



Research article

Investigation of different ingredients affected the flavor changes of Yu-Shiang shredded pork by using GC-IMS and GC-MS combined with E-nose and E-tongue

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ABSTRACT

The objective of this study was to assess and compare the characteristics of Yu-Shiang Shredded Pork made with different ingredients by using physicochemical measurements and intelligent sensory analysis. The study revealed that there were 18 varied amino acids present, with the taste active values (TAVs) of Leu, Glu, Asp, Asn, and Ala all higher than 1.0. Intelligent sensory analysis showed that the samples lacking lettuce and fungus had similar aromas and flavors, while those lacking shredded pork and pickled chillies had distinct aromas and flavors. Moreover, VOCs (volatile organic compounds) were detected in five types of Yu-Shiang Shredded Pork, with 43, 42, 53, 36, and 50 identified in GC-MS (gas chromatography-mass spectrometry), respectively. Olefins (20.62 %–30.93 %) were the most abundant. GC-IMS (gas chromatography-ion mobility spectrometry) detected 68 volatiles flavor compounds, with esters having a significantly higher relative content than other compounds, indicating their significant role in the flavor formation process of Yu-Shiang Shredded Pork. Furthermore, the Orthogonal Partial Least Squares-discriminant analysis (OPLS-DA) model analysis identified 19 marker compounds that could differentiate the five types of Yu-Shiang Shredded Pork. These fundamental results lay the groundwork for future research on the connection between ingredients and the flavor characteristics of Yu-Shiang Shredded Pork.

1. Introduction

Yu-Shiang Shredded Pork is appreciated worldwide not only as a traditional and typical Sichuan dish but also for its perfect combination of colour, aroma, taste, and appearance, whose characteristic is that there is no fish meat but the fish aroma is strong. Flavor contributes to sensory characteristics, and aroma and taste are important factors in the assessment of Yu-Shiang Shredded Pork [1]. Due to the complex physical and chemical changes involved in cooking, the formation mechanism of Yu-Shiang Shredded Pork is

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influenced by multiple factors and it has been considered necessary to study the effects of different ingredients on the flavor of Yu-Shiang Shredded Pork, to provide basic scientific research for industrialized production and standardized quality control of Yu-Shiang Shredded Pork [2].

It is widely known that a dish's sensory characteristics are determined by a delicate balance of various parameters such as its appearance, smell, and taste [3]. Flavor is the most important parameter in the assessment of Yu-Shiang Shredded Pork character and quality, which depends on the composition and origin of ingredients, and factors in the cooking and processing [4]. Research showed the flavor substances of a dish mainly refer to its taste components and aroma substances, traditionally, the aromatic volatiles that made up Yu-Shiang Shredded Pork were usually classified into three categories according to their sources: sugar and vinegar, onion, ginger, garlic and other spices, and food ingredients [5,6]. However, there has not been a systematic study on how food ingredients affect the flavor of Yu-Shiang Shredded Pork.

There were many intelligent sensory evaluation instruments were employed to qualitatively analyze its odour and taste, such as electronic nose (E-nose) and electronic tongue (E-tongue), which offer a fast, comprehensive, and easy-to-handle alternative to assess food quality, with the advantages of stability and intuitive response to taste and odour profiles [7]. The latest research of intelligence evaluation methods showed that sensory evaluation can be affected by many factors, including the food properties and oral physiological parameters [8]. Moreover, the flavor characteristics of food were composed of flavor amino acids, organic acids, volatile substances, etc. [9]. Amino acid analysers, gas chromatography-mass spectrometry (GC-MS), and gas chromatography-ion mobility spectrometry (GC-IMS) can be used to quantitatively analyze free amino acids (FAAs) and volatile components in food [10]. On the one hand, GC-MS is an effective technique for the separation and identification of complex volatile compounds in qualitative and quantitative detection of volatile components in food and has the advantage of high resolution [11,12]. On the other hand, GC-IMS was an innovative technology that can be complemented by GC-MS with many advantages including a high sensitivity to detect low levels of compounds and providing a more comprehensive flavor profile of foods [13,14].

In this work, E-nose, E-tongue, GC-MS, GC-IMS, automatic amino acid analyzer, and other analytical instruments were simultaneously applied to identify and quantify the flavor quality of Yu-Shiang Shredded Pork cooked with different raw materials were characterized through four modes, such as lack of shredded pork, lettuces, fungus, and pickled chillies. Orthogonal Partial Least Squares-discriminant analysis (OPLS-DA) combined with heat map analysis was employed to identify characteristic compounds in differently treated samples, aiming to reveal the interrelationship between the flavor composition and the raw materials. The obtained results will provide a theoretical and scientific basis for the standardized industrial production and quality control of Yu-Shiang Shredded Pork.

2. Materials and methods

2.1. Material and cooking methods

Ingredients and additives needed to make Yu-Shiang Shredded Pork were all purchased from the local market (Chengdu City, Sichuan Province, China), and after cleaning and slitting, on standby for cooking. Referring to the local standard of Sichuan Province, "Sichuan Cuisine Culinary Techniques" (SB/T 10946-2012) and the pre-test determined the following recipes (Table 1).

The preparation process of Yu-Shiang Shredded Pork included pretreatment, seasoning, cooking and plating. The pretreatment for the dish at hand entails the procurance of cutting the pork into 7 cm × 0.3 cm × 0.3 cm julienne strips, marinating them in the marinade for 20 min and cutting the fungus and lettuce into 7 cm × 0.2 cm × 0.2 cm julienne strips. The seasoning process simply involves completely mixing the ingredients required for the gravy in Table 1. After shredding the meat, it was cooked in 160 °C hot cooking oil and stir-fried. Two min later, excipient and pickled chillies are incorporated into the mixture and further stir-fried for 1 min. Then, lettuce and fungus were added to the mixture and stir-fried for 3 min. Finally, added garvy to the mixture and stir-fried until complete amalgamation was achieved.

On the premise of ensuring consistency in other production processes, the samples are divided into the following five groups: Sample A (without shredded pork), Sample B (without lettuce), Sample C (without fungus), Sample D (without pickled chillies), and Sample E (blank control, regular sample) based on the differences in the ingredients added during the cooking process. Prior to the experiment, it was discovered that breaking down the walls post-processing could improve the consistency of the samples. As a result, multifunctional wall breakers were utilized to break down the walls and evaluate the flavor at 4 °C storage temperature.

Table 1
Preparation of ingredients and dosage of samples.

Class	Name	Dosage (g)	Name	Dosage (g)
Main Ingredients	shredded pork	200.00	lettuce	100.00
	fungus	30.00	pickled chillies	50.00
Marinade	salt	3.00	water	30.00
	cooking wine	6.00	farina	20.00
Gravy	sugar	17.00	vinegar	10.00
	soya sauce	4.00	monosodium glutamate	1.00
Excipient	garlic	15.00	spring onion	25.00
	ginger	7.00	cooking oil	70.00

2.2. Determination of physicochemical indicators

2.2.1. Calories analysis

The energy, carbohydrate, protein, and fat contents of different samples were determined using a food calorie composition tester (CA-HM, JWP, Tokyo, Japan). The test was repeated three times for each set of samples and the results were averaged.

