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ORIGINAL ARTICLE

The impact of thermal extraction on the quality of *Phyllanthus emblica* Linn. fruit: A systematic study based on compositional changes

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Abstract *Phyllanthus emblica* (PE) is a well-known tropical crop with a distinct flavor and several health advantages. From the standpoint of the transformation of volatile and nonvolatile components, the purpose of this paper is to investigate the impact of heat extraction on the flavor attributes and biological activity of PE. According to studies, thermal extraction can greatly enhance the amount of the molecule 4,5-epoxy-(E)-2-decanal with a green odor while removing offensive scents like 2-isobutyl-3-methoxy pyrazine. Temporal dominant description evaluation found that with the extension of thermal extraction time, the five flavors of PE are enhanced. According to high-resolution mass spectrometry studies, the primary reaction processes during thermal extraction were the hydrolysis and condensation of tannin and flavonoid glycosidic bonds. The main core groups of compound transformation were galloyl and hexahydroxydibenzoyl (HHDP), which led

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to the final product gallic acid and ellagic acid were significantly increased. Finally, it was found that thermal extraction can significantly enhance the antioxidant and antibacterial activities of PE.

1. Introduction

The fruit of *Phyllanthus emblica* Linn. (PE), which is a popular fruit tree belonging to the *Phyllanthaceae* family widely distributed in tropical and subtropical countries, such as India, Southwest China, Vietnam, Thailand and Indonesia, with thousands of years of edible history. In many Asian regions, PE is a chief source crop of vitamin C and minerals (Variya et al., 2016). As a fruit, its distinctive aftertaste sweetness and sour flavor are its most distinguishing flavor traits. These flavors can alleviate dry mouth and pharyngeal discomfort by providing long-lasting comfort to the tongue after a brief sour and astringent taste (Huang et al., 2021). Polyphenols, flavonoids, and amino acids are also abundant in PE. Particularly, polyphenols can make up as much as 33 % of the dry weight of PE (Avula et al., 2013). It has excellent antioxidant(Jhaumeer Laulloo et al., 2018) properties as well as anti-inflammatory, anti-diabetic, antibacterial, and anti-tumor effects due to the abundance of phenolic hydroxyl groups in it (Huang et al., 2021). PE is one of the three plants that are recommended by the WHO for planting internationally because of its distinctive flavor, abundant nutritional value, and exceptional efficacy. PE is currently widely employed in the production of dietary supplements, foods, pharmaceuticals, beverages, etc. due to its potent health advantages and distinctive flavor.

One of the crucial processes in the industrial processing of PE is thermal extraction. The PE extracting solution (PES) can be directly dried as PE extract (using microwave or spray drying), which can be employed as a step in the manufacturing and processing process. Therefore, PES will experience a thermal treatment process throughout the industrial extraction, drying, or sterilizing stages. The final product's flavor and activity may substantially depending on the process's time and temperature. Considering that PES is a common polyphenol solution (Yang and Liu 2014), PES's chemical reaction can be split into enzymatic and non-enzymatic reactions (thermal conversion), although thermal extraction inactivates the enzyme. The Arrhenius equation states that temperature is the primary determinant of a non-enzymatic reaction's outcome (Huang et al., 2019). Previous studies(Huang et al., 2019) discovered that polyphenols can primarily experience hydrolysis, oxidation, polymerization, and other reactions during the extraction process, which have a significant impact on their appearance, flavor, and activity. For instance, when heating tea soup, the change in appearance and flavor is more noticeable the higher the temperature and the longer the heating time (Zhu et al., 2020). Alcohol and coffee, both popular beverages, have similar reports (Li and Sun 2019). Some non-thermal extraction techniques, like ultrasonic, high hydrostatic pressure, pulsed electric fields, and non-thermal plasma, have recently gained popularity in the quest for authentic flavor and energy conservation. The flavor and biological activity of natural products are greatly influenced by the various extraction techniques. However, it has not been reported how different extraction techniques affect PES's flavor, taste, and bioactivity, and it is unclear how components transform during thermal extraction, which is a pressing issue that needs to be looked into.

To address the aforementioned issues, a rapid sensory evaluation research methodology for PES was established in this paper based on temporal dominant description taste evaluation and HS-SPME/GC-QQQ-MS/MS odor analysis technology. Additionally, the effects of the thermal extraction process on flavor, appearance, and physical and chemical properties were systematically investigated. Secondly, the primary difference indicators before and after heat extraction were discovered, and the primary transformation components and transformation pathways were hypothesized and confirmed based on multivariate statistical analysis. Finally, the component-activity correlation analysis method was utilized to uncover its quality markers after studying the transition laws of PES composition, flavor, taste, and activity. This is important for the processing and quality assessment of PES. In short, the purpose of this paper is to investigate the flavor and activity of PE after thermal processing, such as extraction, drying, or sterilization. We also hope that this study will serve as a guide for PE extraction and preparation in the pharmaceutical, food, and beverage, and other industries.

2. Materials and methods

2.1. Ethics statement

Volunteers were given written informed consent regarding the purpose of the study and their right to keep information confidential. Informed written consent was obtained from all participants.

2.2. Materials and chemicals

Milli Q water purification system (Millipore, Bedford, MA, USA). HPLC-grade methanol Fisher Chemical (Fisher Chemical, Pittsburg, PA, USA). HPLC-grade formic acid, Anhydrous Ethanol (Analytical purity), Vitamin C (Chengdu KeLong Chemical Factory, Chengdu, China). DPPH free radical scavenging ability test kit, ABTS buffer solution (Solaribio biotechnology Co., ltd. Beijing, China), α-Glucosidase (Sigma, USA), 4-Nitrophenyl-β-D-glucopyranoside (PNPG, Sigma, USA) Acarbose (Bayer, Germany). Standards of Citric acid, mucinous acid, malic acid Gallic acid (GA, No. CHB201131), Epicatechin gallate (ECG, No. CHB-B-081), Quercetin(Q, No. CHB-H-040), Corilagin (CR, No. CHB-K-004), Gallocatechin (GC, No.4051109), Catechin (C, No.14051508), Epigallocatechin gallate (EGCG, No.14121608), Gallocatechin gallate (GCG, No.14102009), Ellagic acid (EA, No. CHB-R-039), Chebulagic acid (CLA No. CHB-H-114), Chebulic acid (CA No. CHB-H-140), Chebulinic acid (CBA No. CHB-H-018) were purchased from Chengdu Biopurify Phytochemicals ltd. (Chengdu, China). The purity of the twelve standards was each above 98.0 %.

2.3. Preparation of sample solution

Take an appropriate amount of 6 batches of PE dried fruits (batch number: 190401; 201203; 200601Z; 201209; 210101; 200301) as parallel samples. Accurately weigh an appropriate amount of PE, add 10 times pure water, heat and reflow for 0 h (ultrasonic 30 min, E1), 0.5 h (E2), 1 h (E3), 1.5 h (E4), 2 h (E5), and make up for the weight loss after cooling. Then immediately centrifugate at high speed (9000 r) for 10 min, and take the supernatant as the sample solution.

2.4. Determination method of appearance and physicochemical parameters

Accurately suction 300 μL of the sample and added it to a 96-well plate. Then the flatbed scanner (Epson perfection V370 Photo) was used to obtain the scanned image. And the Photoshop CC was used to obtain the R, G, B values of the and make a color chart, and the data were analyzed by PCA. The surface tension was measured by DCAT-21 surface tension analyzer (DataPhysics, Germany). The temperature was set at 25 ℃, and added 30 mL PES. The surface tension was measured by Wilhelmy hanging plate method after temperature equilibrium. The solution viscosity was determined by LVDV-1 T viscometer (Shanghai Fangrui Instrument Co., ltd.), the temperature was set at 25 ℃, rotor 1 was selected, 20 mL PES was added, and the rotational speed was set at 12 r/min.

2.5. Electronic tongue analysis method

The signal acquisition parameters were set as follows: acquisition temperature 25 ℃, data acquisition time 120 s, acquisition cycle 1 s, stirring speed 1 R/s. The ultrapure water was used as cleaning solution, and the sensor was cleaned for 10 s before each measurement. The above PE solutions was filtered through a 0.45 μm microporous membrane and placed in a 50 mL matching beaker for determination. Each sample was determined 10 times in parallel according to the above method, in order to obtain stable results. For reliable data, the last three times of data are taken as the output value. The average of the three output values of each sample was taken as the post-processing data. The verification results show that RSD was < 2 %, indicating that the instrument was stable. When the sample was measured, the PE sample solution was diluted to a concentration of 6 mg/mL for testing.

2.6. UPLC-QTOF-Mass conditions

2.6.1. Sample preparation

Precisely draw 0.1 mL of the above PE extraction solution to 5 mL volumetric flask, add 50 % methanol–water solution to the scale line and dissolve it by ultrasonic for 30 min as the sample solution. Appropriate amount of each reference substance was weighed and made into reference substance solution respectively. All solutions above were filtered through 0.22 μm membranes (Jinteng, Tianjin, China) before injection.

