table 9: Average increase in visual analysis chart

Column	1	2	3	4	
Factors	Voltage	Frequency	Time	None	Result
Test 1	1	1	1	1	10.500
Test 2	1	2	2	2	11.000
Test 3	1	3	3	3	4.200
Test 4	2	1	2	3	6.300
Test 5	2	2	3	1	9.700
Test6	2	3	1	2	12.500
Test7	3	1	3	2	8.500
Test8	3	2	1	3	-4.300
Test9	3	3	2	1	2.100
Mean 1	8.567	8.433	6.233	7.433	
Mean 2	9.500	5.467	6.467	10.667	
Mean 3	2.100	6.267	7.467	2.067	
Range	7.400	2.966	1.234	8.600	

DISCUSSION

The studies of effect for HPEF to tea aroma components is yet few, exploratory experiment was carried out. ripe Pu'er tea produced in Yunnan, Lincang 2005 vintage Fu Yuan Chang Luxury choose as experimental materials and orthogonal analysis including voltage, frequency and action time in tea leaves HPEF process, then the use of electronic nose system for tea aroma components were detected analysis. The results showed that after treatment by HPEF, tea aroma components changed more significantly, which the alcohols, organic sulfides, short chain hydrocarbons aromatic ingredients with a significant role in promoting. And in the field conditions for 18 kV/180 Hz/30 min, the three types of fragrance ingredients to achieve maximum average increase of 12.5%. Finally, an increase of these three types of aroma components by orthogonal experiment analysis and found that the point is the key factor affecting the field voltage variation of fragrance components, has been within the scope of this experimental condition three aroma components for promoting sexual effect is more ideal conditions were HPEF of 18 kV/120 Hz/60 min, 12 kV/120 Hz/60 min, 18 kV/12 Hz/30 min, the effect of promoting a comprehensive three aroma components HPEF ideal conditions for 18 kV/120 Hz/60 min. This study provides a feasible method to improve the aroma of Pu’er tea and improves the aroma of Pu’er ripe tea without undermining the tea component. Meanwhile, it provides a new approach that can not only reduce the aging time of Pu'er tea but also shorten the cycle to meet with consumers, which can be considered large-scale industrial production.

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