2.2.2. Determination of FAAs

The FAAs were analyzed using an Automatic Amino Acid Analyzer (S433D, Sykam, Munich, Germany), following the method described by Li et al. [15]. Briefly, the samples were sonicated with a 7 % sulfosalicylic acid solution of 9 mL at 55 KHz for 35 min at 45 °C. Following centrifugation at 10000 r/min for 15 min at 35 °C, 1 mL of the supernatant was filtered through a 0.22 μm micropore membrane (Sigma Eldrich Trading Co., Ltd., Shanghai, China) and then loaded into sample bottles for assay using an injection volume of 40 μL. The integrated program of the instrument combined the spectra of the samples and automatically calculated the free amino acid content by using the selected calibration file. The ninhydrin reagent was used at a rate of 0.25 mL/min, with a LCA K07/Li analysis column (150 mm × 4.6 mm) that had a 10 % cross-linking and a reactor temperature of 130 °C.

2.2.3. Analysis of volatile compounds by GC-MS

Refer to the method of Xiao et al. [16]. Briefly, began by obtaining a 2 g sample and placing it in a 15 mL vial. Next, seal the vial with a stir bar and set the magnetic stirring device to 120 °C with a rotation speed of 1.5 r/s. Allow the mixture to reach equilibrium for 10 min. Following this, inserted the aged (250 °C, 10 min) extraction tip into the vial and let it sit for 120 min. Once the time has elapsed, insert the GC-MS inlet and maintain the resolution for 10 min. Then, removed the sampler from the headspace vial and inserted it into the GC injector. Use an Elite-5MS (30 m × 0.25 mm × 0.25 μm) and set the initial temperature to 40 °C for 1 min. Increase the temperature to 170 °C at 5 °C/min, then to 250 °C at 15 °C/min and maintain it at 250 °C for 1 min for the test.

Based on the total ion flow diagram of the volatile compounds, the first 200 peaks with the largest peak areas were integrated, and a similarity search was performed using the NIST05 database and combined with the RI of the compounds for characterization.

2.2.4. Analysis of volatile compounds by GC-IMS

GC-IMS analyzed Volatile compounds in Yu-Shiang Shredded Pork (Flavor Spec, G.A.S., Dortmund, Germany). Five grams of the sample were placed in a headspace vial and sealed. The sample volume was 500 μL, and the incubation time was 15 min. The incubation temperature was 70 °C, the injection needle temperature was 65 °C, and the incubation speed was 500 r/min.

2-Butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, and 2-nonanone were used as external references for calculating the RIs. The volatile compounds were qualitatively determined by comparing their RIs and drift time with those of standards in the GC-IMS library Search (G.A.S., Dortmund, Germany). Qualitative analysis of volatile compounds was performed based on the IMS and the NIST database integrated within the GC-IMS Library Search. As for the quantitative analysis of volatile compounds, it primarily relied on the peak intensity observed in the GC-IMS, which is directly proportional to the content of the volatile compound. The relative content of the VOCs (%) = (The peak volume/Total peak volume) × 100 [17].

2.3. Analysis of intelligent senses

2.3.1. Analysis of E-nose

Overall odour characteristics were obtained using a Fox 4000 E-nose (Alpha MOS, Toulouse, France), which had a metal oxide 122 semiconductor sensor array containing 18 sensor chambers (LY2/LG, LY2/G, LY2/AA, LY2/Gh, LY2/gCT1, LY2/gCT, T30/1, P10/1, P10/2, P40/1, T70/2, PA/2, P30/1, P40/2, P30/2, T40/2, T40/1, TA/2). The main pieces of information granted by each sensor are shown in Table 2. Before injection, each sample (3 g) was placed in a 10 mL airtight glass vial for 5 min at 70 °C (headspace-generation time and temperature), and the measurement phase lasted for 2 min according to the method of Shen et al. [18].

2.3.2. Analysis of E-tongue

Gustatory sense analysis was performed by α-ASTREE E-tongue (Alpha MOS, Toulouse, France), which provided seven sensors for sourness (AHS), saltiness (CTS), umami (NMS), sweetness (ANS), bitterness (SCS), and two reference electrodes (PKS and CPS). For the

Table 2

Performance description of the E-nose sensors.

Sensors	Performance description	Sensors	Performance description
LY2/LG	Sensitive to oxidizing gas	P40/1	Sensitive to fluorine
LY2/G	Sensitive to ammonia, carbon monoxide	T70/2	Sensitive to aromatic compounds
LY2/AA	Sensitive to ethanol	PA/2	Sensitive to ethanol, ammonia/organic amines
LY2/Gh	Sensitive to ammonia/organic amines	P30/1	Sensitive to polar compounds (ethanol)
LY2/gCT1	Sensitive to hydrogen sulfide	P40/2	Sensitive to heteroatom/chloride/aldehydes
LY2/gCT	Sensitive to propane/butane	P30/2	Sensitive to alcohol
T30/1	Sensitive to organic solvents	T40/2	Sensitive to aldehydes
P10/1	Sensitive to hydrocarbons	T40/1	Sensitive to chlorinated compounds
P10/2	Sensitive to methane	TA/2	Sensitive to air quality

experiment, 10 g of the sample was mixed with 100 g of ultrapure water and sonicated for 35 min at 40 °C before filtration. After centrifugation at 10,000×g for 10 min at 25 °C, 80 mL of the supernatant was then filtered and transferred to a specialized beaker for E-tongue analysis. The E-tongue was operated under specific measurement conditions: a data acquisition time of 2 min, no acquisition delay, and each sample was analyzed 5 times, and the stable values from the last 3 measurements were considered as the test results [19].

2.4. Statistical analysis

The samples of E-nose and E-tongue were analyzed in five replicates, while Calorie analysis, FAAs analysis, and GC-IMS were performed in three replicates. All data were analyzed using IBM SPSS Statistics 26.0 (SPSS Inc., Chicago, IL, USA) and one-factor analysis of variance (ANOVA), and $p < 0.05$ was considered to be statistically significant. The characteristic fingerprint and the difference plots were generated by Gallery Plot and Reporter software (G.A.S., Dortmund, Germany). Origin software (Origin Lab Corporation, Northampton, MA, USA) was used for radar plot analysis, line chart analysis, and histogram analysis. PCA analysis performed by GenesCloud Tools. SIMCA 14.1 (MKS Umetrics, Umea, Sweden) was used for chemometrics.

3. Results and discussion

3.1. Physicochemical properties analysis

3.1.1. Calories analysis

Since Calorie values were related to precursors for flavor formation such as fats and proteins, the Calories analysis was used to detect the calories of Yu-Shiang Shredded Pork as a reference [20]. The results of the Calories analysis of samples with different cooking ingredients were presented in Fig. 1, which showed that the energy, protein, and carbohydrates of Yu-Shiang Shredded Pork using different ingredients varied greatly, with the lowest energy without shredded pork (129 g/100 g) and the highest energy without pickled chillies (189 g/100 g) ($p < 0.05$). The protein content was highest without lettuces (20 g/100 g) and lowest without fungus (11.7 g/100 g) ($p < 0.05$). Carbohydrate content was highest without the addition of fungus (5.6 g/100 g) and lowest without the addition of lettuce (2.9 g/100 g) ($p < 0.05$). As the lack of shredded pork can lead to a substantial reduction in fat, this may also lead to a corresponding reduction in the content of other substances, such as energy. From the data, it can be deduced that the lettuce has a relatively low moisture content, with B having the least amount of moisture. A, C, D, and E did not exhibit any significant variations in moisture content. Furthermore, the fat content of the shredded pork had a more substantial impact on the carbohydrate content than the fungus content. The protein and moisture content of the dish showed a positive correlation with the quantity of lettuce utilized. However, the reason for this phenomenon needs further study.

