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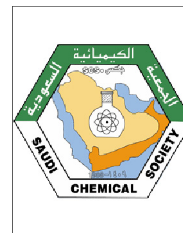

ORIGINAL ARTICLE

Quality analysis combined with mass spectrometry imaging reveal the difference between wild and cultivated *Phyllanthus emblica* Linn.: From chemical composition to molecular mechanism

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KEYWORDS

Phyllanthus emblica;
Wild;
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Abstract In this paper, the quality of Phyllanthus emblica was evaluated by establishing a comprehensive taste index and odor component analysis method, and the formation mechanism of quality difference was revealed by mass spectrometry imaging and metabolomics. Studies have found that the representative substances of bitterness and astringency in cultivated PE, such as amlaic acid, kaempferol, quercetin and their derivatives, have higher content. Compared with wild PE, cultivated PE has a very lower 2-Isobutyl-3-methoxypyrazine content, a kind of odor molecule that destroys the fruits flavor. Biological activity studies showed that the biological activity of wild

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PE was stronger than cultivated PE. Mass spectrometry imaging revealed significant differences in the distribution of multiple components between the two types of PE. Metabonomic studies show that their quality differences may be related to the difference of flavonoids and flavonol biosynthesis in PE.

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1. Introduction

Phyllanthus emblica L. (PE), a common tropical fruit in Southeast Asia, originated in India and Myanmar, which is a tree in the *Phyllanthaceae* family widely distributed in Southwest China, India, Vietnam and Thailand (Perianayagam et al., 2005), with thousands of years of edible history. Its most attractive flavor feature is, aftertaste-sweetness and stimulation of saliva secretion, which can give lasting comfort to the mouth after a short sour and astringent taste (Huang, Qiu, Lin, Li, Ma, Ran, et al., 2021). Moreover, its juice and dried fruits also have a pleasant fresh fragrance. It is particularly a chief source of vitamin C and minerals compared to other citrus fruits (Variya, Bakrania, & Patel, 2016). PE has high nutritional value and is rich in tannins (33% of the dry weight), vitamins, amino acids, mineral elements, phenolic acids and flavonoids, which gives it strong anti-fatigue, antioxidant, anti-inflammatory, antitumor, cardioprotective and antidiabetic abilities(Grover, Tewari, Sharma, Singh, Yadav, & Naula, 2016; Huang, Qiu, et al., 2021; Liu, Cui, Zhao, Wang, Luo, Yang, et al., 2008; Thirunavukkarasu, Selvaraju, Tapias, Sanchez, Palesty, & Maulik, 2015; Zhao, Sun, Marques, & Witcher, 2015). Because of its unique flavor, rich nutrition and outstanding efficacy (Gantait, Mahanta, Bera, & Verma, 2021), PE is one of the three plants recommended by the United Nations for global cultivation and is favored by the food and functional beverage industry (Liu, Ma, Wan, Li, & Ma, 2020).

China is one of the main producing areas of PE in the world, and the demand is increasing year by year. But at present, PE is still a low-improved economic fruit tree in China, and most of it is still in a wild and semi-wild state(Huang, Ran, Tan, Zhang, Li, Fan, et al., 2021). The most representative wild PE is Chuxiong, Yongren, etc in Yunnan, which has high genetic diversity(Liu, Ma, Wan, Li, & Ma, 2020). The PE produced in Yunnan mainly comes from low-latitude wild mountainous areas at an altitude of 1500–2000 m, which is the largest producing area in China. The weather here is hot and dry during the daytime, with strong ultraviolet radiation and 3–6 times evaporation (compared with local precipitation). Wild PE has the characteristics of small fruit, bitter and astringent taste due to rich tannins and flavonoids (Liu, et al., 2020), which is difficult to be accepted by consumers. Therefore, in the process of cultivation, PE with strong adaptability, high yield and quality are selected purposefully. Such as Guangdong, a hot and humid low-altitude area with abundant precipitation and weak ultraviolet radiation, has a large number of cultivated PE. The PEs in these two places are significantly different in appearance, smell, taste and composition (Li, et al., 2020; Liu, Ma, Wan, Li, & Ma, 2020). However, with the human-made destruction and shrinking of wild resources, cultivated PE has a tendency to gradually replace wild resources (Huang, Ran, et al., 2021; Liu, Ma, Wan, Li, & Ma, 2020). This means that the overall quality of PE in the Chinese market is also gradually changing, which may cause changes in related food and beverage markets and industrial chains (Huang, Ran, et al., 2021). However, there are no reports on the comprehensive qualities (including flavor, composition, biological activity, etc.) differences between them and the chemical essence behind them, and the formation mechanism of their quality differences is unknown. In order to further promote the evaluation and control of its flavor quality, cultivation, quality improvement and development, we must first answer these questions.

To fill the above research gaps, this paper established the temporal dominant description method and the odor rapid analysis method based on Headspace Solid Phase Microextraction equipped with Triple Quadrupole Mass Spectrometry (HS-SPME/GC-QQQ-MS/MS) technology (the built-in database contains 150 kinds of odor components MRM ion pair, threshold, retention time and standard curve), and the differences of special flavor substances between wild and cultivated PE were investigated by the above methods respectively. Secondly, t their biological activity differences were investigated. Finally, metabolomics and mass spectrometry imaging techniques were used to study the formation mechanism of their comprehensive mass differences. We hope that this study can provide reference and guidance for PE quality control, cultivation, pharmaceutical industry, food function and dietary supplement development.

2. Materials and methods

2.1. Ethics statement

Volunteers were given written informed consent forms about 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

Water was purified using a Milli Q water purification system (Millipore, Bedford, MA, USA). HPLC-grade methanol was purchased from Fisher Chemical (Pittsburg, PA, USA). HPLC-grade formic acid was obtained from Chengdu KeLong Chemical Factory (Chengdu, China). Anhydrous Ethanol (Analytical purity), Vitamin C was also purchased from Chengdu KeLong Chemical Factory (Chengdu, China). DPPH free radical scavenging ability test kit, ABTS buffer solution was purchased from Solaribio biotechnology Co., Ltd. (Beijing), α-Glucosidase, 4-Nitrophenyl-β-D-glucopyrano side (PNPG, Sigma, USA) and 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. Phyllanthus emblica sample collection

12 batches of PE samples were collected from Yunnan and Guangdong, and identified by associate professor Gao Ji-hai from Chengdu University of TCM, and the samples are kept in the National Seed Resource Bank. The specific sampling

information is shown in Table 1 and Fig. 1. All the 12 batches were fresh PE fruits collected at mature period.

2.4. UPLC-QTOF-Mass conditions

2.4.1. Sample preparation

0.1 g PE freeze-dried powder of each sample was put in clean erlenmeyer flask respectively, added 50 mL of 50% methanol–water solution and ultrasonic for 30 min to dissolve it as a 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.

2.4.2. Chromatographic conditions

Samples were analyzed by Acquity UPLC I-class (Waters) ultra performance 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^{-1}, and the injection volume was 3 μL.

2.4.3. 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 was used for detection and analysis. The capillary voltage was 4 kV, cone voltage 50 V, ion source temperature 150℃. The atomizing gas was

Table 1 Details of 12 Phyllanthus emblica samples.

Batch	Collection place	Origin identification	Altitude (m)	Growing Conditions	Collection time
M1	Chuxiong, Yunnan	*Phyllanthus emblica* L.	1900	Wild	2021.03
M2	Chuxiong, Yunnan	*Phyllanthus emblica* L.	1900	Wild	2021.03
M3	Yuanmou, Yunnan	*Phyllanthus emblica* L.	1800	Wild	2021.03
M4	Yuxi, Yunnan	*Phyllanthus emblica* L.	1800	Wild	2021.02
M5	Yongren, Yunnan	*Phyllanthus emblica* L.	1600	Wild	2021.02
M6	Yongren, Yunnan	*Phyllanthus emblica* L.	1600	Wild	2021.02
F1	Shantou, Guangdong	*Phyllanthus emblica* L.	120	Cultivation	2021.03
F2	Shantou, Guangdong	*Phyllanthus emblica* L.	120	Cultivation	2021.03
F3	Chaozhou, Guangdong	*Phyllanthus emblica* L.	140	Cultivation	2021.03
F4	Chaozhou, Guangdong	*Phyllanthus emblica* L.	140	Cultivation	2021.02
F5	Chaozhou, Guangdong	*Phyllanthus emblica* L.	140	Cultivation	2021.03
F6	Shantou, Guangdong	*Phyllanthus emblica* L.	120	Cultivation	2021.02