2.6.2. Chromatographic conditions

Samples were analyzed by Acquity UPLC I-class (Waters) ultraperformance liquid chromatography system. The Waters ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm) was used for the analysis. The mobile phase A was 0.1 % formic acid aqueous solution, and the mobile phase B was acetonitrile solution. The gradient elution was 0–3 min, 2 %-2% B; 3–5 min, 2 %-7% B; 5–15 min, 7 % −21 % B; 15–20 min, 21 %-78 % B; 20–21 min, 78 %-85 % B; 21 % −24 % min, 85 %-95 % B; 24–26 min, 95 %-95 %B; 26–28 min, 95 %-2%B; 28–30 min, 2 %-2%. The column temperature was set as 40 ℃, and the flow rate was 0.3 mL/min, and the injection volume was 3 μL.

2.6.3. Mass spectrometry conditions

Samples were analyzed by SYNAPT XS (Waters) high-resolution time-of-flight mass spectrometer. The electrospray ion source (ESI) negative ion mode is used for detection and analysis. The spatial resolution was 120 μm, capillary voltage was 4 kV, cone voltage 50 V, ion source temperature 150℃. The atomizing gas was high-purity nitrogen, cone gas flow rate was 50 L/h, desolvention gas flow rate was set as 600 L/h, and the temperature was set as 250 ℃. The mass spectrum data was collected in MS^E mode, ion scanning range was m/z100-1200. Leucine-enkephalin (LE) was used for calibration during data acquisition. LE $[M-H]^-$ accurate relative molecular mass was calculated as m/z 554.2615 in negative ion mode.

2.6.4. Data processing and multivariate analysis

Masslynx 4.1 was used to collect data, and the original data was imported into progenesis Qi (Waters, V2.0) for processing. The quality error parameter $|ppm| < 5$ was set, and the peak comparison, selection and normalization were performed to obtain the retention time, m/z and peak intensity of each sample. The above information was imported into EZinfo 3.0 for principal component analysis (PCA) and partial least squares discriminant analysis (OPLS-DA) to find the different compounds. Finally, compounds with VIP > 1and $P < 0.05$ were selected as differential metabolites.

2.7. Temporal dominant description method

To evaluate the taste difference of PES, a human sensory test using the visual analog scale (VAS) was proposed to verify the results(Han et al., 2018). With the approval of the medical ethics committee of the Affiliated Hospital of Chengdu University of TCM, 10 well-trained and healthy volunteers (4 males and 6 females, aged 21–28) were selected. Volunteers were selected from graduate students at Chengdu University of Traditional Chinese Medicine. They had no smoking, drinking and other bad habits, no genetic history, no recent oral and throat diseases, and normal taste. All volunteers were voluntary and signed informed consent before the trail.

PES has five basic flavors, which are astringency, bitterness, sourness, saliva secretion and aftertaste-sweetness. It is necessary to establish a special method for PES taste and flavor evaluation, which is called temporal dominant description method(Li et al., 2019). During the training sessions, volunteers were trained with different concentrations of model solutions (Sucralose, 3.0, 5.0,7.0 mg/mL; Tannic acid, 0.5, 1.0,2.0 mg/mL; Citric acid, 0.5, 0.8,1.0 mg/mL; Quinine, 0.1, 0.2, 0.3 mg/ml), so they were accustomed to the evaluation scales and bitterness intensities. A drop of approximately 10 mL of each solution was applied to the upper surface of the tongue for 10 s. Then, the test solution was expectorated. Volunteers were asked to score the "bitterness, sweetness, astringency, sourness" using the 100 mm VAS by placing a mark along a 100 mm line 23. Between each test interval, the mouth was rinsed well with distilled water so that no bitter taste remained. Volunteers were given a break (at last 1 h or more) between each sample.

The taste intensity retention time was used to define the flavors duration (TIRT). The intensity of the PES's bitterness, astringency, and sourness (I) has been decreasing throughout

the entire drinking process, whereas the aftertaste-sweetness and salivary secretion have been rising and then lowering throughout. To comprehensively describe TIRT, a coefficient reflecting time needs to be established to reduce the data dimension.

It was found through fitting calculation that the sensory intensity and retention time were linearly fitted ($y = kx + b$), and the fitting results were shown in Table 1. Thus, the slope k value could be easily obtained. The k value has the connotation that the shorter the taste retention period, the higher the absolute value of k must be. To characterize the flavor retention time, we now present its retention time coefficient K. The formula is as follows:

$$K_{A,S,B} = \frac{1}{|k|}; K_{AS} = \frac{1}{k1 - k2}$$

K represents the TIRT coefficient of 5 tastes, which are defined as: bitterness (K_B), astringency (K_A), sourness (K_S), and aftertaste-sweetness (K_{AS}). Salivary secretion is calculated by the sum of salivary secretion times. In the fitting process, the data starting from 5 s until the taste intensity is greater than or equal to 0.5 are regarded as fitting objects. This rule applies to sourness, bitterness and astringency, and the TIRT coefficient K and R^2 can be directly obtained ($k < 0$, $R^2 > 0.800$). However, the aftertaste-sweetness intensity increased first and then decreased. Their K value is the reciprocal of rising slope k_1 minus the falling slope k_2 ($k_1 > 0\, k_2 < 0$). Finally, Multiply the above-mentioned sensory TIRT coefficient K by the sum of various sensory intensities (SI) at each time point to obtain the comprehensive taste coefficient T of each taste. The calculation formula is as follows:

$$T = \sum_{1}^{n} SI \times K$$

n is the number of time points in the evaluation process.

2.8. HS-SPME/GC-QQQ-MS/MS conditions

2.8.1. HS-SPME conditions

The lyophilized sample was crushed into fine powder (passed through a No. 3 sieve). Accurately weighed 0.5 g PE fine powder and placed in a 20 mL inert headspace bottle, and then equilibrated at 50 °C for 40 min. Before and after sample injection, the Solid phase microextraction (SPME) head was automatically aged for 3 min in the 270 ℃ aging device, inserted into the headspace via a PTFE septum, without contact the sample. After extraction and adsorption at a constant temperature of 50 ℃ for 10 min, the SPME head quickly insert the GC–MS injection port in the pre-operation state, desorb at 250 ℃ for 2 min, and then perform GC–MS/MS analysis.

Table 1 K and linear fitting R^2 of different taste.

Batch	K_B	R^2	K_S	R^2	K_A	R^2	K_{AS}	R^2
E1	2.667	0.996	1.132	0.911	1.499	0.985	2.001	1.000
E2	1.600	0.989	1.062	0.879	1.350	0.885	1.538	0.903
E3	1.613	0.953	1.126	0.848	1.541	0.954	1.018	1.000
E4	1.395	0.929	1.013	0.833	1.301	0.973	1.177	1.000
E5	1.163	0.936	0.841	0.833	1.270	0.972	1.500	0.984

2.8.2. Chromatography and mass spectrometry conditions

The PE samples (lyophilized powder) were analyzed by a TQ8050 NX triple quadrupole GC–MS equipped with Aoc-6000 automatic sampler and an electron bombardment ion source (EI), a PAL heating magnetic stirring module and a PAL SPME Arrow solid phase microextraction sampler (1.5 mm × 120 μm × 20 mm, PN: ARR15-DVB/C-W*R*-120/20CT, CTC Analytics AG, Switzerland). The inertcap pure wax capillary column (30 m × 0.25 mm × 0.25 μm) was used as chromatographic column during analysis. The chromatographic conditions were set as follows: injection temperature was 250 ℃, split ratio was 5:1, injection pressure was 83.5 kPa; carrier gas was high purity helium, carrier gas control mode was constant pressure mode; purge flow was 3.0 % mL/min. The temperature program was set as follows: the initial temperature was 50 ℃ for 5 min, then raised from 10 ℃ to 250 ℃ for 10 min; the column equilibrium time was 2.0 min. The mass spectrometry conditions were set as follows: the ionization energy was 70 EV, the ion source temperature was 200 ℃, the mass spectrum transmission interface temperature was 250 ℃, the collision gas is argon; the mass spectrum monitoring mode is multi reaction monitoring (MRM), the detector voltage is + 0.3kv relative to the tuning result, and the solvent delay time is 1.3 min. In order to improve the sensitivity of the detection, the compounds were monitored by time segment.

2.8.3. Qualitative and quantitative method

Precisely draw 1 μL of a mixed solution (0.1 μg/mL) containing 3 kinds of internal standard substances for analysis to obtain the peak area of the internal standard substance, and finally measure the sample according to the above conditions. The qualitative of the target compound is confirmed by the qualitative and quantitative transition. The quantification of the target compound is quantified by the standard curve of 150 compounds built in the Shimadzu TQ8050 reanalysis software (The method parameters and sensory information (odor characteristics and odor threshold, etc.) of about 150 odor compounds were registered in the database.) combined with the measured peak area of the internal standard. Through the method package and database, it is very convenient to establish a variety of odor compounds screening methods, and use the built-in standard curve to semi quantify the detected compounds, and confirm the odor causing substances by comparing the results with their odor threshold.

The term 'odor threshold' describes the least concentration (pg/mg) of a substance that irritates people's sense of smell. The content and threshold work together to determine how well odor components perform in PE, rather than just the content alone. The ratio of the concentration to the threshold is called the odor activity values (OAV):

$$\mathrm{OAV} = \frac{C}{M * Th}$$

In the formula: C represents the component content (pg); M for the sample mass(mg); Th for the component's odor threshold (pg/mg). Generally, the components with OAV > 0.1 should be considered to have an obvious impact on their odor.