3.1.2. Free amino acids analysis

In addition to protein and carbohydrates, Yu-Shiang Shredded Pork was also rich in amino acids, inorganic acids, mineral substances, and so on, playing a role in its organoleptic properties. Table 3 shows that this dish contained a total of 18 amino acids, with six of them being essential (Thr, Ser, Val, Ile, Leu, Tyr, Phe, Lys). The content of FAAs varies across samples, ranging from 155.93 to 85.94 mg/g in descending order (D > A > E > C > B). The FAAs can be classified into four taste categories: umami amino acids (Asp, Asn, and Glu), sweet amino acids (Ala, Gly, Pro, Thr, and Ser), bitter amino acids (His, Lys, Arg, Ile, Leu, and Val), and astringent amino acids (GABA). Yu-Shiang Shredded Pork contained a high percentage of umami amino acids (34.97 %–70.66 %), which may be due to the Maillard reaction between FAAs and reducing sugars [21]. During the cooking process, various aroma compounds in Yu-Shiang

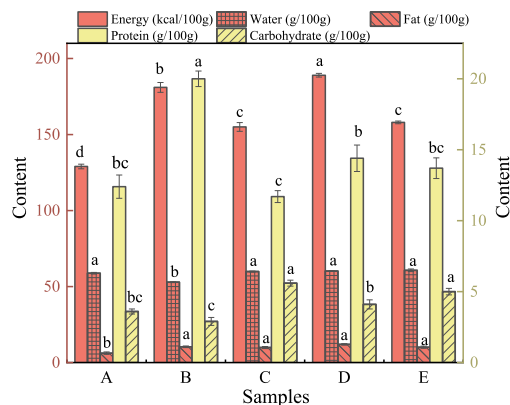


Fig. 1. Calories analysis of Yu-Shiang Shredded Pork. Values marked with different letters in the five columns were significantly different ($p < 0.05$, $n = 3$).

Table 3
Composition of amino acids in different ingredients of Yu-Shiang Shredded Pork.

	Component	Sample				
		A	B	C	D	E
Total	Asn	5.91 ± 0.86 ^a	2.91 ± 0.48 ^c	3.85 ± 0.98 ^b	4.70 ± 0.85 ^{ab}	4.16 ± 0.75 ^{ab}
	Glu	22.82 ± 1.89 ^a	6.60 ± 1.15 ^c	14.38 ± 1.54 ^b	14.40 ± 1.84 ^b	15.13 ± 1.68 ^b
	/	62.38 ± 2.10 ^b	20.00 ± 2.98 ^d	25.77 ± 2.29 ^d	90.51 ± 3.85 ^a	45.31 ± 3.05 ^c
Sweet AA	/	91.11	29.52	44.00	109.61	64.60
	Gly	1.65 ± 0.43 ^b	3.84 ± 0.54 ^a	2.32 ± 0.19 ^{ab}	2.42 ± 0.28 ^{ab}	2.44 ± 0.25 ^{ab}
	Ala	8.02 ± 1.43 ^{ab}	10.18 ± 1.08 ^a	7.50 ± 0.64 ^b	8.63 ± 1.25 ^{ab}	7.41 ± 1.05 ^b
	Pro	3.66 ± 0.54 ^a	3.47 ± 0.92 ^b	2.91 ± 0.46 ^c	2.03 ± 0.56 ^c	2.78 ± 0.27 ^c
	Thr	3.81 ± 0.63 ^a	2.14 ± 0.73 ^b	2.65 ± 0.38 ^b	2.76 ± 0.82 ^b	2.73 ± 0.73 ^b
	Ser	6.42 ± 0.98 ^a	4.11 ± 0.90 ^b	4.44 ± 0.78 ^b	4.64 ± 0.92 ^b	4.38 ± 0.83 ^b
	/	23.57	23.74	19.83	20.48	19.73
Bitter AA	Val	3.89 ± 0.77 ^a	3.52 ± 0.80 ^b	3.20 ± 0.96 ^{bc}	2.42 ± 0.72 ^c	3.16 ± 0.49 ^{bc}
	Ile	2.37 ± 0.43 ^a	2.39 ± 0.60 ^a	1.93 ± 0.21 ^b	1.80 ± 0.25 ^b	2.03 ± 0.37 ^b
	Leu	3.42 ± 0.99 ^a	3.98 ± 0.70 ^a	2.78 ± 0.34 ^b	2.25 ± 0.24 ^c	2.92 ± 0.48 ^b
	Tyr	1.06 ± 0.28 ^b	1.29 ± 0.32 ^a	0.92 ± 0.18 ^b	0.82 ± 0.19 ^c	0.97 ± 0.24 ^b
	Phe	1.99 ± 0.38 ^a	1.83 ± 0.38 ^b	1.64 ± 0.55 ^c	1.40 ± 0.35 ^d	1.85 ± 0.85 ^b
	Lys	3.35 ± 0.96 ^a	0.83 ± 0.29 ^b	0.55 ± 0.53 ^{bc}	0.36 ± 0.21 ^c	0.53 ± 0.35 ^{bc}
	Arg	15.65 ± 1.98 ^a	15.33 ± 2.13 ^a	14.57 ± 2.53 ^b	14.69 ± 2.35 ^b	13.88 ± 2.95 ^c
	/	31.74	29.16	25.58	23.74	25.35
	Astringent taste	GABA	2.90 ± 0.21 ^a	2.05 ± 0.38 ^{ab}	1.89 ± 0.53 ^b	1.30 ± 0.45 ^c
Tasteless taste	Hyp	1.42 ± 0.36 ^a	1.43 ± 0.21 ^b	0.96 ± 0.31 ^{ab}	0.49 ± 0.16 ^c	0.99 ± 0.29 ^{ab}
	Orn	0.37 ± 0.15 ^a	0.04 ± 0.01 ^c	0.17 ± 0.08 ^b	0.31 ± 0.06 ^b	0.24 ± 0.10 ^b
Total	/	1.79	1.47	1.13	0.80	1.23
Total AA	/	151.11	85.94	92.43	155.93	112.86

Values marked with lower case letters in the same line were significantly different ($p < 0.05$).

Shredded Pork were formed through specific reactions involving FAA, thus giving foods their distinctive flavor and nutrient content [22]. TAV was used to evaluate the overall taste contribution. When TAV is greater than 1.0, the substance was considered to contribute more to the flavor of the sample, and when TAV is less than 1.0, it is considered that the substance does not contribute to the flavor [23]. As shown in Fig. 2 the amino acids with TAV greater than 1.0 in the samples were Leu, Glu, Asp, Asn, and Ala, indicating that the umami flavor amino acids in all the samples contributed more, meanwhile, sweet amino acids contributed to the overall flavor presentation of Yu-Shiang Shredded Pork.