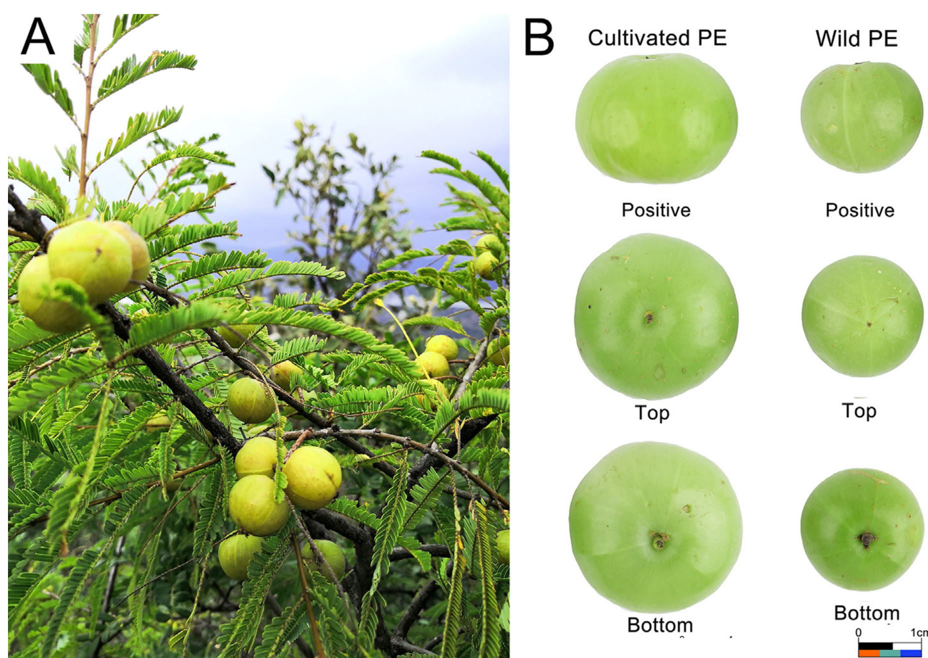

Fig. 1 Typical figures of PE plant (A), appearance of wild and cultivated PE (B).

high-purity nitrogen, cone gas flow rate was 50 $L \cdot h^{-1}$, desolvention gas flow rate was set as 600 $L \cdot h^{-1}$, 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.

2.4.4. Data processing and multivariate analysis

Masslynx 4.1 was used to collect data, and the original data was imported into progenesis Qi (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 times, m/z and peak intensities 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 greater than 1and $P < 0.05$ were selected as differential metabolites.

2.4.5. Metabolic pathway and function analysis

The selected differential compounds were imported into the MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/) website for pathway analysis Analysis. This website can conduct pathway analysis of compounds through KEGG and HMDB values.

2.5. Mass imaging conditions

PE was cut into even sheet and frozen In −80 degreesC refrigerator. After removal, the slide was covered and compressed, and then freeze-dried as the mass spectrum imaging sample. Samples were analyzed by SYNAPT XS, with DESI ion source. Mass spectrometry conditions were set as follows: negative ion scanning, scanning range100-1500 m/z, spatial resolution 120 um, spray voltage 4.5KV; sampling cone voltage 40 V; sprayer (nitrogen pressure) 0.5 MPa; solvent methyl formic acid (1000: 1), volume flow 2 μL/min; sprayer angle of incidence 70 degrees; collector angle 10 degrees; ion source temperature 150℃; sampling voltage was −40 V. The data acquisition software was Masslynx V4.2 (Waters company, USA) and data processing software was Masslynx HDI V 1.5 software (waters company, USA).

2.6. Establishment of temporal dominant description method

To evaluate the taste difference of PE, a human sensory test using the visual analog scale (VAS) was proposed to verify the results (Han et al., 2018) (Han, Jiang, Han, Xiong, He, Fu, et al., 2018). VAS was a measurement instrument for subjective characteristics or attitudes that cannot be directly measured. It was initially widely used in pain scoring, and then used in the sensory evaluation. 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 TCM. They have no smoking, drinking and drug abuse, no genetic history, no recent oral and throat diseases, and normal taste. All the volunteers were voluntary and signed the informed consent before the experiment.

PE has four basic flavors, which are astringency, bitterness, sourness, aftertaste-sweetness, and one sensations called saliva secretion. Among them, aftertaste-sweetness and salivary secretion are important sensory characteristics of PE. These flavors and sense will appear in sequence during the chewing process, and the last one is sweetness, which is called aftertaste-sweetness. Its precise meaning is that in a certain period of time, a certain taste will occupy an absolute advantage, which makes the flavor of PE have a sense of hierarchy. Previous sensory descriptions cannot accurately evaluate the temporally hierarchical taste of PE. Therefore, it is necessary to establish a special method for PE taste and flavor evaluation, which is called temporal dominant description method (P. Li, Zhang, Lin, Han, Ke, Han, et al., 2019). During the training sessions, volunteers were trained with different concentrations of model solutions (Table 2) so they were accustomed to the evaluation scales and bitterness intensities. After that, the samples were evaluated. A drop of approximately 10 mL of each solution was applied to the upper surface of the tongue for 15 s. Then, the test solution was expectorated. Volunteers were asked to score the "bitterness, sweetness, astringency, acidity" 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 between each session.

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

2.7.1. 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 degreesC 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 (Niu, Wang, Xiao, Zhu, Sun, & Wang, 2019).

Table 2 concentration of model solution (mg/mL).

Level	Sucralose (sweet)	Tannic acid (astringent)	Citric acid (sour)	Quinine (bitter)
Slight	3.0	0.5	0.5	0.1
Obvious	5.0	1.0	0.8	0.2
Serious	7.0	2.0	1.0	0.3

2.7.2. 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-WR-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 was argon; the mass spectrum monitoring mode was multi reaction monitoring (MRM), the detector voltage was + 0.3kv relative to the tuning result, and the solvent delay time was 1.3 min. In order to improve the sensitivity of the detection, the compounds were monitored by time segment.

2.7.3. Qualitative and quantitative method

Precisely draw 1 μL of the mixed solution of 4 compounds (Acetophenone solution (2 μg/mL, Cat:48292, Sigma), Naphthalene (5 μg/mL, Cat:40053, Sigma), 2,6-Dichlorophenol (2 μg/mL, Cat:40302, Sigma), 2,4,6-Trichloroanisole (2 μg/mL, Cat:47526-U, Sigma) for analysis to evaluate the applicability of the instrument system (Add 5 mL of methanol to a 10 mL volumetric flask, use a pipette to draw 10 μL of each of the 4 compound standard solutions into the volumetric flask, dilute to the mark with methanol, shake well, and use a 0.22 μm microporous filter membrane to filter the solution). Then precisely draw 1 μL of the mixed solution (0.1 μg/mL) containing three internal standards for sample analysis to obtain the peak area of the internal standard. Finally, the samples were measured according to the above conditions. The qualitative of the target compound is confirmed by the m/z ratio and the ion pair. 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 substance. 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 the threshold.

2.8. Biological activity determination methods

Take the α-glucosidase and PNPG reaction system as a model for testing, and the specific operations were as follows: Added 10 μL of α-glucosidase solution (2U/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 incubated for 30 min. Finally, added sodium carbonate solution to stop the reaction. Each sample has 3 replicate wells. Measured the absorbance at 405 nm as soon as possible by a multifunctional 96-well plates reader, and repeat for 3 times. 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 50, 100, 150, 250, 350, 500, 750, and 1000 μg/mL and calculate the inhibition rate.

According to the DPPH free radical scavenging ability test kit (Solebo biotechnology Co., Ltd.) instructions, prepared the solution and required reagents. Vitamin C was used as a positive control, the concentration was 0.01, 0.05, 0.20, 0.40, 0.60, 0.80, 1.00, 2.00 mg/mL; the samples were set with 8 concentration gradients of 0.20, 0.60, 1.00, 2.00, 4.00, 6.00, 8.00, and 10.00 mg/mL and calculated the scavenging rate. The calculation formula was as follows:

$$\text{Inhibitionrate}(\%) = \frac{A0 - (A1 - A2)}{A0} \times 100\%$$

In the formula: A_0 was the absorbance value of blank group; A_1 was the absorbance value of inhibition group; A_2 was the absorbance value of background group. All data were imported into Graphpad prism7.0 software to calculate half inhibitory concentration (IC_{50}).