2.9. Antioxidant and antihyperglycemic activities

The α-glucosidase and PNPG reaction system was used as a model for testing, and the specific operations were as follows: Added 10 μL of α-glucosidase solution (2 U/mL) and 10 μL of the sample solution to each reaction well in turn, mixed well and incubated in a 37 ℃ water bath for 15 min. Then, added 50 μL of PNPG (1 mmol/L), placed it in a 37 ℃ water bath and incubate for 30 min, and finally added sodium carbonate solution to stop the reaction. Each sample had 3 replicate wells. The absorbance was measured at 405 nm as soon as possible by a multifunctional microplate reader. Acarbose was used as a positive control, the concentration was 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 mg/mL; the samples were set with 8 concentration gradients of 10, 50, 100, 250, 500, 750, 1000 and 2500 μg/ml and calculate the inhibition rate.

DPPH, ABTS radical scavenging activity (IC_{50}) and FRAP total antioxidant capacity of the sample were measured according to the instructions of the kit (Solebo biotechnology Co., ltd.).

2.10. Determination of antibacterial and antifungal activity

Staphylococcus aureus, Escherichia coli, Aspergillus variegatus and Aspergillus flavus (purchased from Baina Biological Co., ltd., China) were inoculated and cultured for 3 generations. Under aseptic conditions, take 0.2 mL of bacterial suspension (the best concentration of bacterial solution is 1×10^6 CFU/mL) and spread it evenly on the surface of the agar plate. The positive group was gentamicin sulfate injection (diluted four times, 10 mg/mL). Take 2 mL of each sample solution (10 times water extract, filter sterilization) into a sterilized EP tube, and then put a 6 mm diameter neutral filter paper into the EP tube to soak for 4 h. Place the filter paper clockwise on the same plate, parallel three groups, and measure the average value after incubation at 37 ℃ for 24 h.

2.11. Data processing and analysis

Analysis was performed by using Heat map and Cor-Heatmap tools in Hiplot (https://hiplot.com.cn), a comprehensive web platform for scientific data visualization. Statistical analyses were performed using SPSS 22.0 package (SPSS Inc., Chicago, IL, USA) and Oringin 2018 (OriginLab, Hampton, Massachusetts, USA). PCA and OPLS-DA were analyzed by SIMCA-P11.0 (Umetrics AB, Umea, Sweden).

3. Results

3.1. PES physicochemical properties changes during thermal extraction

The physical properties and appearance of PES changed significantly during the extraction process, and the results are shown in Fig. 1 (E1 represent ultrasonic extraction or thermal extraction for 0 h; E2, E3, E4 and E5 stand for heating reflux extraction for 0.5 h, 1 h, 1.5 h and 2 h respectively).

The results in Fig. 1 demonstrated that the color of PES deepened as extraction time increased, surface tension signifi-

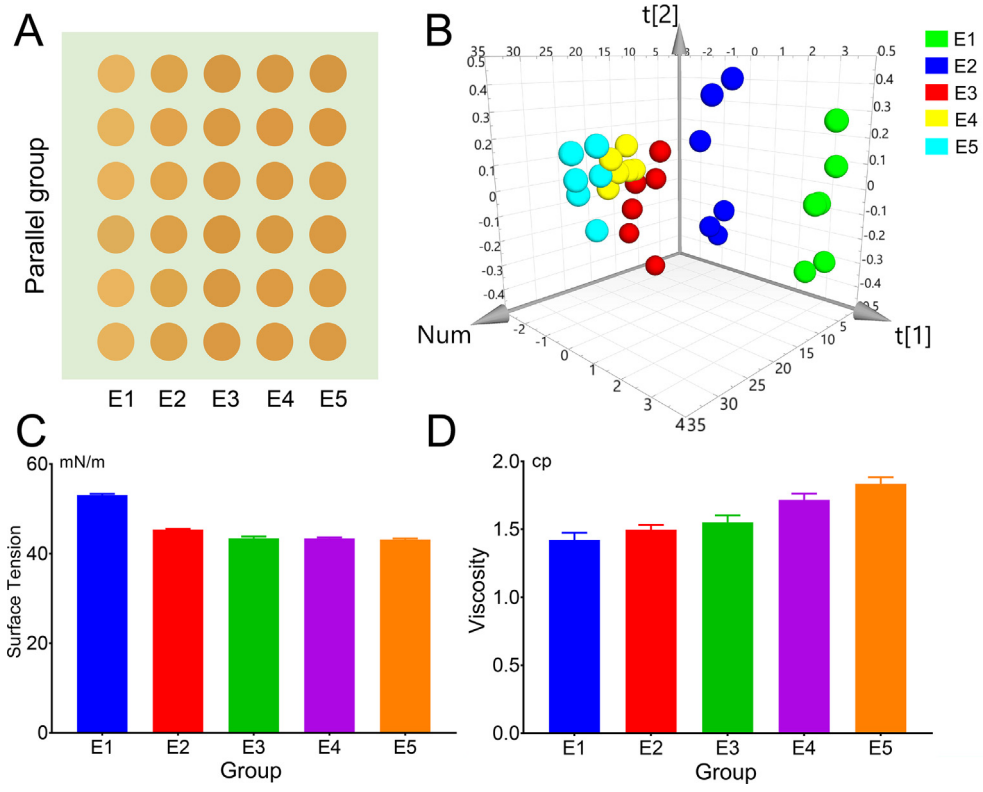

Fig. 1 Appearance and physical properties results, A the appearance of different extraction time, B PCA analysis of R, G, B values of different extraction time appearance, C variation of surface tension and D viscosity at different extraction time.

cantly decreased, and viscosity was favorably connected with extraction time. The metamorphosis of internal parts is intimately tied to this change in appearance and physical characteristics. Indicating that some components created after transformation have higher surface activity than the original components, such as polysaccharides and polyphenols (Zhan et al., 2018). Therefore, its physical and chemical properties are closely related to the transformation of its components.

3.2. Sensory evaluation results

3.2.1. Temporal dominant description results

A temporal dominating description method (Li et al., 2019) to assess the sensory qualities of PES was established based on its taste characteristics, and it is capable of precisely and dynamically describing the flavor changes that occur in PES during the thermal extraction process.

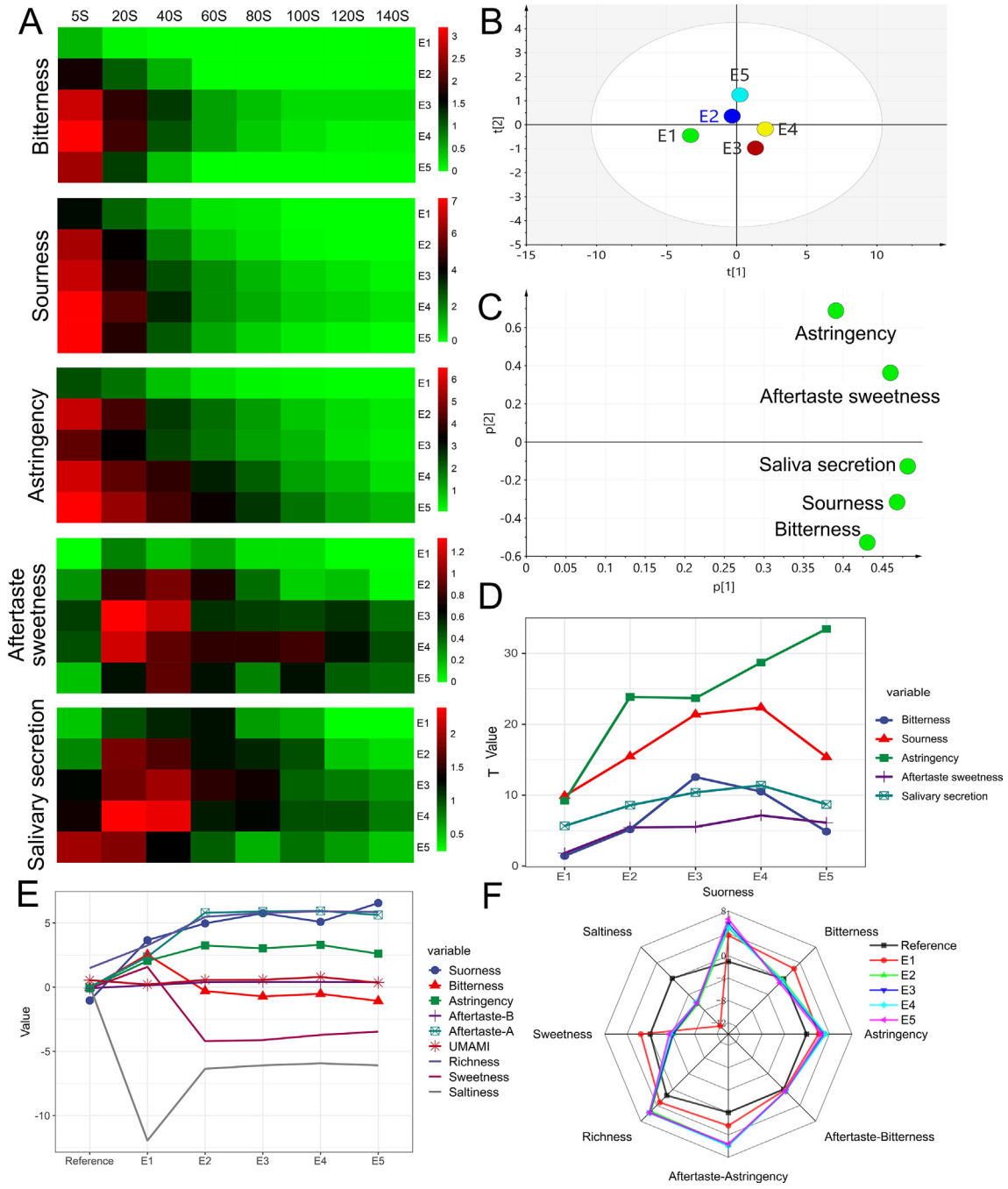

Fig. 2 Temporal dominant description method and electronic tongue results, A the VAS scores heat map of PES samples (E1 to E5) at different extraction time points, B PCA analysis results of *T* value, C loading scatter plot, D change tendency of *T* values, E, F electronic tongue measurement results.