3.2. GC-MS analysis

An important organoleptic characteristic that influenced the preference and acceptance of Yu-Shiang Shredded Pork was its VOCs content, which varied among different processed ingredients [24,25]. In Fig. 3a, the mean relative area percentages and number of volatiles in each class of various Yu-Shiang Shredded Pork samples were presented, a total of 116 VOCs were detected in these samples. The VOCs were classified into eight groups based on their chemical properties, including alcohols, alkanes, pyrazines, olefins, aldehydes, ketones, esters, and furan. The numbers of volatile components identified in the samples obtained using different ingredients

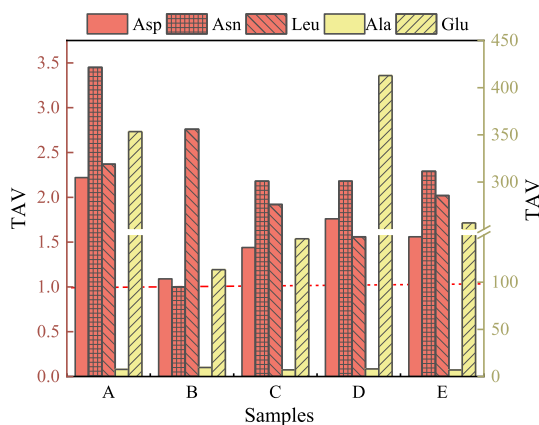


Fig. 2. Taste activity values (TAVs) of free amino acids. TAVs of free amino acids were calculated by the ration of the concentration of a compound to its taste threshold.

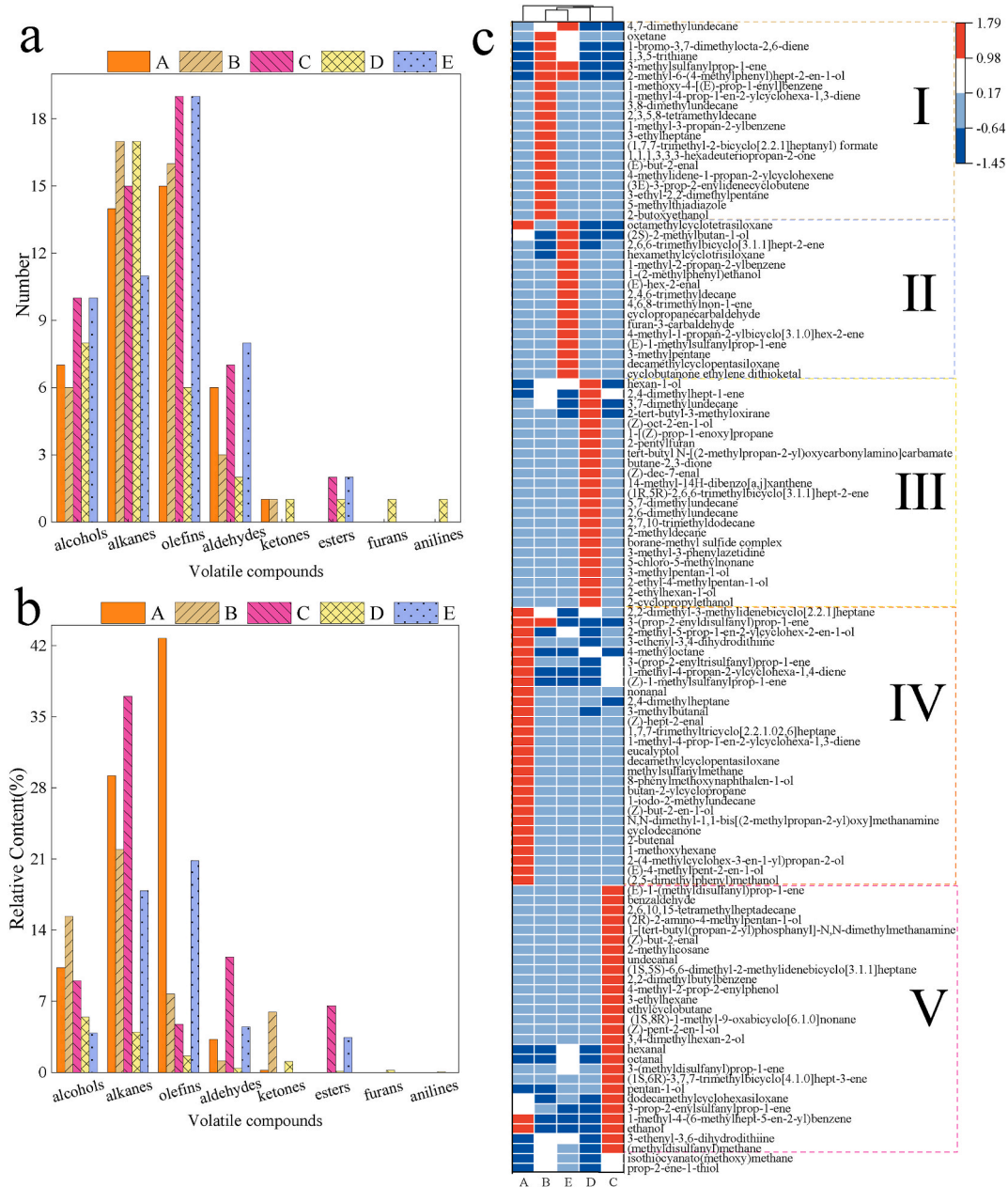


Fig. 3. Analysis of volatile compounds of Yu-Shiang Shredded Pork identified by GC-MS. The number (a) relative content (b) and heatmap visualization (c) of VOCs among different samples.

were as follows: 43 (A), 44 (B), 53 (C), 36 (D) and 50 (E). Compared to D, the number of compound species increased significantly after the use of the different ingredients, with the highest number of olefins in C and E, the highest number of alkanes in B and D, and aldehydes most abundant in E. The components with higher proportions were olefins, alkanes, alcohols and aldehydes. In addition, the content of the volatile component classes varied between ingredients (Fig. 3b). The relative content of volatile components identified in the samples obtained using different ingredients were as follows: 79.81 % (A), 52.02 % (B), 68.65 % (C), 12.97 % (D) and 50.48 % (E). Olefins were most abundant in Yu-Shiang Shredded Pork without shredded pork (42.75 %) while the lowest content in Yu-Shiang Shredded Pork without pickled chillies (1.63 %); Alkanes were most abundant in Yu-Shiang Shredded Pork without fungus (37.01 %) while the lowest content in Yu-Shiang Shredded Pork without pickled chillies (1.63 %). There were significant differences in the types and contents of volatile compounds in the samples with different ingredients but the effect of specific flavour compounds needs to be further analyzed in conjunction with the GC-IMS results.

For a more intuitive presentation, as shown in Fig. 3c, samples were clustered into four subclasses, showing high relevance in

Table 4
Volatile compounds spotted from different ingredients of Yu-Shiang Shredded Pork.