The antibacterial activity of PE is one of its important biological activities. It has a wide range of antibacterial activities, especially against oral bacteria and common pathogenic bacteria(Staphylococcus aureus, Candida albicans and Aspergillus flavus)(Khan, Hassan, Ullah, Karim, Baseer, Abid, et al., 2013; Thaweboon & Thaweboon, 2011). Staphylococcus aureus, Candida albicans 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). Take 2 mL of each sample solution (12 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.

2.9. Data processing and analysis

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 and discussion

3.1. Temporal dominant description evaluation results

PE has 5 basic taste senses, which are bitterness, sourness, astringency, aftertaste-sweetness and stimulate saliva secretion. These five sensations represent the five main kinds of

compounds, such as the sourness and saliva secretion comes from organic acids, astringency comes from tannins, aftertaste-sweetness is more complex may come from the comprehensive effect of sugars, amino acids and catechins(Das, Sasmal, & Arora, 2021). Meanwhile, these five sensations will appear in sequence at different times after PE enters the oral cavity. Based on the PE taste characteristics, this paper established a temporal dominant description methods to evaluate the sensory characteristics of wild and cultivated PE(P. Li, et al., 2019). First, 10 volunteers' VAS scores were used to evaluate 5 sensory scores at different time points. The average scores of 12 batches at different time points were shown by heat map, and the results are shown in Fig. 2 A. It can be clearly seen from the results of the heat map that the five senses of taste are obviously time-dependent. In 0–20 s, sourness, bitterness and astringency were the dominant taste senses, while in 20–40 s were aftertaste and saliva secretion. The tastes appearance time is defined as the taste intensity retention time (TIRT). During the whole tasting process, the bitterness, astringency and sourness intensity (I) of PE showed a downward trend, the rest showed a trend of rising first and then falling. It can be intuitively found from the heat map (Fig. 2 A) that the intensity and duration of bitterness, sourness and astringency of wild PE are significantly stronger than that of cultivated PE. However, many evaluation methods like single oral taste evaluation method, fuzzy mathematics comprehensive evaluation method, sensory simulation evaluation method, etc. cannot accurately reflect the taste dynamic changes in the mouth during chewing. Therefore, it is necessary to establish a method to describe its TIRT.

It is found through fitting calculation that the sensory intensity and retention time are linearly fitted ($y = kx + b$), and the fitting results are shown in Table 3. Thus, the slope k value can be easily obtained. The meaning of k value is that the greater the absolute value of k, the shorter the taste retention time. Therefore, it is necessary to establish a function to characterize its retention time. Since the highest taste intensity is 10, the maximum value of the slope (k) is also 10 (the first time point is 0, the second time point is 10, so $0 < |k| \langle 10$). There are eight time points in the experiment, and the maximum value is 8. Using the above data, the conversion function $f(x) = -0.8x + 8$ can be obtained. Here, we introduce its retention time coefficient K to characterize the taste retention time, the formula is as follows:

$$K = f(x) = -0.8|k| + 8$$

K represents the TIRT coefficient of 5 tastes, which is defined as: bitterness (K_B), astringency (K_A), sourness (K_S), aftertaste-sweetness (K_{AS}) and stimulate saliva secretion (K_{SS}). 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, and the rest are discarded. As can be seen from Fig. 2 A, this rule applies to sourness, bitterness and astringency, so the TIRT coefficient K and R^2 can be directly obtained ($k < 0$, R^2 greater than 0.800). However, the intensity of salivary secretion and aftertaste-sweetness increased first and then decreased. Therefore, they have two K values. K_{AS1} and K_{SS1} represent the rising stage of taste, and K_{AS2} and K_{SS2} correspond to the falling stage (Table. 3). 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 index T of each taste. The aftertaste-sweetness and saliva secretion are slightly complex. K_{AS1} and K_{SS1} is multiplied by the sum of taste rising stages, K_{AS2} and K_{SS2} is multiplied by the sum of taste falling stages. The two are added to get the T value. The calculation formula is as follows:

$$\text{Bitterness, astringency, sourness}: T = \sum_{1}^{n} SI \times K_{A,B,S}$$

$$\text{Sweetnessandsalivasecretion}: T = \sum_{1}^{n1} SI1 \times K_1 + \sum_{n1}^{n2} SI2 \times K_2$$

n is the number of time points in the evaluation process; SI_1, n_1 represents the rising stage of taste; SI_2, n_2 is the falling stage. After calculating the T values of 5 taste senses of 12 PE batches, cluster analysis and PCA analysis were performed, and the results are shown in Fig. 2 B. According to the PCA results (Fig. 2 A $R^2 = 0.804$), cultivated and wild PE can be clearly distinguished on the comprehensive taste index, indicating that the model has better discriminative ability. Due to the large difference in the wild environment, it can be clearly found from the PCA result that the dispersion degree of the comprehensive taste index of wild PE is significantly greater than that of the cultivated PE, which indicates that the internal flavor quality of the samples is quite different. In addition, according to the loading scatter plot (Fig. 2 C), cultivated PE is most affected by the aftertaste sweetness, while wild PE has strong bitterness, acidity and astringency, which shows that the aftertaste sweetness intensity is an important factor to distinguish their taste. As the unique flavor of PE, aftertaste sweetness is widely welcomed by consumers. Many related beverages use it as a means of sales promotion. From the results, the reason why cultivated PE can be widely popular also lies in its stronger effect of returning sweet. From the cluster heat map of the comprehensive taste index in Fig. 2 D, it can be found that wild and cultivated can be divided into two groups, further indicating that the model has good discrimination ability. The data will be further correlated with key compounds to screen potential compounds that affect the overall taste of PE.

3.2. HS-SPME/GC-QQQ-MS/MS result

The unique aroma of PE is one of its sensory characteristics. Experienced suppliers will judge its quality according to the strength of its aroma. At present, there is no research on the chemical nature of PE aroma. Based on HS-SPME/GC-QQQ-MS/MS odor analysis technology, combined with retention time, parent ion and MRM monitoring ion pair and built in calibration curve, it can accurately and rapidly identify 150 odorants and analyze their concentration. This paper established a rapid method to identify the chemical components in the special odor of PE, and explored the key differences in the odor components of different types of PE.

Odor threshold refers to the minimum concentration of substances causing human olfaction (pg/mg). The performance of the odor components in PE is not entirely determined by the content, but also the threshold of the components. The threshold indicates the lowest concentration at which the human body can smell the substance. The ratio of the concentration to the threshold is called the odor activity values (OAV):