Table 2 The change law of the average content of differential odor markers (n = 3).

No	Compounds	Odor description	Threshold *Th* (pg/mg)	*Rt* (min)	Average content of different time points *C* (pg)					Difference marker
					E1	E2	E3	E4	E5	
1	Ethyl acetate	Pineapple fragrance	1000	2.104	17679.810	4978.099	4170.190	4812.597	3084.240	***, VIP > 1
2	Diacetyl	The smell of butter	10	2.844	2780.087	3044.103	3013.911	3565.254	4027.459	***,
3	Mesityl oxide	Sweet, chemical	10	5.885	219.310	114.199	109.183	58.015	97.774	***
4	Octanal	Sharp and powerful aromas of green and pungent fat and wax, with fruity and jasmine flavor	100	9.505	5042.463	3720.061	5249.689	3089.878	4270.557	**
5	*trans*-2-Heptenal	Fat, soap, almond	10	10.062	7576.881	6416.928	8467.292	2316.654	5600.729	***
6	Acetic acid	Sour taste	1000	12.214	155781.700	144864.600	165983.400	151018.900	146999.400	***, VIP > 1
7	*n*-Decanal	Soap, fat and wax, orange peel	1	13.100	1934.932	857.739	1010.537	647.460	914.326	***
8	Benzaldehyde	Almond, caramel	1000	13.311	17749.770	10876.730	23326.900	13415.560	15396.800	***
9	2-isobutyl-3-methoxypyrazine	Soil flavor, spice flavor, green pepper flavor	0.01	13.452	115.295	0.00	0.00	1.482	2.984	***
10	Propionic acid	Putrid, spicy, soy sauce	1000	13.494	28489.240	27113.080	32335.810	28386.72	27966.280	**
11	2-Nonenal	Paper smell	1	13.593	712.872	444.596	274.554	193.8355	364.596	***
12	Linalool	Fragrance of flowers and lavender	10	13.748	168.231	77.238	67.215	93.078	66.678	***
13	1-Octanol	Metal, burnt, chemical	100	13.890	2446.057	1112.965	1629.611	1039.398	1519.007	***
14	5-Methyl furfural	Almond, caramel	1000	14.045	18341.990	18410.610	30691.11	28451.61	27409.110	***, VIP > 1
15	2-Methylisoborneol	Stale and mouldy	0.1	14.412	55.292	21.092	16.1185	25.302	43.0823	**
16	Butyric acid	Putrid, cheese, sweat	1000	14.783	11648.820	10312.040	14454.66	12758.38	12293.490	***
17	Phenylacetaldehyde	Sweetness and honey	10	15.005	5273.081	11327.300	18975.170	15960.59	14878.980	***, VIP > 1
18	Isovaleric acid	Putrid, sweat, sour	100	15.333	8692.676	8011.605	10624.230	9697.376	9639.278	***
19	Salicylaldehyde	Herbal, toasted	1	15.477	421.378	474.004	1599.766	1080.489	1108.472	***
20	*trans*-2,4-Nonadienal	Green fragrance, wax and fat fragrance	10	15.830	140.112	67.627	121.844	37.979	56.982	***
21	Borneol	Stale and mouldy	1	15.843	115.335	145.7845	68.821	83.160	56.425	***
22	*n*-Dodecanal	It has a strong aroma similar to pine leaf oil and orange oil	10	15.973	934.002	910.518	1065.818	983.073	519.857	***
23	Methyl salicylate	mint	1	16.737	1200.007	843.041	2697.523	1253.449	1897.136	***
24	Isocaproic acid	Putrid, sweat, sour	100	17.007	1407.514	1540.538	1826.279	1804.966	1618.833	***
25	Caproic acid	The smell of sweat	100	17.485	23065.970	20280.700	28414.050	27251.1	25879.550	***
26	Geraniol	Geranium, rose	1	17.578	890.972	365.687	210.674	182.1175	322.966	***
27	Guaiacol	Sweetness, medicine, smoke	1	17.683	425.341	385.427	962.766	685.9205	749.733	***
28	Benzyl acetone	Fruity, ethereal	0.1	17.695	47.115	114.831	113.401	22.477	58.058	***
29	Benzyl alcohol	Sweet, fragrant	100	17.859	4497.089	2934.417	5527.015	3635.269	4164.503	***
30	gamma-Octalactone	Coconut aroma	1	18.339	171.964	114.652	152.928	63.7055	136.989	***
31	Dibutylhydroxytoluene	Phenol smell	10	18.373	283.703	143.2315	204.395	330.4785	361.382	***
32	beta-Ionone	There are aromas of violet, raspberry and seaweed	0.1	18.662	14.278	0.00	0.00	0.00	6.2183	***
33	Enanthic acid	Green, orange, soap, gasoline	10	18.718	3795.854	4339.398	4582.191	4744.185	3963.703	
34	4,5-Epoxy-(E)-2-decenal	Green fragrance, metal smell	0.01	19.363	341.767	245.0385	491.539	261.4515	427.676	
35	p-Ethylguaiacol	Spice and clove oil aroma	0.1	19.601	92.2110	97.9505	134.066	115.211	111.543	***
36	p-Cresol	It has the smell of smoke and herbal medicine	1	20.099	490.315	462.228	935.463	650.2635	673.653	***
37	m-Cresol	Plastic, faeces smell	0.1	20.184	605.872	562.249	1211.434	835.6695	865.460	
38	2,3-Xylenol	Gasoline smell	1	20.789	2.356	14.049	20.419	15.59	10.696	

(*continued on next page*)

Table 2 (*continued*)

No	Compounds	Odor description	Threshold *Th* (pg/mg)	*Rt* (min)	Average content of different time points *C* (pg)					Difference marker
					E1	E2	E3	E4	E5	
39	Pelargonic acid	Green fragrance, oil fragrance	100	20.992	3084.561	3663.357	2449.716	3167.952	2207.319	***
40	Capric acid	Greasy, stale	10	22.030	747.462	974.287	561.2085	818.2395	434.442	***
41	Coumarin	Sweet and green	1	23.753	14.947	11.956	16.089	13.9845	14.070	***
42	Phenylacetic acid	Floral, honey	10	24.646	1686.522	1674.168	1839.024	1568.55	1629.066	***
43	Vanillin	Vanilla	1	24.681	490.291	448.2035	1731.433	785.6225	1445.416	***
44	Cinnamic acid	It has the fragrance of cinnamon	100	27.562	25344.500	35321.860	62970.090	49,981	69568.300	***, VIP > 1

** t < 0.05, ***t < 0.01.

Heat maps were used to examine the average scores of PES samples (E1 to E5) at various time intervals, and the results are displayed in Fig. 2A. Sourness, bitterness, and astringency dominated taste perception in the first 0–20 s, followed by aftertaste sweetness and saliva secretion in the second 20–60 s. As seen in Fig. 2B, PCA can clearly distinguish PES at five locations (R^2 = 0.989, Q^2 = 0.87), demonstrating that the model is capable of evaluating the sensory time intensity in its whole. The loading scatter plot can also show how extraction time affect PES tastes (The smaller the Euclidean distance, the greater the impact). The result of Fig. 2A and the distance of E1 from each point in Fig. 2C both indicate that E1′s tastes intensity are the weakest. Additionally, E4 has a stronger taste of saliva secretion and aftertaste-sweetness, whereas E3 is more noticeably impacted by bitterness and sourness. E5 has the most noticeable astringency. The smallest bitterness and astringency make E1 seem like the best option, but the aftertaste-sweetness is not obvious. The strength of the astringency and sourness soon decreased after spitting out PES, and the aftertaste-sweetness and salivation now predominate. The major elements that had the biggest influence on customer preferences is aftertaste-sweetness, a flavor that is most characteristic to PE. Considering the biological activity and flavor, E2 and E4 are the best choices.