No	Component Name	CAS	RI	Rt [sec]	Relative amount/%					Odour description
					A	B	C	D	E	
1	1- butanol-D	C71363	1140.5	565.607	0.40 ± 0.10b	0.68 ± 0.10a	0.70 ± 0.06a	0.25 ± 0.06c	0.44 ± 0.08d	medicine, fruit
2	1- butanol-M	C71363	1141.2	567.245	0.65 ± 0.13c	0.85 ± 0.08ac	1.04 ± 0.14b	1.44 ± 0.11a	0.88 ± 0.12b	medicine, fruit
3	2- butanol	C78922	1025.9	379.646	0.76 ± 0.11ab	0.86 ± 0.08a	0.83 ± 0.10ab	0.56 ± 0.17b	0.96 ± 0.24a	wine
4	1-Pentanol-D	C71410	1258.1	898.345	0.44 ± 0.07b	0.51 ± 0.14bc	0.85 ± 0.07b	3.43 ± 0.38a	0.79 ± 0.22bc	balsamic
5	1-Pentanol-M	C71410	1260.1	904.9	1.93 ± 0.17c	2.12 ± 0.27c	2.79 ± 0.07b	7.82 ± 0.26a	2.76 ± 0.28b	balsamic
6	1-Propanol, 2-methyl-D	C78831	1097.8	468.252	0.12 ± 0.01b	0.23 ± 0.04a	0.26 ± 0.04a	0.10 ± 0.02b	0.12 ± 0.04b	wine, solvent, bitter
7	1-Propanol, 2-methyl-M	C78831	1096.3	465.504	0.61 ± 0.07b	0.79 ± 0.01ab	0.89 ± 0.06a	0.76 ± 0.12ab	0.66 ± 0.16b	wine, solvent, bitter
8	1-propanethiol	C107039	857.6	263.608	0.50 ± 0.05a	0.46 ± 0.02a	0.55 ± 0.05a	0.16 ± 0.02b	0.47 ± 0.18a	NA
9	1 -hexanol-D	C111273	1362.2	1219.889	0.08 ± 0.01b	0.12 ± 0.04b	0.08 ± 0.01b	1.23 ± 0.63a	0.09 ± 0.01b	resin, floral aroma
10	1 -hexanol-M	C111273	1362.9	1221.952	0.42 ± 0.12b	0.62 ± 0.20b	0.42 ± 0.04b	3.31 ± 0.86a	0.33 ± 0.07b	resin, floral aroma
11	2-ethyl hexanol	C104767	1501.7	1637.99	4.73 ± 0.28b	5.40 ± 0.23a	3.28 ± 0.33c	0.8 ± 0.14d	4.19 ± 0.53b	rose, green
12	1-Propanol-D	C71238	1037.3	393.615	1.63 ± 0.17b	2.44 ± 0.18a	2.30 ± 0.03ab	0.92 ± 0.26c	2.04 ± 0.77ab	alcohol, pungent
13	1-Propanol-M	C71238	1035.5	391.349	0.85 ± 0.05b	0.95 ± 0.12b	1.03 ± 0.11b	2.00 ± 0.34a	1.03 ± 0.27b	alcohol, pungent
14	2-Propanol	C67630	935.9	308.658	0.59 ± 0.08a	0.58 ± 0.10a	0.52 ± 0.01a	0.67 ± 0.06a	0.65 ± 0.14a	NA
15	1-Butanol, 3-methyl-D	C137326	1208.7	737.581	0.54 ± 0.08c	0.96 ± 0.20a	0.75 ± 0.02b	0.33 ± 0.08d	0.66 ± 0.05bc	wine, onion
16	1-Butanol, 3-methyl-M	C123513	1209.9	741.371	0.97 ± 0.10b	1.61 ± 0.14a	1.59 ± 0.15a	1.58 ± 0.31a	1.57 ± 0.07+a	whiskey, malt, burnt
17	2-Methyl-2-propanol	C75650	918.7	298.738	0.74 ± 0.16a	0.84 ± 0.09a	0.92 ± 0.15a	0.91 ± 0.17a	0.66 ± 0.26a	NA
18	(E)-2-Heptenal	C18829555	1325.1	1108.615	1.82 ± 0.05c	1.05 ± 0.15d	2.29 ± 0.26b	3.64 ± 0.15a	1.97 ± 0.36bc	soap, fat, almond
19	(E)-2-octenal	C2548870	1437.3	1445.006	0.4 ± 0.02b	0.23 ± 0.04c	0.38 ± 0.06b	0.54 ± 0.02a	0.36 ± 0.07b	fatty, orange
20	3-Methyl butanal	C590863	919	298.944	1.10 ± 0.26a	1.05 ± 0.10a	1.13 ± 0.21a	1.03 ± 0.18a	0.87 ± 0.45a	malt
21	Heptaldehyde	C111717	114.2	1186.1	0.90 ± 0.04c	0.89 ± 0.04c	1.05 ± 0.09bc	1.93 ± 0.07a	1.46 ± 0.58ab	fat, citrus, rancid
22	(E)-2-Pentenal-D	C1576870	1130.3	542.467	0.36 ± 0.00a	0.15 ± 0.03c	0.28 ± 0.05b	0.20 ± 0.01bc	0.24 ± 0.08b	NA
23	(E)-2-Pentenal-M	C1576870	1130.1	541.998	0.63 ± 0.01c	0.52 ± 0.02d	0.76 ± 0.10b	0.98 ± 0.02a	0.67 ± 0.05bc	NA
24	1-hexanal-D	C66251	1085.3	452.133	2.02 ± 0.45b	1.91 ± 0.37b	2.36 ± 0.48b	9.72 ± 1.86a	2.40 ± 0.93b	grass, tallow, fat
25	1-hexanal-M	C66251	1085.7	452.561	1.02 ± 0.12c	1.32 ± 0.13bc	1.52 ± 0.22b	2.98 ± 0.31a	1.19 ± 0.33bc	grass, tallow, fat
26	(E)-2-hexen-1-al-D	C6728263	1219	771.063	0.93 ± 0.04a	0.45 ± 0.09b	0.60 ± 0.06b	0.27 ± 0.03c	0.60 ± 0.19b	green, fruit
27	(E)-2-hexen-1-al-M	C6728263	1221.1	778.064	1.37 ± 0.01a	1.10 ± 0.04b	1.37 ± 0.14a	1.51 ± 0.09a	1.53 ± 0.13a	green, fruit
28	acrolein	C107028	832.7	249.314	0.17 ± 0.03c	0.24 ± 0.02b	0.20 ± 0.04bc	1.24 ± 0.04a	0.19 ± 0.02bc	NA
29	Butanal-D	C123728	886.3	280.146	0.76 ± 0.06a	0.53 ± 0.11b	0.61 ± 0.06ab	0.34 ± 0.03c	0.73 ± 0.18a	pungent, green
30	Butanal-M	C123728	886.3	280.107	0.22 ± 0.02c	0.29 ± 0.02b	0.26 ± 0.02b	0.73 ± 0.03a	0.29 ± 0.03b	pungent, green
31	n-Pentanal-D	C110623	993.7	341.892	2.59 ± 0.04c	1.67 ± 0.15d	3.11 ± 0.17b	7.23 ± 0.36a	3.35 ± 0.36b	almond, malt, pungent
32	n-Pentanal-M	C110623	981.8	335.04	0.12 ± 0.00bc	0.16 ± 0.02a	0.14 ± 0.02ab	0.13 ± 0.01bc	0.11 ± 0.01c	almond, malt, pungent
33	(Z)-2-Methylpent-2-enal	C623369	1148	582.744	0.57 ± 0.09a	0.62 ± 0.08a	0.24 ± 0.08b	0.08 ± 0.01c	0.17 ± 0.02bc	NA