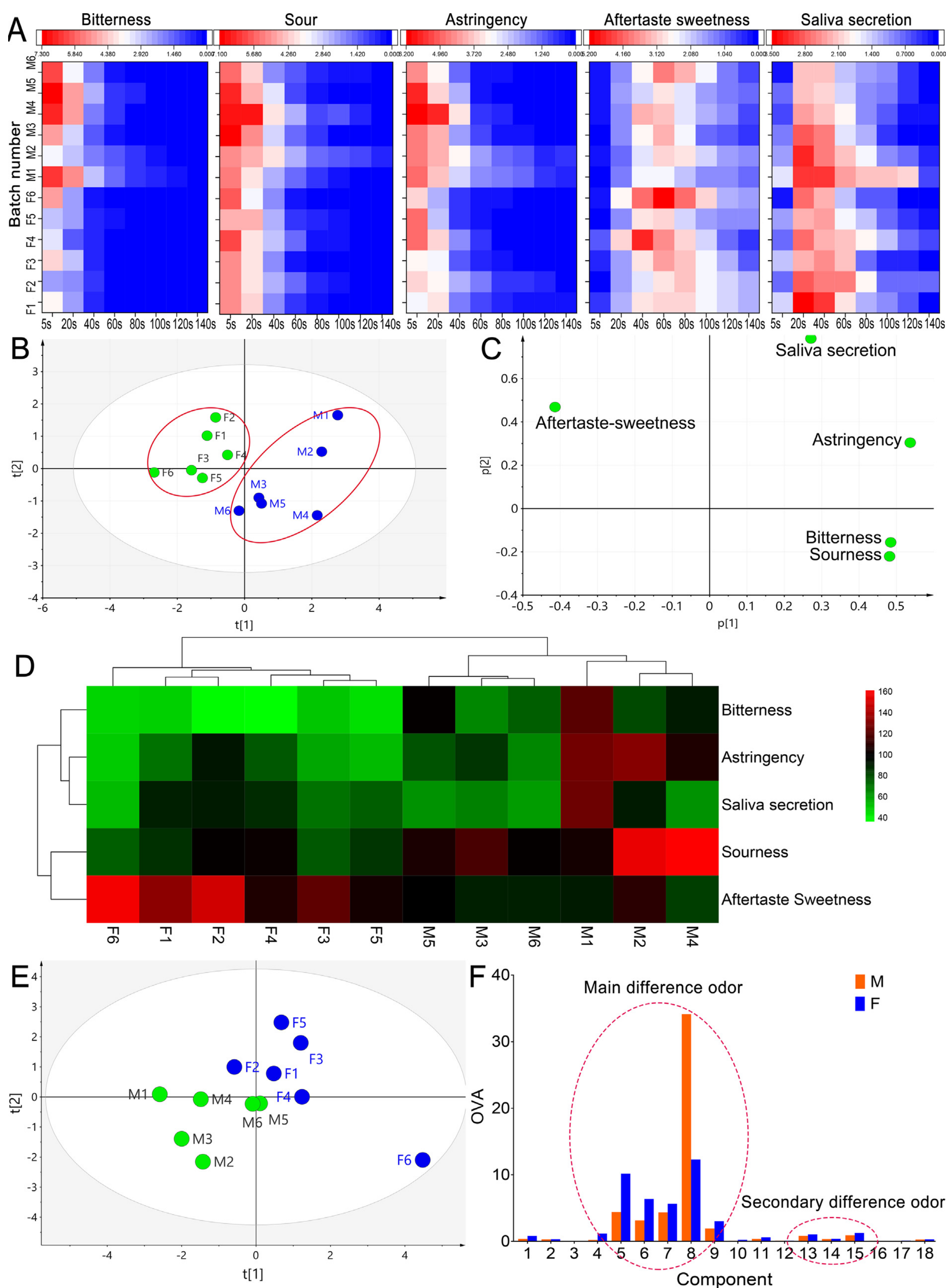

Fig. 2 Temporal dominant description evaluation analysis and odor component analysis results, (A) the heatmap of average abundance for the volunteer's VAS scores at 8 time points, (B) PCA result of comprehensive taste index, (C) loading scatter plot of PCA, (D) clustering heat map of T value, (E) PAC analysis result, (F) comparison result of odor intensity mean value of each component.

Table 3 K and linear fitting R^2 of 5 sensory.

Batch	K_B	R^2	K_S	R^2	K_A	R^2	K_{AS1}	R^2	K_{AS2}	R^2	K_{SS1}	R^2	K_{SS2}	R^2
F1	6.760	0.997	6.792	0.979	6.984	0.970	7.324	0.939	7.640	0.999	6.400	1.000	7.509	0.944
F2	7.360	0.923	7.120	0.938	7.408	0.976	7.408	0.907	7.656	0.936	6.400	1.000	7.600	0.822
F3	6.560	0.991	6.712	0.995	7.216	0.940	7.144	0.977	7.472	0.977	7.520	0.871	7.280	0.871
F4	6.920	0.928	6.872	0.984	6.840	0.989	6.42	0.988	7.311	0.993	6.400	1.000	7.696	0.968
F5	7.120	0.962	6.520	0.968	6.400	0.986	7.36	0.82	7.688	0.848	7.480	0.807	7.560	0.992
F6	6.120	0.988	6.584	0.994	6.840	0.999	6.704	0.972	7.256	0.955	6.000	1.000	7.416	0.976
M1	6.992	0.964	7.084	0.983	7.465	0.924	7.06	0.999	7.736	0.955	6.800	1.000	7.676	0.792
M2	7.440	0.977	7.468	0.948	7.429	0.969	7.24	0.9923	7.656	0.973	6.320	1.000	7.595	0.992
M3	6.736	0.971	6.712	0.998	6.920	0.964	7.088	0.987	7.432	0.902	6.480	1.000	7.504	0.968
M4	6.384	0.910	7.083	0.841	6.528	0.940	6.744	0.989	7.5808	0.981	6.480	1.000	7.624	0.965
M5	6.256	0.996	6.696	0.982	6.592	0.998	7.344	0.928	7.656	0.928	6.800	1.000	7.712	0.952
M6	6.416	0.982	6.976	0.951	6.872	0.985	6.520	0.999	7.4928	0.986	6.480	1.000	7.704	0.907

$$\mathrm{OVA} = \frac{F}{M * T}$$

In the formula: OAV is the odor component intensity; F represents the content of the component (pg); M represents sample mass(mg); T represents the odor threshold of the component (pg/mg). Generally, the components with OAV value greater than 0.1 should be regarded as the components with obvious contribution to their odor, which can affect the flavor of samples in varying degrees. According to the above formula, all batches of PE lyophilized-dried powder were analyzed, and 18 components with OAV greater than 0.1 were found. Their content and OAV are listed in Table 4 and Table 5 respectively.

PCA analysis showed that the odor components of wild and cultivated PE could be clearly distinguished (Fig. 2 E). The OAV values of 18 kinds of odor components were compared in pairs (Fig. 2 F). When OAV $\geq$ 1, it indicates that it is a key odor substance, while 0.1 $\leq$ OAV $<$ 1 indicates that it plays an important role in changing the overall odor of the sample. The difference components were screened by the following principles: 1. The average OAV value of odor was greater than 0.1; 2. The OAV of odor component was significantly higher than that of other groups; 3. The average value of group F was significantly different from that of group M. Based on the above principles, it was found that No.4–9 and No.13–15 compositions are the substances with the highest odor contribution and the maximum difference in two groups. According to the OVA, it can be divided into the Main and second difference odor components (Fig. 2 F).

Although hundreds of compounds have been identified in PE, only a few compounds actually contribute to sensory of PE flavor. From the results of Fig. 2 F, most of the aroma components are pleasant in cultivated PE, and the OAV is higher than wild PE. For example, *trans*-2-heptenal has a greasy, fresh, and fruity fragrance, and decanal has an orange peel fragrance. But cultivated PE also mixed with some bad smell, such as dimethyl trisulfide, which described as smelling like cabbage. It should be noted that the OAV of compound 8 in wild PE is much higher than that in cultivated PE. Compound 8, 2-Isobutyl-3-methoxypyrazine (IBMP) has a strong aroma of soil, spices and green pepper, which has a great impact on the overall flavor of PE. IBMP is very common in the food industry, especially in wine and beverage, which will have an adverse effect on the overall flavor of the product (Ling, Zhou, & Lan, 2021). IBMP accumulates during the early growth of fruits, and the content will be affected by photodegradation after the maturity period and the content will decrease rapidly (Sidhu, Lund, Kotseridis, & Saucier, 2015). In the process of fruit ripening, external factors such as climate, environmental conditions and canopy dressing will affect the light and temperature conditions, thus affecting the content of IBMP in the fruit (Gregan et al., 2012); Pickering, Karthik, Inglis, Sears, & Ker, 2008). For example, high temperature may cause the accumulation of IBMP in PE (Lei, Xie, Guan, Song, Zhang, & Meng, 2018). Artificial cultivation and climate change may be an important reason for the great difference of IBMP content between wild and cultivated PE. Therefore, IBMP may be a potential quality marker to distinguish wild PE from cultivated PE. Dimethyl trisulfide, another odor substance with high content in cultivated PE, has a certain meat flavor, which is not conducive to the flavor of PE. According to previous reports, dimethyl trisulfide may come from Strecker degradation of cysteine and methionine(Cho, Roman, Yeboah, & Konishi, 2007). In addition, No. 13, No. 14 and No. 15 compounds have a low contribution rate, but they can change the overall smell of PE and give it a unique aroma. The combination of these odor components makes the overall smell of PE very special, and some people describe cultivated PE as having an apple-like aroma. In short, the smell of cultivated PE is more pleasant. In brief, the smell of cultivated PE is more likely to be pleasant.

3.3. Mass imaging result

MS imaging technology provides a visual method for the characterization of metabolites in PE tissue, which can deeply reveal the component distribution and metabolic differences between wild and cultivated PE from the level of spatial distribution, and provide valuable information for the inner quality and the study of metabolic differences. In the experiment, the compositions were identified based on the literature and HMDB database (Yang, Kortesniemi, Liu, Karonen, & Salminen, 2012). Table.6 shows the main compounds identified in the MS imaging results (Cultivated PE on the left).

3.3.1. Distribution characteristics of organic acids

Organic acids are another kind of important secondary metabolites in plants. They play an important role in plant

Table 4 The basic information of eighteen selected compounds above the threshold.