Electronic tongue was employed as a benchmark to further validate the temporal dominant description method's findings. In all tastings, E1 could be recognized from other samples according to the radar map (Fig. 2 F). The aftertaste-sweetness and bitterness showed the highest value at the E1 time point, and then decreased to 0 or negative value with the extension of the extraction time, which was slightly different from the volunteers' results. The trend of sourness and astringency was consistent with the volunteer evaluation results (Fig. 2 E, F). The majority of participants believed PES to have very little bitterness, according to the results of the volunteer evaluation stage. The complexity of PES's components and the possibility that some bitter compounds go unrecognized by the electronic tongue may play a significant role in this discrepancy (Reis et al., 2020). Additionally, the cause of the aftertaste-sweetness in PES is unknown, however it is hypothesized that it may be due to ingredients like EC or EGC, which is similar to tea soup (Zhang et al., 2016). According to certain research (McBurney and Bartoshuk 1973), the aftertaste's sweetness could be a contrast effect and an oral cavity illusion. When the bitterness, sourness and astringency of PES continue to rise, the contrast effect is significantly enhanced, leading the brain to perceive the sweetness as stronger(Liu et al., 2023). This may be why the aftertaste-sweetness cannot be detected by the electronic tongue. As a result, the temporal dominant description method can be used to collect more plentiful sensory data.

3.2.2. Transformation of volatility components (Odor components)

One of the sensory qualities of PES is its distinct fragrance. It typically has a sweetness and aroma similar to caramel after thermal extraction, which may primarily be the result of the Maillard reaction during the heating and extraction process. Based on HS-SPME/GC-QQQ-MS/MS odor analysis technology, this paper established a rapid method to identify the chemical components in the special odor of PES, and explored

the key differences in the odor components of different extraction time of PES.

According to the OAV calculation formula, the odor components of different batches of PES were analyzed, and 44 components with OAV > 0.1 were found (Table.2). The primary difference markers before and after heating extraction can be quickly identified using the OAV combined with multivariate statistical analysis method (OPLS-DA, Fig. 3A), and significantly difference markers (*VIP* > 1, *t* < 0.05 and OAV > 0.1) were found in Fig. 3B and C, namely ethyl acetate, acetic acid, 5-methyl furfural, phenylacetaldehyde and cinnamic acid.

To precisely describe the odor differences, here we introduce the odor characteristic spectrum (OCS) to quickly and intuitively describe the odor profile of the sample. The OCS of PES is obtained in Fig. 3D by using the odor components as the abscissa and the OAV (Intensity) as the ordinate, and this allows us to quickly identify the components that are crucial in causing odor alterations. According to Fig. 3D, 2-isobutyl-3-methoxypyrazine is the distinctive odor of E1, which provides a clue as to whether it was extracted through heating. Wine often contains 2-isobutyl-3-methoxy pyrazine (IBMP), a substance that should be avoided because it is mostly produced by unripe grapes and can adversely impact the wine's overall flavor (Ling et al., 2021). Among them, 4,5-epoxy-(E)-2-decanal occupies the most conspicuous position. This substance has a green flavor, and its OAV keeps rising with the extension of heat extraction time. *N*-decanal and methyl salicylate are two more noteworthy components. While the latter's OAV value continuously rises, the former's falls during the extraction process (Table.3). Methyl salicylate is described as mint smell, which is frequently added to cosmetics to improve their aroma. Other flavor substances, such as methyl salicylate, geraniol, phenylacetaldehyde and vanillin, increase dynamically with the extension of extraction time, giving PES more distinctive flavor.

3.3. Transformation of non-volatile components

Heated extraction usually involves a non-enzymatic chemical reaction that facilitates the hydrolysis of tannins to produce low molecular weight molecules, including polyols, gallic acid, ellagic acid, and saccharides. However, under conditions of continued heating, these small molecular products will con-

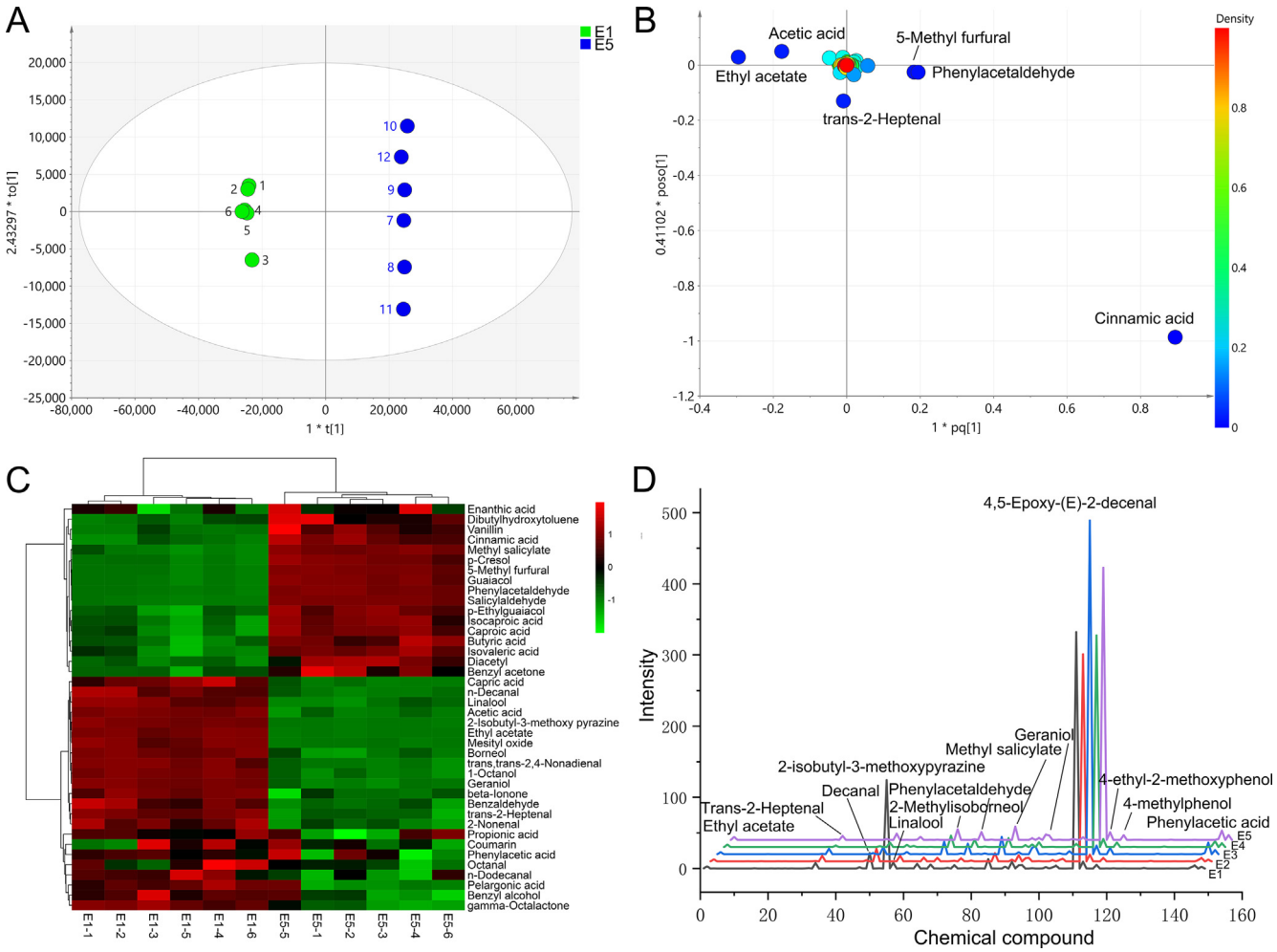

Fig. 3 Analysis results of odor components, A, OPLS-DA results for E1 and E5, B loading scatter plot (the marked components indicate VIP > 1), C difference compounds (t < 0.05, the threshold > 0.1), D odor characteristic spectrum (OCS) of PES.

tinue to react, leading to complex end products (Lu et al., 2008). PES has a molecular structure that has numerous active sites and active groups, including phenolic hydroxyl and carboxyl groups, acyl groups, etc. that allow for a range of complex reactions. According to results (Fig. 4), this process has the following characteristics: 1. The reaction process is intri-

Table 3 OVA average value of PES odor components (n = 3).