(continued on next page)

Table 4 (continued)

No	Component Name	CAS	RI	Rt [sec]	Relative amount/%					Odour description
					A	B	C	D	E	
34	1-octanal	C124130	1293.8	1014.49	0.22 ± 0.04b	0.17 ± 0.02b	0.20 ± 0.01b	0.67 ± 0.06a	0.23 ± 0.11b	mushroom, fat
35	2-Methyl propanal	C78842	812.5	237.694	1.14 ± 0.02b	0.41 ± 0.04c	1.14 ± 0.07b	2.01 ± 0.06a	1.00 ± 0.45b	pungent, malt, green
36	2-Methyl butanal	C96173	890.1	282.326	0.14 ± 0.01b	0.45 ± 0.07a	0.42 ± 0.03a	0.36 ± 0.01a	0.42 ± 0.20a	wine, onion
37	Decanal	C112312	1497.8	1626.354	0.85 ± 0.01b	0.64 ± 0.14c	0.85 ± 0.02b	0.29 ± 0.05d	1.00 ± 0.06a	soap, orange peel, tallow
38	(E, E)-2,4-heptadienal	C4313035	1476	1561	0.49 ± 0.03bc	0.55 ± 0.09ab	0.40 ± 0.03c	0.20 ± 0.02d	0.67 ± 0.13a	nut, fat
39	(E, E)-2,4-hexadienal	C142836	1405.6	1349.965	0.50 ± 0.11a	0.32 ± 0.03bc	0.19 ± 0.06d	0.39 ± 0.07ab	0.21 ± 0.02cd	NA
40	Acetic acid ethyl ester	C141786	889.2	281.833	1.76 ± 0.14a	1.15 ± 0.12b	1.57 ± 0.06a	0.52 ± 0.02c	1.66 ± 0.43a	pleasant, sweet, fruity
41	Acetic acid propyl ester	C109604	996.2	343.402	0.23 ± 0.02c	0.28 ± 0.04bc	0.29 ± 0.06bc	1.11 ± 0.03a	0.33 ± 0.05b	NA
42	Butanoic acid, 3-hydroxy-, ethyl ester-D	C5405414	1501.3	1637.06	9.04 ± 0.78b	10.62 ± 0.36a	6.20 ± 0.65d	1.11 ± 0.32e	7.65 ± 1.23c	marshmallow
43	Butanoic acid, 3-hydroxy-, ethyl ester-M	C5405414	1503.2	1642.648	9.57 ± 0.42b	10.86 ± 0.98a	11.24 ± 0.87a	10.10 ± 0.25b	11.60 ± 0.29a	marshmallow
44	Butanoic acid ethyl ester	C105544	1046.9	405.318	5.67 ± 0.32a	5.10 ± 0.07ab	4.69 ± 0.26b	0.90 ± 0.20c	4.50 ± 0.98b	apple
45	Methyl hexoate	C106707	1182.2	660.824	0.42 ± 0.05b	0.35 ± 0.03c	0.33 ± 0.01cd	0.56 ± 0.01a	0.28 ± 0.03d	fruit, fresh, sweet
46	Isovaleric acid, methyl ester	C556241	1019.3	371.644	0.10 ± 0.01b	0.11 ± 0.02b	0.10 ± 0.01b	0.35 ± 0.03a	0.20 ± 0.16b	apple
47	1-Octen-3-one	C4312996	1323.7	1104.376	0.54 ± 0.05b	0.24 ± 0.06c	0.65 ± 0.11ab	0.79 ± 0.06a	0.50 ± 0.18b	mushroom, metal
48	2-Pentanone	C107879	977.6	332.609	0.46 ± 0.03c	0.57 ± 0.01b	0.62 ± 0.02a	0.42 ± 0.01c	0.46 ± 0.04c	ether, fruit
49	2-methyl-2-hepten-6-one	C110930	1342.3	1160.306	0.59 ± 0.12a	0.35 ± 0.02b	0.27 ± 0.03bc	0.19 ± 0.05cd	0.14 ± 0.02d	pepper, rubber
50	2-Butanone	C78933	907	292.057	1.75 ± 0.48b	2.48 ± 0.25ab	2.96 ± 0.63a	1.59 ± 0.44b	1.79 ± 0.99b	ether
51	Acetoin	C513860	1290	1002.008	1.42 ± 0.06c	2.26 ± 0.10a	1.93 ± 0.11b	0.34 ± 0.00d	1.91 ± 0.03b	butter, cream
52	2,3 Butanedione	C431038	983.2	335.844	0.27 ± 0.01c	0.32 ± 0.03bc	0.32 ± 0.05bc	0.74 ± 0.04a	0.34 ± 0.03b	butter
53	Cyclohexanone	C108941	1285.3	986.903	1.47 ± 0.21b	1.86 ± 0.06a	1.04 ± 0.15c	0.15 ± 0.02d	1.33 ± 0.36bc	NA
54	triethylamine	C121448	824.8	244.77	0.61 ± 0.01b	0.71 ± 0.22ab	0.64 ± 0.11ab	0.82 ± 0.09ab	0.97 ± 0.29a	NA
55	1-(3,4-Dihydro-2H-pyrrol-5-yl) ethanone	C85213225	1335.7	1140.301	0.28 ± 0.01b	0.09 ± 0.02c	0.30 ± 0.03b	0.83 ± 0.03a	0.32 ± 0.04b	nut, roast
56	alpha-Pinene-D	C80568	1021.9	374.738	1.93 ± 0.16c	2.65 ± 0.13b	3.05 ± 0.18a	0.51 ± 0.09d	2.85 ± 0.06b	pine, turpentine
57	alpha-Pinene-M	C80568	1018.5	370.585	1.09 ± 0.20a	0.75 ± 0.07b	0.99 ± 0.10a	0.61 ± 0.02b	0.68 ± 0.07b	pine, turpentine
58	beta-Pinene	C127913	1114.8	506.92	1.64 ± 0.17ab	1.53 ± 0.01b	1.81 ± 0.07a	0.27 ± 0.05c	1.56 ± 0.21d	pine, resin, turpentine
59	(+)-limonene-D	C138863	1208.2	736.001	2.10 ± 0.73a	0.66 ± 0.02b	1.00 ± 0.30b	0.41 ± 0.04b	0.45 ± 0.09b	lemon, orange
60	(+)-limonene-M	C138863	1202.8	718.417	7.22 ± 0.74a	4.54 ± 0.22b	5.40 ± 0.48b	2.56 ± 0.89c	3.38 ± 0.03c	lemon, orange
61	beta-myrcene-D	C123353	1156.9	603.02	0.36 ± 0.14a	0.18 ± 0.03b	0.23 ± 0.04b	0.24 ± 0.01ab	0.14 ± 0.01b	balsamic, must, spice
62	beta-myrcene-M	C123353	1155.1	598.916	0.54 ± 0.18a	0.36 ± 0.05ab	0.52 ± 0.09a	0.14 ± 0.02c	0.25 ± 0.05bc	balsamic, must, spice
63	alpha-terpinolene	C586629	1285	985.712	0.28 ± 0.08ab	0.38 ± 0.03a	0.20 ± 0.04bc	0.11 ± 0.02c	0.24 ± 0.07b	pine, plastic
64	2-Ethylfuran	C3208160	995.1	342.647	0.56 ± 0.02c	0.63 ± 0.05bc	0.71 ± 0.02b	0.80 ± 0.04a	0.68 ± 0.05b	smoky flavor
65	2-pentyl furan	C3777693	1231	810.231	0.46 ± 0.04a	0.36 ± 0.06b	0.39 ± 0.01ab	0.33 ± 0.01b	0.37 ± 0.04b	green bean, butter
66	Tetrahydrofuran	C109999	866	268.487	1.73 ± 0.14a	0.84 ± 0.15b	0.95 ± 0.28cd	1.27 ± 0.16bc	1.40 ± 0.14ab	NA
67	2-Methylpyrazine	C109080	1269	933.73	0.22 ± 0.01c	0.25 ± 0.01c	0.28 ± 0.00b	0.17 ± 0.02d	0.33 ± 0.03a	popcorn