Serial number	Compounds	Molecular formula	Odor description	Threshold *T* (pg/mg)	Retention time (min)	Content *F* (pg)											
						M1	M2	M3	M4	M5	M6	F1	F2	F3	F4	F5	F6
1	2,3-butanedione	$C_4H_6O_2$	Butter scent	10.00	2.844	1928.005	0.000	0.000	4014	2405.856	2943.299	1507.128	5908.682	6829.979	2637.664	4205.740	3452.763
2	Valeraldehyde	$C_5H_{10}O$	Almond flavor, spicy flavor, malt flavor	100.00	2.904	15148.190	25988.630	8497.559	16062.26	0.000	20015.940	13592.03	20811.450	0.000	25019.49	0.000	34593.370
3	α-pinene	$C_{10}H_{16}$	Solvent smell	10.00	3.498	850.857	367.991	198.740	149.664	21.54	171.937	34.682	45.751	58.174	666.949	26.248	559.498
4	Octanal	$C_8H_{16}O$	Sharp and powerful aromas of green and pungent fat and wax, with fruity and jasmine flavor	100.00	9.503	3051.376	20583.17	12610.7	5813.635	10972.24	7764.241	9344.215	6202.305	13409.99	89479.16	8467.164	218757.800
5	Trans-2-Heptenal	$C_7H_{12}O$	Fatty, soap, almond	10.00	10.060	0.000	0.000	0.000	0.000	106189.5	26076.04	52622.45	0.000	65300.28	0.000	24586.04	162342.9
6	Dimethyl trisulfide	$C_2H_6S_3$	Cabbage, fish, sulfur	0.10	11.064	13.558	143.95	133.921	27.476	245.869	374.392	237.499	196.927	160.608	463.967	170.419	679.289
7	Decanal	$C_{10}H_{20}O$	Soap, waxy, orange peel	1.00	13.093	793.008	3282.745	2442.16	2087.352	2636.374	1804.386	3192.379	1659.468	3749.547	2355.544	2295.315	3641.778
8	2-isobutyl-3-methoxypyrazine	$C_9H_{14}N_2O$	Earthy, spice, green pepper	0.01	13.443	30.002	551.732	266.862	49.027	58.394	68.652	83.193	34.823	94.688	6.061	136.472	13.675
9	Trans-2-Nonenal	$C_9H_{16}O$	Papery	1.00	13.584	554.872	1998.556	1164.004	371.951	1063.255	662.602	2327.227	455.993	2038.206	725.823	1968.747	1564.791
10	Linalool	$C_{10}H_{18}O$	Floral, lavender	10.00	13.739	650.754	364.046	433.588	209.754	192.378	434.326	909.033	276.518	297.109	1915.447	714.896	2467.23
11	2-Methylisoborneol	$C_{11}H_{20}O$	Earthy, musty	0.10	14.400	18.36	0.000	0.000	0.000	0.000	92.961	74.801	17.431	0.000	0.000	93.094	0.000
12	Dodecanal	$C_{12}H_{24}O$	Fatty aroma, with strong aroma similar to pine leaf oil and orange oil	10.00	15.958	0.000	608.232	377.437	615.942	562.941	511.419	649.855	435.348	498.888	323.552	489.484	369.019
13	Methyl salicylate	$C_8H_8O_3$	Mint flavor	1.00	16.718	1121.976	130.924	180.853	359.083	534.918	165.117	101.29	131.404	170.336	66.625	2582.055	105.118
14	Geraniol	$C_{10}H_{18}O$	Geranium aroma, rose aroma	1.00	17.556	56.243	255.276	198.44	265.569	59.385	200.793	27.766	22.959	65.366	46.843	226.662	752.436
15	Beta-Ionone	$C_{13}H_{20}O$	Aroma of violets, raspberries, seaweed	0.10	18.633	9.461	60.156	36.595	25.236	64.355	75.336	48.716	32.381	62.491	78.009	62.867	92.292
16	Heptanoic acid	$C_7H_{14}O_2$	Green, orange, soap, gasoline	10.00	18.689	0.000	681.212	579.43	0.000	530.774	472.729	518.78	561.254	573.792	0.000	0.000	487.891
17	1-Dodecanol	$C_{12}H_{26}O$	Waxy aroma	1.00	18.943	0.000	0.000	0.000	95.009	112.87	0.000	0.000	118.662	0.000	108.78	0.000	80.144
18	M−Cresol	C_7H_8O	Plastic, fecal smell	0.10	20.151	9.885	22.678	15.058	0.000	18.816	12.419	6.844	14.997	23.718	9.99	18.798	11.589

Table 5 OVA of 12 batches of PE.

Serial number	Component	M1	M2	M3	M4	M5	M6	Average	F1	F2	F3	F4	F5	F6	Average
1	2,3-butanedione	0.386	0.000	0.000	0.803	0.481	0.589	0.376	0.301	1.182	1.366	0.528	0.841	0.691	0.818
2	Valeraldehyde	0.303	0.520	0.170	0.321	0.000	0.400	0.286	0.272	0.416	0.000	0.500	0.000	0.692	0.313
3	α-pinene	0.170	0.074	0.040	0.030	0.004	0.034	0.059	0.007	0.009	0.012	0.133	0.005	0.112	0.046
4	Octanal	0.061	0.412	0.252	0.116	0.219	0.155	0.203	0.187	0.124	0.268	1.790	0.169	4.375	1.152
5	Trans-2-Heptenal	0.000	0.000	0.000	0.000	21.238	5.215	4.409	10.524	0.000	13.060	0.000	4.917	32.469	10.162
6	Dimethyl trisulfide	0.271	2.879	2.678	0.550	4.917	7.488	3.131	4.750	3.939	3.212	9.279	3.408	13.586	6.362
7	Decanal	1.586	6.565	4.884	4.175	5.273	3.609	4.349	6.385	3.319	7.499	4.711	4.591	7.284	5.631
8	2-Isobutyl-3-methoxypyrazine	6.000	110.346	53.372	9.805	11.679	13.730	34.156	16.639	6.965	18.938	1.212	27.294	2.735	12.297
9	Trans-2-Nonenal	1.110	3.997	2.328	0.744	2.127	1.325	1.938	4.654	0.912	4.076	1.452	3.937	3.130	3.027
10	Linalool	0.130	0.073	0.087	0.042	0.038	0.087	0.076	0.182	0.055	0.059	0.383	0.143	0.493	0.219
11	2-Methylisoborneol	0.367	0.000	0.000	0.000	0.000	1.859	0.371	1.496	0.349	0.000	0.000	1.862	0.000	0.618
12	Dodecanal	0.000	0.122	0.075	0.123	0.113	0.102	0.089	0.130	0.087	0.100	0.065	0.098	0.074	0.092
13	Methyl salicylate	2.244	0.262	0.362	0.718	1.070	0.330	0.831	0.203	0.263	0.341	0.133	5.164	0.210	1.052
14	Geraniol	0.112	0.511	0.397	0.531	0.119	0.402	0.345	0.056	0.046	0.131	0.094	0.453	1.505	0.381
15	Beta-Ionone	0.189	1.203	0.732	0.505	1.287	1.507	0.904	0.974	0.648	1.250	1.560	1.257	1.846	1.256
16	Heptanoic acid	0.000	0.136	0.116	0.000	0.106	0.095	0.075	0.104	0.112	0.115	0.000	0.000	0.098	0.071
17	1-Dodecanol	0.000	0.000	0.000	0.190	0.226	0.000	0.069	0.000	0.237	0.000	0.218	0.000	0.160	0.103
18	M−Cresol	0.198	0.454	0.301	0.000	0.376	0.248	0.263	0.137	0.300	0.474	0.200	0.376	0.232	0.286

energy metabolism and plant response to external stresses. For example, malic acid is widely involved in photosynthesis and respiration of plants; citric acid, a common plant organic acid, has biological activities such as oxidation resistance and antibacterial activity (Chang, Foo, Loh, Lim, & Abdul Mutalib, 2020). In the mass imaging experiment, 9 organic acids and their distribution characteristics were identified. Among them, aconitic acid, citric acid, malic acid and fumaric acid are the typical components involved in the tricarboxylic acid cycle. As shown in Fig. 3, the contents of all 6 organic acids content are lower in the core and fruit compartment diaphragm (the obvious black lines in the fruit in Fig. 3), but increased significantly near the epidermis. This suggests that the metabolism of tricarboxylic acid in PE may be more vigorous in the tissue structure close to the epidermis. In particular, citric acid and malic acid are the most significant, their content in the epidermis is extremely high, which makes the sour substances preferentially released during the chewing process, causing their sour taste to be prominent. In addition, it is not difficult to find that the content of citric acid and malic acid in this two PEs is significantly different. The content of cultivated PE is lower and even distribution. This is one of the main reasons why the wild tastes extremely sour and difficult to eat.