No	Compounds	Odor description	E1	E2	E3	E4	E5
1	beta-Ionone	Aroma of violets, raspberries, seaweed	1.428	0.473	0.617	0.710	0.622
2	Acetic acid	Sour	1.558	1.449	1.660	1.510	1.470
3	Phenylacetic acid	Floral scent, honey	1.687	1.674	1.839	1.569	1.629
4	gamma-Octalactone	Coconut aroma	1.720	1.147	1.529	0.637	1.370
5	Cinnamic acid	With cinnamon aroma	2.534	3.532	6.297	4.998	6.957
6	Caproic acid	Sweat smell	2.307	2.028	2.841	2.725	2.588
7	Diacetyl	Butter scent	2.780	3.044	3.014	3.565	4.027
8	Enanthic acid	Green, orange, soap, gasoline	3.796	4.339	4.582	4.744	3.964
9	Vanillin	Vanilla	4.903	4.482	17.314	7.856	14.454
10	Guaiacol	Sweet, medicinal, smoke	4.253	3.854	9.628	6.859	7.497
11	Salicylaldehyde	Herbal flavor, toast flavor	4.214	4.740	15.998	10.805	11.085
12	Benzyl acetone	Fruity, ethereal	4.712	11.483	11.340	2.248	5.806
13	p-Cresol	Smoky, herbal smell	4.903	4.622	9.355	6.503	6.737
14	Phenylacetaldehyde	Sweet, honey,	5.273	11.327	18.975	15.961	14.879
15	2-Methylisoborneol	Earthy, musty	5.529	2.109	1.612	2.530	4.308
16	*trans*-2-Heptenal	Fatty, soap, almond	7.577	6.417	8.467	2.317	5.601
17	Trans-2-nonanal	Papery	7.129	4.446	2.746	1.938	3.646
18	Geraniol	Geranium aroma, rose aroma	8.910	3.657	2.107	1.821	3.230
19	p-Ethylguaiacol	With spice and clove oil aroma	9.221	9.795	13.407	11.521	11.154
20	Methyl salicylate	Mint	12.000	8.430	26.975	12.534	18.971
21	*n*-Decanal	Soap, waxy, orange peel aroma	19.349	8.577	10.105	6.475	9.143
22	m-Cresol	Plastic, fecal smell	60.587	56.225	121.143	83.567	86.546
23	2-Isobutyl-3-methoxy pyrazine	Earthy, spice, green pepper	115.296	0.000	0.000	1.482	2.984
24	4,5-Epoxy-(E)-2-decenal	Green scent, metallic scent	341.767	245.039	491.539	261.452	427.676

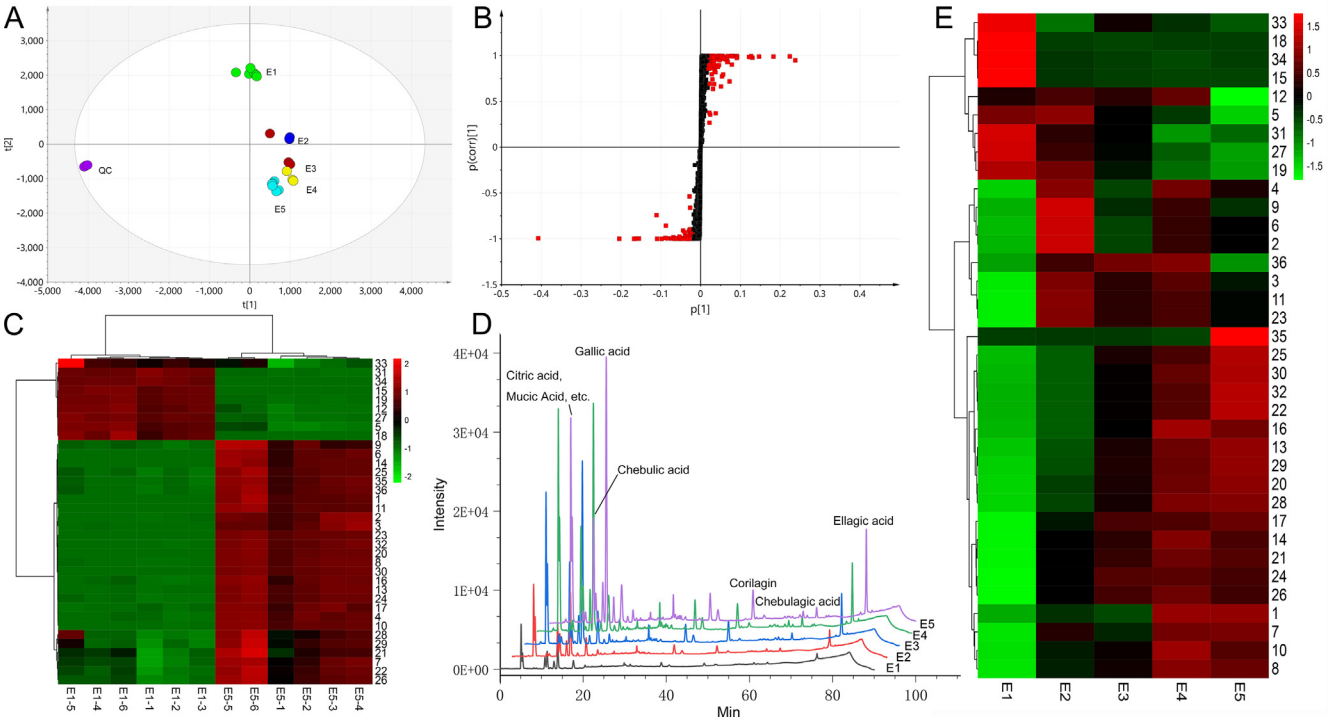

Fig. 4 Analysis results of differential markers, A. PCA results, B S-plot, C difference marker heat map, D, HPLC chromatogram at different extraction times, E. change trends of differential markers.

cate, involving both hydrolysis and polymerization; 2. The reaction is significant and rapid after heating; 3. A variety of end products with intricate structures are produced. With the help of high-resolution mass spectrometry, we focus on the following aspects:1. Determine the chemical change profile, such as reaction type, general reaction rules, etc.; 2. Identify the

Table 4 Differential markers in different extraction methods.

No	Compound	Retention time (min)	Molecular formula	Measured (*m*/*z*)	Molecular ion	Error (ppm)	Isotope similarity	Content change
1	Quercetin	14.9703	$C_{15}H_{10}O_7$	301.0344	M−H	−3.3317	91.4662	↓
2	3,4,8,9,10-pentahydroxy-6-oxo-6H-benzo[*c*] chromene-1-carboxylic acid	14.3442	$C_{14}H_8O_9$	300.9983	M−H_2O−H	−2.0450	89.5665	↑
3	Granatin B	14.3299	$C_{41}H_{28}O_{27}$	951.0747	M−H	0.1397	81.9781	↑
4	Tellimagrandin II	14.1306	$C_{41}H_{30}O_{26}$	937.0958	M−H	0.6183	83.46731	↑
5	Quercitrin	14.0949	$C_{21}H_{20}O_{11}$	447.0923	M−H, 2 M−H	−2.1753	92.5691	↓
6	Bicornin	12.5511	$C_{48}H_{32}O3_0$	1087.091	M−H	0.7384	75.2856	↑
7	Ellagic acid	11.2482	$C_{14}H_6O_8$	300.9982	M−H	−2.7505	95.0828	↑
8	Tercatain	10.5721	$C_{34}H_{26}O_{22}$	785.0844	M−H	0.1668	82.8016	↑
9	Sanguiin H2	10.4656	$C_{48}H_{32}O_{31}$	1103.086	M−H	0.1781	80.9349	↑
10	m-Trigallic acid	9.9388	$C_{21}H_{14}O_{13}$	473.0362	M−H	0.1163	84.2970	↑
11	1-O-Galloylpedunculagin	9.5901	$C_{41}H_{28}O_{26}$	935.0795	M−H	−0.1203	89.0098	↑
12	1,3,4-trigalloyl-beta-d-glucopyranose	9.5901	$C_{27}H_{26}O_{19}$	635.0886	M−H_2O−H	−0.5601	83.5092	↓
13	Corilagin	8.6509	$C_{27}H_{22}O_{18}$	633.0738	M−H	0.7252	95.2451	↑
14	4,4,5,5,6,6′-hexahydroxy- [1, 1′-biphenyl]-2, 2-dicarboxylic acid	8.2307	$C_{14}H_{10}O_{10}$	337.0193	M−H	−2.3119	90.7627	↑
15	6-[4-({[7,8,8,12,13,22-hexahydroxy-19-(hydroxymethyl)-3,6,16-trioxo-2,17,20,23-tetraoxapentacyclo[16.3.1.1^{7},^{11}.04,^{9}.0^{10},^{15}] tricosa-4,10,12,14-tetraen-21-yl] oxy}carbonyl)-2,6-dihydroxyphenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid	5.6405	$C_{33}H_{30}O_{25}$	807.0906	M−H_2O−H	0.9501	91.6979	↓
16	6-({1-carboxy-3,8,9,10-tetrahydroxy-6-oxo-6H-benzo[*c*]chromen-4-yl}oxy)-3,4,5-trihydroxyoxane-2-carboxylic acid	4.7151	$C_{20}H_{16}O_{15}$	495.0418	M−H	0.2367	87.2027	↑
17	3,4,5-trihydroxy-6-(3,4,5-trihydroxybenzoyloxy)oxane-2-carboxylic acid	4.7222	$C_{13}H_{14}O_{11}$	327.035	M−H_2O−H	−2.2045	89.54094	↑
18	Succinylacetoacetate	4.4806	$C_8H_{10}O_6$	183.0294	M−H_2O−H	−2.5031	95.4474	↓
19	1,2-Digalloyl-beta-D-glucopyranose	4.2812	$C_{20}H_{20}O_{14}$	483.078	M−H, 2 M−H	−0.1006	89.7035	↑
20	3,4-dihydroxy-5-(3,4,5-trihydroxybenzoyloxy)benzoic acid	3.7045	$C_{14}H_{10}O_9$	321.0246	M−H	−1.8258	91.0399	↑
21	4-[(6-carboxy-3,4,5-trihydroxyoxan-2-yl) oxy]-4′,5,5′,6,6′-pentahydroxy-[1,1′-biphenyl]-2,2′-dicarboxylic acid	1.5344	$C_{20}H_{18}O_{16}$	513.0519	M−H	−0.6480	89.0181	↑
22	6-Methyl 2-galloylgalactarate	1.1723	$C_{14}H_{16}O_{12}$	751.1207	2 M−H	−0.5153	98.4140	↑
23	Sanguiin H4	1.1366	$C_{27}H_{24}O_{19}$	633.0735	M−H	0.2529	79.0543	↑
24	2-O-Galloyl-1,4-galactarolactone	1.0295	$C_{13}H_{12}O_{11}$	343.0307	M−H	−0.0179	94.0372	↑
25	Gallic acid	1.0017	$C_7H_6O_5$	169.0141	M−H	−0.7509	98.7058	↑
26	1-Methyl 2-galloylgalactarate	0.9517	$C_{14}H_{16}O_{12}$	375.0566	M−H	−0.7721	89.8624	↑
27	2-Galloylglucose	0.8453	$C_{13}H_{16}O_{10}$	331.0666	M−H, 2 M−H	−1.3246	96.4136	↓
28	5-O-Galloyl-1,4-galactarolactone	0.6953	$C_{13}H_{12}O_{11}$	687.0699	M−H, 2 M−H	1.4303	93.2576	↑
29	Citric acid	0.6953	$C_6H_8O_7$	191.0195	M−H	−1.1884	97.2197	↑
30	3,4,5,11,12,13,21,22,23-nonahydroxy-9,14,17-trioxatetracyclo[17.4.0.0^{2},^{7}.0^{10},^{15}] tricosa-1(23),2,4,6,19,21-hexaene-8,18-dione	0.6674	$C_{20}H_{18}O_{14}$	481.0642	M−H	3.8702	88.7237	↑
31	Malic acid	0.5675	$C_4H_6O_5$	133.0141	M−H	−1.4131	95.5124	↓
32	Chebulic acid	0.5389	$C_{14}H_{12}O_{11}$	355.0314	M−H_2O−H, M−H	1.2365	92.7179	↑
33	2-O-Galloylgalactaric acid	0.5318	$C_{13}H_{14}O_{12}$	361.0419	M−H, 2 M−H	1.71817	94.8857	↓
34	Galactinol	0.5039	$C_{12}H_{22}O_{11}$	341.1097	M−H	2.2280	90.6854	↓
35	D-2-Hydroxyglutaric acid	0.5039	$C_5H_8O_5$	147.0296	M−H	−1.7772	96.0944	↑
36	2-Hydroxybutyric acid	0.5039	$C_4H_8O_3$	85.02942	M−H_2O−H	−0.7952	98.0449	↑