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Table 4 (continued)

No	Component Name	CAS	RI	Rt [sec]	Relative amount/%					Odour description
					A	B	C	D	E	
68	Allyl sulfide	C592881	1142.9	571.124	4.21 ± 0.43a	4.19 ± 0.34a	3.36 ± 0.04b	1.25 ± 0.32c	4.18 ± 0.19a	NA

Odour description is taken from the Chemical Book database. M = monomer, D = dimer, RI = retention index, Rt = retention time, NA = no aroma description. Values marked with lower case letters in the same column were significantly different ($p < 0.05$).

similar attributions. Subsequently, B and E samples formed a large category, indicating relatively similar VOCs within each group. The relative contents of VOCs in part I were highest in B, with a total of 20 VOCs. Among them, olefin substances accounted for the main proportion. Meanwhile, the relative contents of 16 VOCs in part II were highest in the E, among which olefins and alkane accounted for a large proportion. The relative contents of VOCs in part III were highest in D, with a total of 23 VOCs, among which alcohols accounted for a higher proportion. Similarly, VOCs in Part IV are relatively more abundant in A with a total of 28 VOCs, and alcohol and alkenes had the highest concentration. The relative contents of VOCs in part V were highest in C, with a total of 27 VOCs, among which aldehydes accounted for a higher proportion. Notably, the alcohol and alkenes identified were 2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol (fresh, spearmint, caraway aromas), 1-methyl-4-prop-1-en-2-ylcyclohexa-1,3-diene (turpentine aromas), 1-methyl-4-propan-2-ylcyclohexa-1,4-diene (gasoline, turpentine aromas) which played a more significant role in the overall flavor of A. The detected aldehydes including benzaldehyde (almond, burnt sugar aromas) and octanal (fat, soap, lemon, green aromas) were recognised as significant contributors to C owing to their low odour thresholds and intricate flavor characteristics [26]. Song et al. [27] found that the aromatic flavor of A, B, C, and E was largely due to the presence of certain olefin compounds, including 3- (methyl-disulfanyl) prop-1-ene, 3-prop-2-enylsulfanylprop-1-ene, and 3- (prop-2-enyltrisulfanyl) prop-1-ene. Notably, D contained very little of these compounds. In addition, the horizontal comparison demonstrated the decreased aldehydes and alkanes in A and D samples which lacked pork and pickled chillies, while ketones were more abundant. Overall, the analysis of volatile matter revealed a significant difference in flavor between A and D. This suggested that the absence of shredded pork and pickled chillies played a bigger role in the flavor of Yu-Shiang Shredded Pork.

3.3. GC-IMS analysis

3.3.1. Volatile compounds analysis

The volatile compounds of Yu-Shiang Shredded Pork with different ingredients were well separated by GC-IMS, and the differences could be visualized. The 2D spectra of the aroma compounds in Yu-Shiang Shredded Pork at the five sampling stages were depicted in Fig. 4a, clearly demarcating the aroma compounds. The colour represented the aroma compound concentration, with white denoting low concentration and red indicating high concentration. Generally, the colour depth/intensity escalated with increasing concentration [28]. To clarify the VOCs alterations, as presented in Fig. 4b, the 3D topography (Y = drift time, X = retention time, and Z = peak intensity) revealed that different groups' volatilities had different peak intensities [29,30]. Based on the results of the GC-IMS, a total of 68 peaks (including monomers and dimers) were detected and identified in this study, including 22 aldehydes, seven esters, seven ketones, 17 alcohols, one pyrazine, three furans, one pyrrole, one thioether, eight olefins, and one amine (Table 4). As shown in Fig. 4c, the results showed that the volatile flavor compounds of the five fish flavor shreds were mainly composed of esters, aldehydes and alcohols, which accounted for 15.94%–31.84%, 16.46%–39.67% and 17.58%–28.58%, respectively. However, pyrrole and pyrazine were relatively low, accounting for 0.1%–0.9% and 0.18%–0.37%, respectively. The relative contents of esters were highest in B, aldehydes in D and alcohols in D. All sample compound component signal peaks were selected using the Gallery Plot plug-in that comes with the instrument to form a visual fingerprint that can practically reflect the overall characteristics of the sample, which was widely used in food flavor analysis [31,32]. Three times in parallel for each sample horizontally and the concentration content of each compound vertically, with darker colours indicating higher concentration content and lighter colours indicating lower concentration content (Fig. 4d), whereas the difference in flavor substances between sample B and sample C and the blank control was not significant. (+)Limonene-D, (E)-2-pentenal-D, (E, E)-2, 4-heptadienal, 2-methyl-2-hepten-6-one, tetrahydrofuran, alpha-pinene-M, 2-methyl-2-propanol, ethyl acetate, 3-methylbutylaldehyde, beta-myrcene-D, butyraldehyde-D, (E)-2-hexene-1-aldehyde-D, (Z)-2-methyl-pent-2-enal were more abundant in sample A than other, and these compounds may be derived from unsaturated fatty acid oxidation, the Maillard reaction, and free amino acid degradation [33,34]. Propyl acetate, 1-hexanol-D, 1-hexanol-M, and acrolein were presented only in high levels in sample D. Therefore, it can be assumed that these compounds were the characteristic flavor substances of Yu-Shiang Shredded Pork without pickled chillies peppers. From the above data, it can be inferred that pork and pickled chillies contributed more to the flavor formation of Yu-Shiang Shredded Pork, while fungus and lettuce had a relatively small effect.