3.3.2. Distribution characteristics of Tannin and Phenolic acid

Tannin is the second major component in PE fresh fruits, and its highest content can reach 30%-40% of its dry weight (Huang, Ran, et al., 2021), which has strong antioxidant, anti-tumor, antibacterial and anti-hyperglycemic activities (Variya, Bakrania, & Patel, 2016). Like organic acids, they are mainly distributed near the epidermis. From Fig. 4, the distribution of tannins in the two PEs are obviously different, such as ellagic acid, malic acid gallate, mucic acid lactone gallate and galloylglucose.

Tannins are important secondary metabolites produced by plants in response to the growth environment. Wild PE grows in a harsh environment of high altitude, drought, heat, and less rain; while-cultivated PE generally grows in a warm and humid low-altitude environment. In the hot and dry environment, plants produce a lot of tannins as defense substances to resist external invasion and prevent the loss of water and other nutrients. Under dry heat stress, plants will promote phenylpropane biosynthesis and flavonoid biosynthesis, and make polyphenols and flavonoids accumulate. They can effectively resist the damage caused by dry heat and ultraviolet radiation, which is the performance of plants against external stress (Kumar et al., 2016). The wild PE epidermis is directly affected by solar radiation and drought, leading to more active metabolism and more accumulation of metabolites. This may explain why it is highly distributed in epidermis (Chen, Liu, Cui, Lu, Wang, Wu, et al., 2018; Morris, Carter, Hauck, Lanot, Theodorou, & Allison, 2021).

3.3.3. Distribution characteristics of nutrients

The nutrition of PE is one of the important properties of its edible value. It not only has high health benefits, but also affects its taste. In the experiment, 8 kinds of nutrients were identified (Fig. 5), including saccharides, vitamins, amino acids and fatty acids. It can be clearly seen from Fig. 5 that the nutrient content of cultivated PE is generally higher, indicating that

Table 6 Desorption electrosprayionization-mass spectrometry imaging (DESI-MSI) component identification results.

No	Compound (Or isomers)	Classification	Molecular formula	Theoretical (m/z)	Measured (m/z)	Molecular ion	Error (ppm)	Intensity
1	Malonic acid	Organic acid	C3H4O4	103.0031	103.0036	$[M-H]^-$	4.8	432
2	Citric acid (or glutamate)	Organic acid	C5H6O4	111.0082	111.0087	$[M-H_2O]^-$	4.5	1106
3	Glutaric acid	Organic acid	C5H8O4	113.0238	113.0239	$[M-H_2O]^-$	0.9	352
4	Fumaric acid	Organic acid	C4H4O4	115.0031	115.0037	$[M-H]^-$	4.9	1473
5	Malic acid	Organic acid	C4H6O5	133.0137	133.0142	$[M-H]^-$	3.7	4904
6	Glutamic acid	Amino acid	C5H9NO4	146.0453	146.0458	$[M-H]^-$	3.4	291
7	Gallic acid	Phenolic acid	C7H6O5	169.0137	169.0143	$[M-H]^-$	3.5	646
8	Aconitate	Organic acid	C6H6O6	173.0086	173.0092	$[M-H]^-$	3.4	421
9	Vitamin C	Vitamin	C6H8O6	175.0248	175.0242	$[M-H]^-$	0.0	4344
10	Glucose	Saccharides	C6H12O6	179.0555	179.0560	$[M-H]^-$	2.8	1056
11	Citric acid	Organic acid	C6H8O7	191.0191	191.0196	$[M-H]^-$	2.6	17,827
12	Mucic acid	Organic acid	C6H10O8	209.0297	209.0303	$[M-H]^-$	2.8	14,209
13	Glucose(Or fructose, etc)	Saccharides	C6H12O6	215.0322	215.0327	$[M + Cl]^-$	2.3	2972
14	linoleic acid	Fatty acid	C18H32O2	279.2324	279.2327	$[M-H]^-$	1.1	1410
15	Oleic acid	Fatty acid	C18H34O2	281.2481	281.2485	$[M-H]^-$	1.4	2571
16	Malic acid gallate	Phenolic acid	C11H10O9	285.0246	285.0256	$[M-H]^-$	3.5	714
17	Ellagic acid	Phenolic acid	C14H6O8	300.9984	300.9990	$[M-H]^-$	1.9	758
18	Galloylglucose	Phenolic acid	C13H16O10	331.0665	331.0669	$[M-H]^-$	1.2	7998
19	Sucrose	Saccharides	C12H22O11	341.1083	341.1083	$[M-H]^-$	0	461
20	Mucic acid lactone gallate	Phenolic acid	C13H12O11	343.0301	343.0303	$[M-H]^-$	0.5	6110
21	Ellagic acid	Phenolic acid	C14H6O8	347.0039	347.0051	$[M + FA-H]^-$	3.4	403
22	2-O-Galloylgalactaric acid	Phenolic acid	C13H14O12	361.0407	361.0409	$[M-H]^-$	0.5	17,968
23	Vitamin B	Vitamin	C16H20O9	377.0853	377.0854	$[M + Na-2H]^-$	0.2	1670
24	Chebulic acid	Tannin	C14H12O11	355.0302	355.0321	$[M-H]^-$	5.3	286
25	Ellagic acid pentose	Phenolic acid	C19H14O12	433.0407	433.0407	$[M-H]^-$	0	347
26	Epicatechin gallate	Flavonoids	C22H18O10	441.0822	441.0821	$[M-H]^-$	−0.2	203
27	Ellagic acid hexose	Tannin	C20H16O13	463.0513	463.0518	$[M-H]^-$	1.1	475
28	Digalloylglucose	Tannin	C20H20O14	483.0775	483.0782	$[M-H]^-$	1.5	925
29	Mucic acid lactone digallate	Phenolic acid	C20H16O15	495.0411	495.0423	$[M-H]^-$	2.4	497
30	Corilagin	Tannin	C27H22O18	633.0728	633.0732	$[M-H]^-$	0.6	254
31	Trigalloylglucose	Tannin	C27H24O18	635.0885	635.0876	$[M-H]^-$	−1.4	241
32	Digalloyl-HHDP-Glucose	Tannin	C34H26O22	785.0838	785.0836	$[M-H]^-$	−0.2	259
33	Chebulagic acid	Tannin	C41H30O27	953.0896	953.0898	$[M-H]^-$	0.2	220

it is rich in nutrients and more suitable for consumption. For example, the content of vitamins and sugars is higher, which can make the sweetness and aftertaste-sweetness of cultivated PE better. Surprisingly, the distribution of suspected fatty acids was found in the epidermis, which was usually found in the seed nucleus. Based on the above results, most of the components of PE are higher in the part near the epidermis, and from the perspective of the spatial distribution and content of the components, it explains the generation of the taste level of PE and the reason why cultivated PE tastes better than wild PE.

3.4. Metabolomics and taste correlation analysis

In order to further reveal the chemical differences and biological background between wild and cultivated PE, metabonomics method based on UPLC-QTOF-MS was used to study the main differences of their metabolic pathways. The quality control samples (QCS) were prepared by mixing the reference materials. QC samples gathered together showed that the repeatability of the system was good, and the data can be further studied. Through OPLS-DA analysis, it was found that there were significant differences in chemical composition and metabolites between group F and group M in negative ion mode ((Fig. 6 A $R^2Y = 0.99$, $Q^2 = 0.73$). To explore the potential variables, S-plot was carried out. In S-plot (Fig. 6 B), the farther the variable deviates from the origin, the higher the value of the variable importance plot obtains. According to the accurate m/z and fragment characteristics (Ion fragment prediction matching) of the ion characteristic metabolites, and compared with the reference substance, a total of 33 characteristic metabolites were identified in the sample (Fig. 6 G). The results showed that the contents of tannins and flavonoids in wild PE were significantly higher than those in cultivated PE, which may be an important reason for the taste difference. Furthermore, the differential marker data were imported into metaboanalyst 5.0 for pathway enrichment (Fig. 6 C).