compounds with the most significant transformation; 3. Identify some representative basic transformation pathways; 4. Pay attention to the transformation of important active ingredients in PES, such as gallic acid and ellagic acid.

Through Progenesis QI software, standard substance and literature information, 36 differential markers (VIP > 1, $p < 0.05$) were identified (Fig. 4 B, C), which represented the most significant transformed compounds in PES (Table.4). In differential markers, the decreased components may participate in transformation. The reaction pathway can be determined by analyzing the dynamic change trend of the

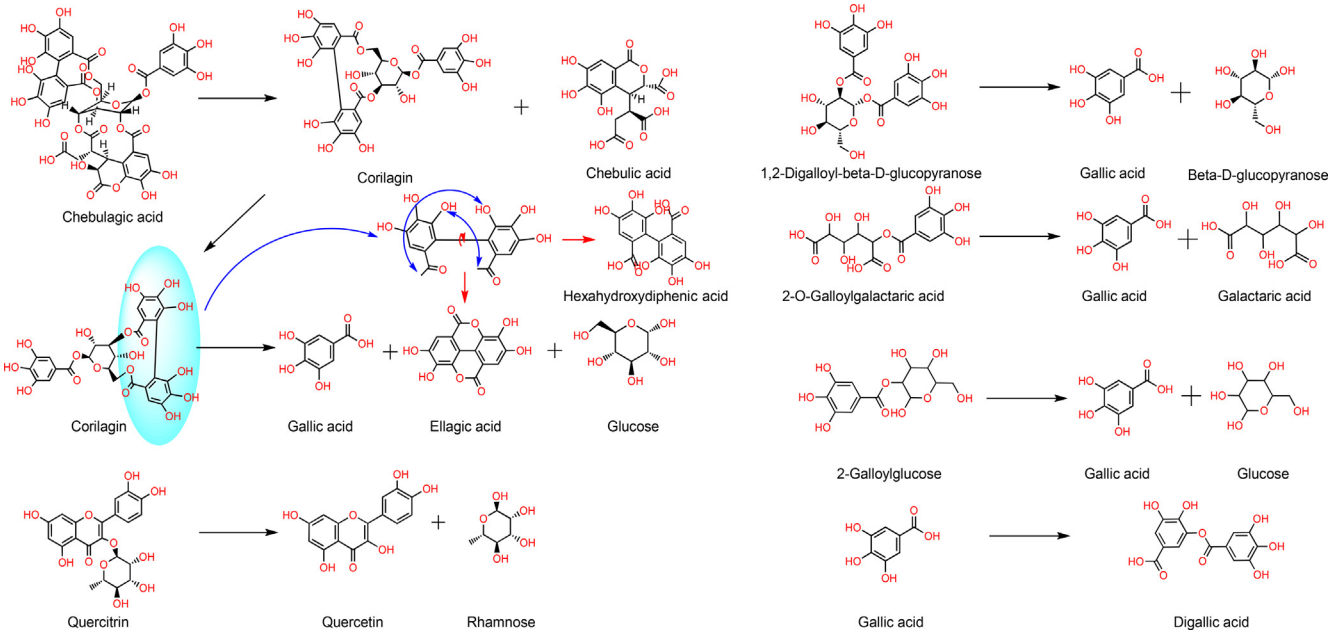

Fig. 5 Main differential compounds transformation pathways.

Table 5 Correlation analysis of difference markers and sensory evaluation results.

Compound number	Suorness	Bitterness	Astringency	Aftertaste-Sweetness	Salivary secretion	Hits
4			0.89			1
7	0.95		0.98		0.90	3
8	0.99	0.95	0.89		0.97	4
10	0.98	0.95			0.98	3
13	0.94	0.91			0.89	3
14	0.99	0.97	0.88	0.89	0.99	5
15	−0.95	−0.89	−0.90	−0.91	−0.91	5
16	0.90	0.90			0.90	3
17	0.98	0.96		0.88	0.94	4
18	−0.94	−0.88	−0.89	−0.91	−0.90	5
19	−0.89					1
20	0.97	0.92	0.92		0.91	4
21	0.99	0.97	0.89	0.89	0.98	5
22	0.89					1
24	0.97	0.97		0.94	0.96	4
25	0.90					1
26	0.98	0.97		0.93	0.98	4
27	−0.95	−0.87	−0.88			3
28	0.97	0.94			0.93	3
29	0.95	0.92			0.90	3
30	0.91					1
31	−0.97	−0.93	−0.87		−0.96	4
32	0.89					1
33			−0.97			1
34	−0.95	−0.89	−0.90	−0.91	−0.91	5

Blank means not significant.

compound in combination with literature reports and experiments.

From the content change (peak area) before and after thermal extraction (Fig. 4 E), it is easy to find several components with the strongest transformation during the thermal extraction process. They are No. 27, 33, 28, 15, 19, 25, 13, 21, 7, 24, 29, 22 and 5 (from high to low). They almost all belong to Gallotannins and Ellagitannins (Lu et al., 2008). It is easy to deduce that aglycone (polyol), gallic acid, and hexahydroxybiphthalic acid are the core components of this reaction. They increase continuously in thermal extraction and become the end products of hydrolyzable tannins. Through the verification of standard model solutions, Fig. 5 shows the hydrolysis process of representative difference markers in PES during thermal extraction. It was shown that the content of some tannins increased during the heat extraction process, indicating that the polymerization event took place concurrently. For instance, during thermal treatment, 2-O-galloylgalactaric acid decomposes into gallic acid and galactic acid. It also reveals an increase of 5-O-galloyl-1,4-galactarolactone, which may be the result of the molecular rearrangement of 2-O-galloylgalactaric acid following hydrolysis.

The pearson correlation analysis was used to examine the relationship between the differential markers and the sensory evaluation data of volunteers (highest value at each time point) at various extraction time points for the screening and identification of flavor compounds. Table.5 reveals that the majority of substances, including citric acid, gallic acid, and its deriva-

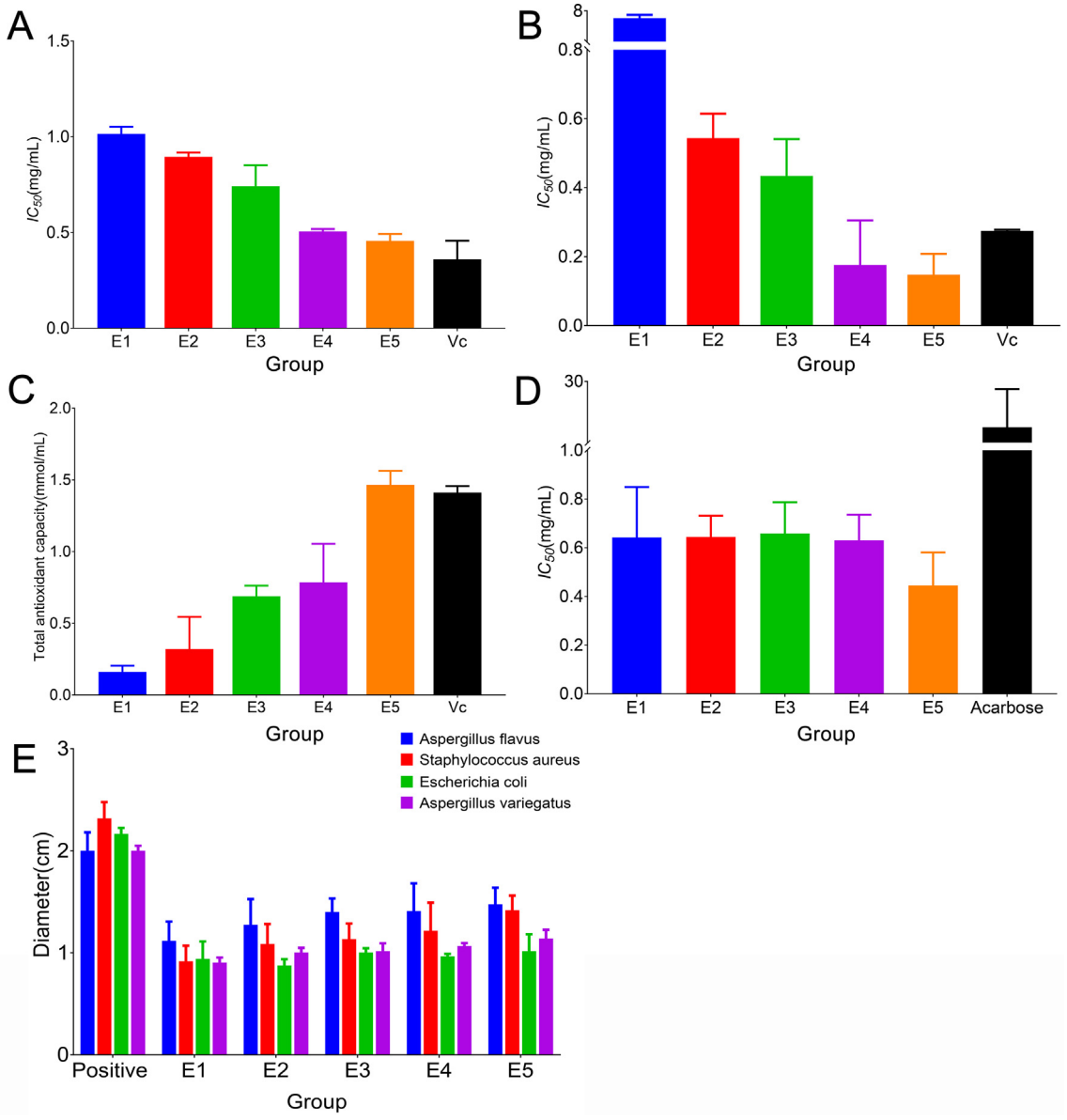

Fig. 6 The biological activity changes of PES with extraction time, A, B and C are ABTS, DPPH, and FRAP antioxidant capacity respectively, D, α Glucosidase inhibitory activity, E antibacterial activity, F correlation heat map of biological activity and differential markers.

tives, strongly correlate with sourness. Tellimagrandin II, ellagic acid, tercatain, 4,4,5,5,6,6′-hexahydroxy-[1,1′-biphenyl]-2, 2-dicarboxylic acid, and other tannins are the primary astringency-related substances. More sophisticated factors, such as flavor contrast, may play a role in the development of the aftertaste's sweetness. Due to the fact that sour can produce salivation, sour components have a strong correlation with salivary secretion.

3.4. Study on PES activity changes and correlation analysis

The hypoglycemic activity of PE is primarily manifested in its inhibitory effect on α-glucosidase; while PES has strong antioxidant and antibacterial properties thanks to the abundance of phenolic hydroxyl groups. This study examines how relevant biological activities alter dynamically as a result of the PES heat extraction process. Fig. 6 makes it very evident that as extraction time is increased, several biological activities are also noticeably improved. Studies revealed that after hydrolysis, some hydrolyzed tannins will have dramatically increased biological activity, such as antibacterial activity (Aguilar-Galvez et al., 2014). Additionally, it is believed that hydrolysable tannins acquire their antibacterial properties from the presence of hexahydroxydiphenoyl and nonahydroxyterphenoyl moieties (Ekambaram et al., 2016). According to Taguri et al. (Taguri et al., 2006), the pyrogallol group is a crucial structural component of polyphenols' antibacterial action. The extra free galloyl group appeared to boost the ellagitannin's inhibitory actions on E. coli (Puljula et al., 2020). Under the conditions of heat extraction, rutin can hydrolyze to quercetin. But quercetin's weak water solubility might lessen its antibacterial power. In addition, polymerization also occurs during the extraction process and has the potential to result in the production of new hydrolyzed tannins. Therefore, the biological activity is always changing, but ultimately leads to the increase of the overall activity of PES.

The changes in biological activity and differential markers are strongly connected. The pearson correlation heat map analysis was utilized for correlation analysis in order to explore prospective biological activity indicators. The empty space denotes a poor significance test result ($p > 0.05$). It should be noted that the activity is stronger the lower the IC_{50} value. In Table 6, the more components hit, the more critical it is. For instance, the compounds Nos. 8, 13, 19, 20, 22, 25, 27, 29, 30 and 32 contain certain well-known bioactive substances, such as corilagin and gallic acid, which are now the most reported substances (Yang and Liu 2014). Escherichia coli has no correlation with all components, so it is not listed.

Table 6 Correlation analysis between bioactivity and differential markers.

Compound number	Aspergillus flavus	Staphylococcus aureus	Aspergillus variegatus	DPPH	ABTS	FRAP	Anti hyperglycemia	Hits
1		0.89	0.92		−0.95			3
3				−0.90				1
5		−0.92	−0.89		0.93	−0.98		4
7			0.92	−0.91				2
8	0.95	0.87	0.92	−0.91	−0.93			5
10	0.91		0.88	−0.90	−0.91			4
11				−0.92				1
12							0.96	1
13	0.98	0.94	0.95		−0.98	0.90		5
14	0.93			−0.96				2
15	−0.88			1.00				2
16	0.90		0.88		−0.98			3
17	0.98		0.90	−0.96				3
18				1.00				1
19	−0.95	−0.93	−0.94		0.99	−0.93		5
20	0.98	0.94	0.96		−0.97	0.89		5
21	0.94		0.87	−0.97				3
22	0.95	0.98	0.97		−0.98	0.96		5
23				−0.92				1
24	0.94			−0.97				2
25	0.97	0.97	0.96		−0.97	0.96		5
26	0.93			−0.97				2
27	−0.97	−0.97	−0.99		0.97	−0.92		5
28	0.97	0.92	0.94		−0.97			4
29	0.98	0.94	0.96		−0.97	0.90		5
30	0.96	0.96	0.97		−0.99	0.95		5
31	−0.92		−0.91		0.95			3
32	0.95	0.98	0.97		−0.98	0.96		5
33				0.93				1
34	−0.87			1.00				2
35							−0.99	1

Blank means not significant.

4. Conclusion

Due to the colloid that its polysaccharides produce, PES's surface tension decreases and its color and viscosity increase as the thermal extraction time lengthens. According to the temporal dominating description approach developed in this study, all taste intensities grew as heating time increased, but the comprehensive taste index revealed that E4 and E2 have better flavor. Additionally, it was discovered through OCS analysis that thermal extraction can get rid of unpleasant smells like IBMP, a type of green pepper flavor that ruins the flavor of foods and beverages, and increase significantly the intensity of the compound 4,5-epoxy-(E)-2-decanal, which gives PES a green smell. The hydrolysis and condensation of tannins were the two fundamental steps in the difficult transformation of non-volatile compounds. And practically all of them are connected to the hexahydroxydibenzoyl (HHDP) and galloyl chemical structures. This is a typical reaction that occurs during the thermal extraction of polyphenols, which are significant elements that affect PES's activity and flavor. In the examination of biological activity, heat extraction was helpful in enhancing biological activity, which may be attributed to an increase in gallic acid, ellagic acid, corilagin, 2-galloylglucose, citric acid, and chebulic acid, among other compounds. In conclusion, PE's flavor and biological activity can be greatly enhanced by thermal treatment.

CRediT authorship contribution statement

Haozhou Huang: Methodology, Data curation, Writing – original draft. **Mengqi Li:** Methodology, Data curation, Writing – original draft. **Qinchu Tan:** Methodology, Data curation. **Ce Tang:** . **Jihai Gao:** . **Xiaoming Bao:** . **Sanhu Fan:** . **Taigang Mo:** . **Li Han:** Conceptualization, Supervision, Validation, Writing – review & editing. **Dingkun Zhang:** Conceptualization, Supervision, Validation, Writing – review & editing. **Junzhi Lin:** Conceptualization, Supervision, Validation, Writing – review & editing.

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Data Availability Statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

Author Sanhu Fan and Taigang Mo were employed by company Sanajon Pharmaceutical Group. All other authors declare no competing interests.

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