The difference markers were marked as HMDB-ID, and imported into MetaboAnalyst5.0 for pathway analysis. The result (Fig. 6 C) shows that the selected metabolites mainly

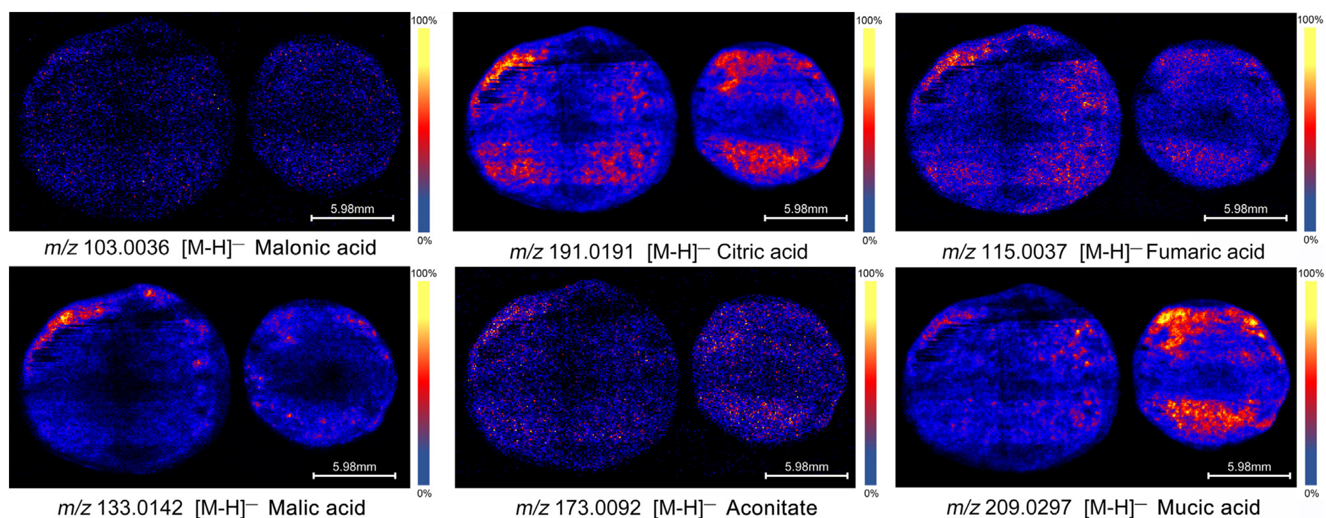

Fig. 3 Distribution characteristics of main organic acids in PE tissue.

m/z 169.0143 [M-H]⁻ Gallic acid
m/z 285.0256 [M-H]⁻ Malic acid gallate
m/z 300.9990 [M-H]⁻ Ellagic acid
m/z 331.0665 [M-H]⁻ Galloylglucose
m/z 343.0303 [M-H]⁻ Mucic acid lactone gallate
m/z 355.0321 [M-H]⁻ Chebulic acid
m/z 463.0518 [M-H]⁻ Ellagic acid hexose
m/z 483.0782 [M-H]⁻ Digalloylglucose
m/z 495.0423 [M-H]⁻ Mucic acid lactone digallate
m/z 633.0732 [M-H]⁻ Corilagin
m/z 635.0876 [M-H]⁻ Trigalloylglucose
m/z 953.0898 [M-H]⁻ Chebulagic acid

Fig. 4 Distribution characteristics of tannin and phenolic acid.

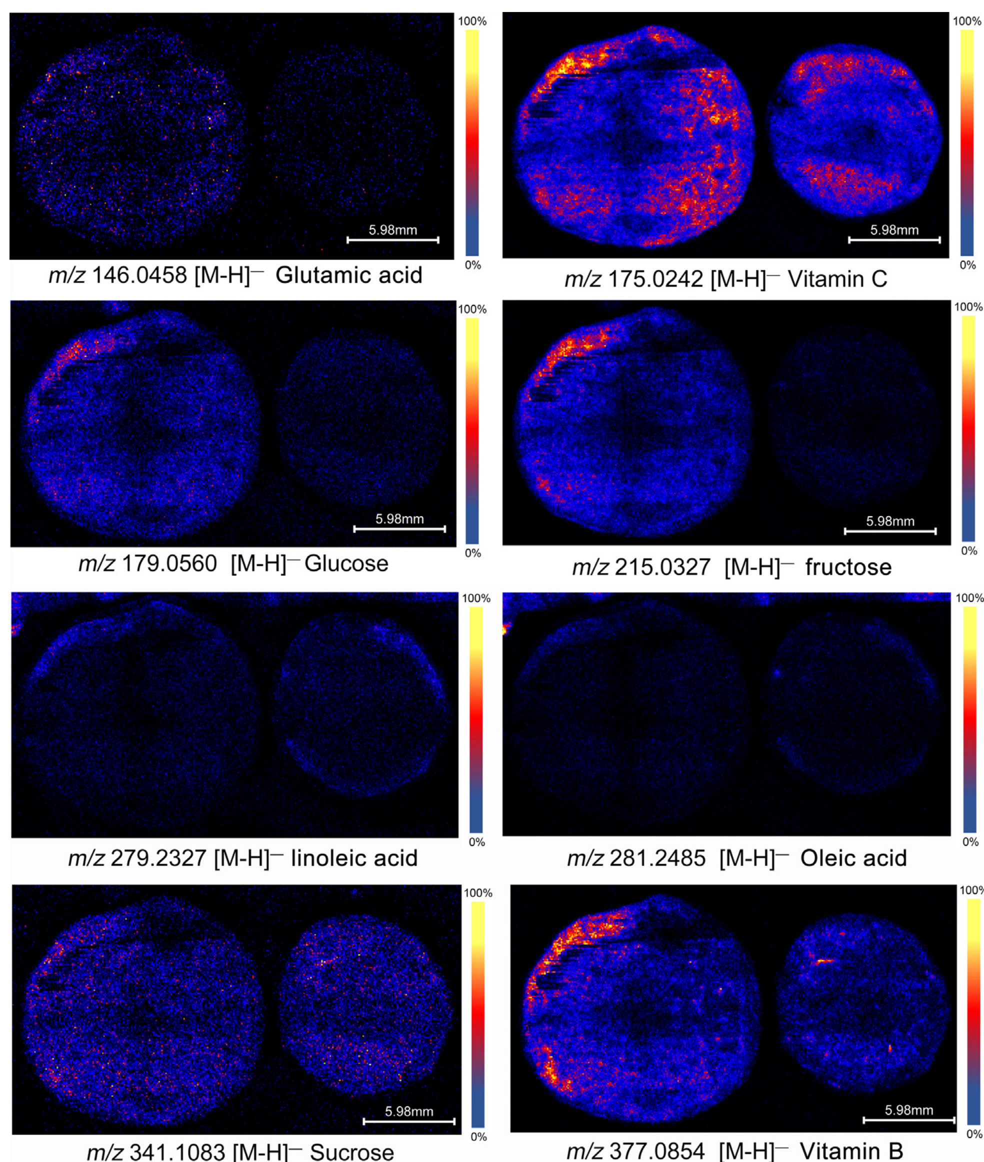

Fig. 5 Distribution characteristics of nutrients.

involve flavone and flavonol biosynthesis, flavonoid biosynthesis, histidine metabolism, arginine biosynthesis, pentose phosphate pathway, carbon fixation in photosynthetic organisms, pyrimidine metabolism, cysteine and methionine metabolism. Among them, the enrichment pathway with $p < 0.05$ was the flavone and flavonol biosynthesis, and flavonoid biosynthesis. Obviously, the generation of this metabolic difference is closely related to its different planting geographical environment. The results showed that the accumulation of phenolic metabolites of wild PE was significantly higher than that of cultivated PE, and the former grew in drought. Phenolic accumulation is very crucial to counteract the negative impacts of drought stress in plants(Ali, Ganai, Kamili, Bhat, Mir, Bhat, et al., 2018). Transcriptomic and metabolomic studies carried out on Arabidopsis plants confirmed that increased flavonoid accumulation under drought stress is very helpful to provide resistance. Accumulation and Biosynthesis of flavonols were also stimulated in plants under water deficit conditions accompanied by enhanced resistance against drought stress (Nakabayashi, Yonekura-Sakakibara, Urano, Suzuki, Yamada, Nishizawa, et al., 2014). For example, contents of flavonoids like kaempferol and quercetin were enhanced in tomato plants accompanied by enhanced drought tolerance (M. Li, Li, Zhang, Li, Gao, Ai, et al., 2018; Sánchez-Rodríguez, Moreno, Ferreres, Rubio-Wilhelmi Mdel, & Ruiz, 2011).

It is well known that plants growing in high altitude areas accumulate more flavonoid phenolics than plants in temperate regions. Under high light/UV radiation, the enhancement of flavonoid accumulation is due to the stimulated flavonoid biosynthesis pathway and its corresponding gene transcription level (Sharma, Shahzad, & Rehman, 2019). Flavonoids also act as light screens due to their capability of absorbing both visible (anthocyanins) and UV radiations (anthocyanins and colorless flavonoids), hence protecting plants from these harm-

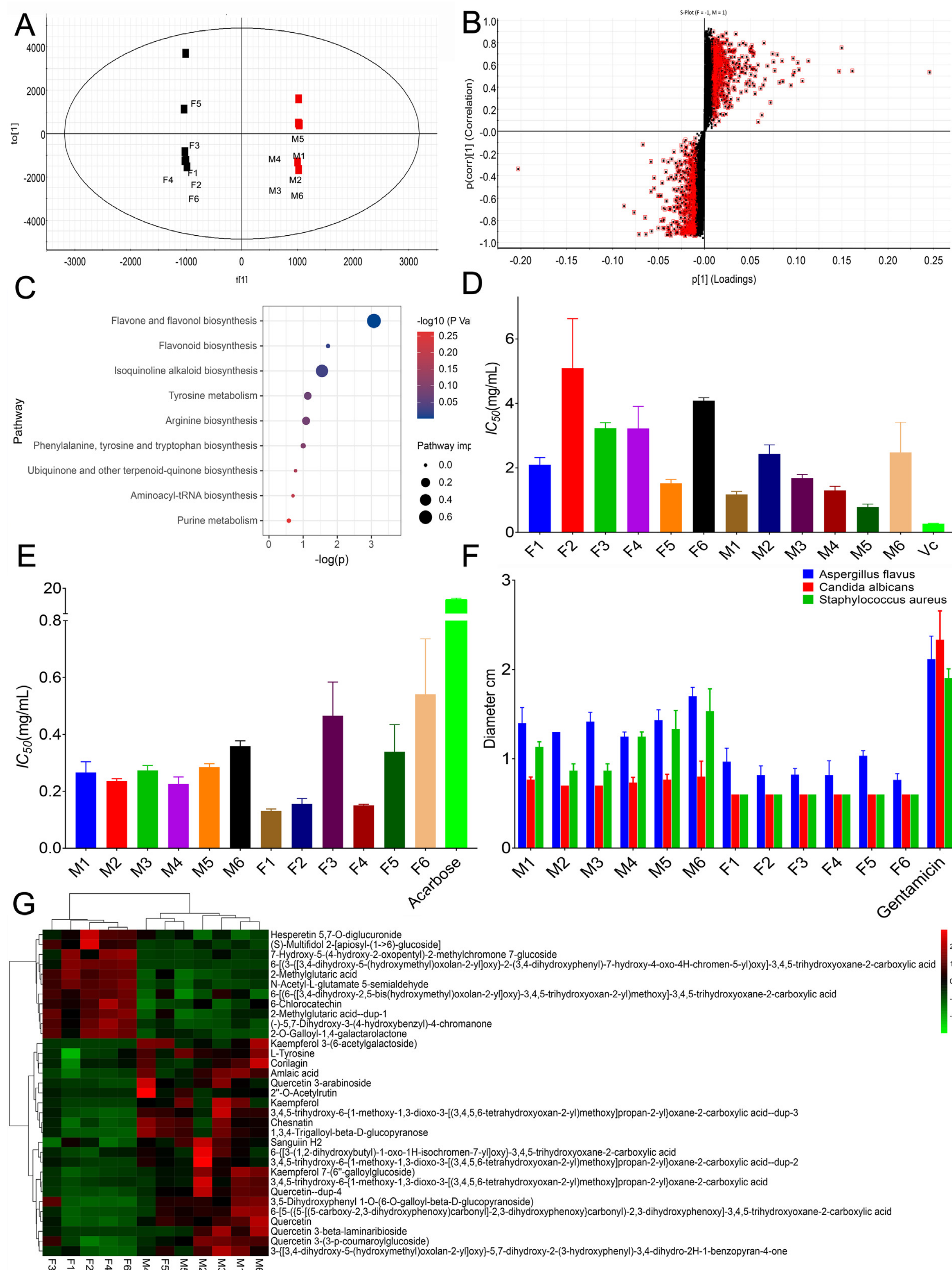

Fig. 6 Results of OPLS-DA(A), S-plot(B), pathway enrichment analysis bubble plot (C), antioxidant activities (D), anti-hyperglycemic activities (E), antibacterial activity (F), heat maps of differential markers (G).

ful radiations (Agati, Brunetti, Di Ferdinando, Ferrini, Pollastri, & Tattini, 2013). This also explains why mass spectrometry imaging shows higher levels of secondary metabolites near the epidermis of wild PE. However, the enhanced synthesis of flavonoids and flavanols will increase their bitterness and worsen the taste, which is a phenomenon that should be avoided in beverages.

In order to further clarify the relationship between different compounds and taste, reveal the chemical properties of their taste, and provide a reference for quality control, this paper explored the correlation between differential markers and comprehensive taste index, and initially screens some quality markers that affect taste. Pearson correlation analysis was used in the data processing. It can be found that bitterness substances are closely related to tannins and flavonoids, such as quercetin (0.62), amlaic acid (0.76) and kaempferol (0.62). This further illustrates that the difference in biosynthesis of flavonoids and flavanols is the cause of the difference in flavor. The sourness and astringency are related to tannins such as sanguiin H2 (0.67) and amlaic acid (0.64). The formation of aftertaste sweetness is more complicated, and it has been reported that it is related to the catechins EC and EGC (Zhang, Yin, Chen, Wang, Du, Jiang, et al., 2016). But at present, the findings in this paper are related to some amino acid derivatives and glycosides, such as hesperetin 5,7-O-diglucuronide (0.64) and N-Acetyl-L -glutamate 5-semialdehyde (0.84). It is worth noting that amlaic acid is relevant to all bad tastes, which may be an important potential flavor quality marker. In addition, quercetin and related derivatives can also be used as quality markers that affect flavor.

3.5. Activity evaluation results

As a dietary supplement and functional beverage, PE has higher health benefits, which is due to its strong antioxidant activity and hypoglycemic effect. The anti-hyperglycemic activity of PE is mainly manifested in its anti-hyperglycemic activity α Inhibition of glucosidase (Majeed, Majeed, & Mundkur, 2020). Therefore, the antioxidant and anti-hyperglycemic activities of two PEs were determined respectively to clarify their difference in activity. As shown in Fig. 6 D, due to the different content of polyphenols, flavonoids and organic acids, the antioxidant activity of wild PE was significantly stronger than cultivated PE ($p < 0.05$). And surprisingly, both wild and cultivated PE in Fig. 6.E showed strong anti-hyperglycemia ability compared with the positive group. Although there are certain differences in activity within the cultivated group, there is no significant difference between the wild and cultivated groups. The antibacterial experiment uses the ultrasonic extract of freeze-dried powder (12 times water, commonly used amount) as the sample solution, which is the commonly used extraction dose of PE in the beverage industry. The results (Fig. 6 F) showed that the antibacterial activity of wild PE was significantly stronger than cultivated PE, and the latter had almost no other antibacterial effect except Aspergillus flavus. This may be closely related to the accumulation of secondary metabolites such as flavonoids and tannins. In addition, because the experiment used a water extract, which made many lipophilic tannins and flavonoids with strong antibacterial activity difficult to dissolve in it, limiting their biological activity (Liu, Zhao, Luo, Yang, & Jiang, 2009). Based on the above results, wild PE has better biological activity.

4. Conclusion

The research shows that cultivated PE has a lower IBMP, a kind of green pepper flavor that destroys the flavor of the foods and beverages. The aftertaste-sweetness is the biggest flavor characteristic of PE, followed by bitterness and astringency, which can directly affect the preference of consumers. In addition, the flavor quality of wild PE is quite different, which makes it difficult to control the quality of the final product. The component distribution of wild PE is mostly concentrated in the epidermis. Combined with metabolomics research, this may be due to the strong ultraviolet radiation in its growth environment, which caused its resistant secondary metabolites such as flavonoids and tannins (quercetin and its derivatives, amlaic acid and kaempferol, etc.) to accumulate bitter and astringent components near the epidermis. This distribution leads to more obvious bad taste such as bitterness and astringency, which further causes the taste of wild PE to decrease. Correspondingly, the content of these components in cultivated PE is low, and the components related to aftertaste-sweetness are significantly higher, making its overall taste better. Although the flavor and taste of wild PE are not as good as cultivated PE, it has stronger antioxidant and antibacterial activities, and it is believed that it also has stronger other biological activities. The mechanism of this phenomenon may involve the metabolic differences of flavone and flavonol biosynthesis, flavonoid biosynthesis, histidine metabolism, arginine biosynthesis, pentose phosphate pathway, etc. This study investigated the quality difference between wild and cultivated Amaryllis, and revealed the chemical nature and biological mechanism of the difference, which provided a reference for artificially-guided cultivation, resource grading and comprehensive utilization of resources in the future.

CRediT authorship contribution statement

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

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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