From the heat map of volatile compound concentrations (Fig. 4e), 2-ethyl hexanol, Butanoic acid, 3-hydroxy-, ethyl ester-D, Butanoic acid ethyl ester and Allyl sulfide were characteristic flavor substances of A, while 1-Pentanol (monomer and dimer), (E)-2-Heptenal and n-Pentanol-D were characteristic flavor substances of D. In addition, the concentration of some compounds was increasing or decreasing with feedstock differences, which was visualized in the volatile compound concentration heat map. For example, concentrations of 2-methyl-2-hepten-6-one with peppery, mushroom, and rubbery aromas, beta-myrcene-D with balsamic, mustard, and spice aromas, and (E)-2-hexene-1-al-D with green, fruity aromas were significantly higher in the absence of shredded pork in Yu-Shiang Shredded Pork compared to the control. and n-pentanal-M, with almond, malty, and spicy flavors, and 1-butanol-3-

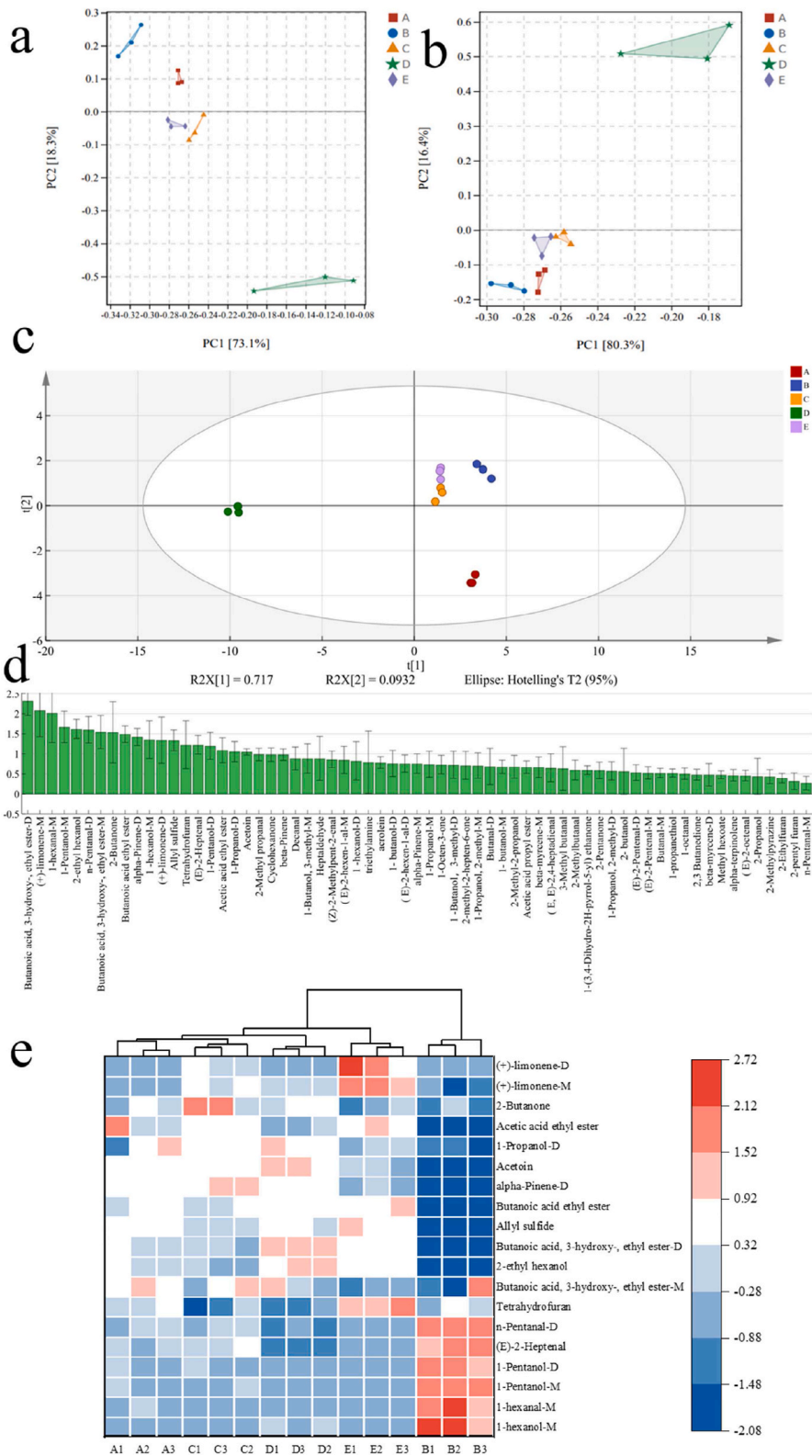


Fig. 5. (a) PCA scatter map of 68 volatile substances, (b) PCA scatter map of 19 differential volatile substances, (c) OPLS-DA scatter plot, (d) VIP distribution from five Yu-Shiang Shredded Pork samples, (e) clustering heatmap of the differential volatile compounds screened from five Yu-Shiang Shredded Pork samples. PCA, principal component analysis; OPLS-DA, Orthogonal Partial Least Squares-discriminant analysis.

hydroxy with butter and creamy flavors, and 1-butanol-3-methyl-D with winey, oniony flavors were significantly higher in Yu-Shiang Shredded Pork compared to E. Acetoin with butter and creamy flavors, 1-butanol-3-methyl-D with wine and onion flavors were significantly more in the absence of lettuce, 1-octen-3-one with ether and fruity flavors, 1-butanol-M with medicinal and fruity flavors, and 1-(3,4-Dihydro-2H-pyrrol-5-yl) ethanone with nutty and baking flavors were significantly more in the absence of pickled chillies [35]. This suggests that the flavor profile formed by the various balanced aroma combinations of the fish-flavor can be disrupted depending on the raw material, resulting in a characteristic flavor.

3.3.2. Principal component analysis and orthogonal partial least squares-discriminant analysis with cross-validation

The GC-IMS data consisting of peak intensity information of 68 compounds was analyzed by applying PCA to highlight the differences in volatile compounds treatment. As shown in Fig. 5a, the five groups achieved a good separation. The variance contributions of PC1 and PC2 were 73.1 % and 18.3 %, respectively, with a cumulative variance contribution of 91.4 %, much higher than the 80 % confidence values, on PC1, D was farther away from A, B, C, and E. The same trend can be observed in the clustered heat map (Fig. 5e), where the volatilization profile of D was significantly different from that of the other four Yu-Shiang Shredded Pork. Therefore, PCA and cluster similarity analysis can be used to differentiate the odour profiles of the five Yu-Shiang Shredded Pork.

To elucidate the active odour-contributing ingredients and understand their contributions, the OPLS-DA method is very effective in performing sample classification and discriminant modelling. The goodness of fit (R^2) and predictive power (Q^2) of the OPLS-DA model are used to discriminate between Yu-Shiang Shredded Pork samples with significant differences [36]. Values closer to 1.0 indicated greater predictive and explanatory power. As shown in Fig. 5c, the model parameters R^2X (cum), R^2Y (cum), and Q^2 (cum) were 0.989, 0.984, and 0.902, respectively. In addition, the intersection of the Q^2 (cum) regression line with the horizontal axis had a negative intercept (Fig. 5b), which proved the reliability of the model. These results showed a good model fit and acceptable predictable accuracy.

To characterize the key differential compounds obtained from different ingredients, the VIP was investigated [37]. In this model, a

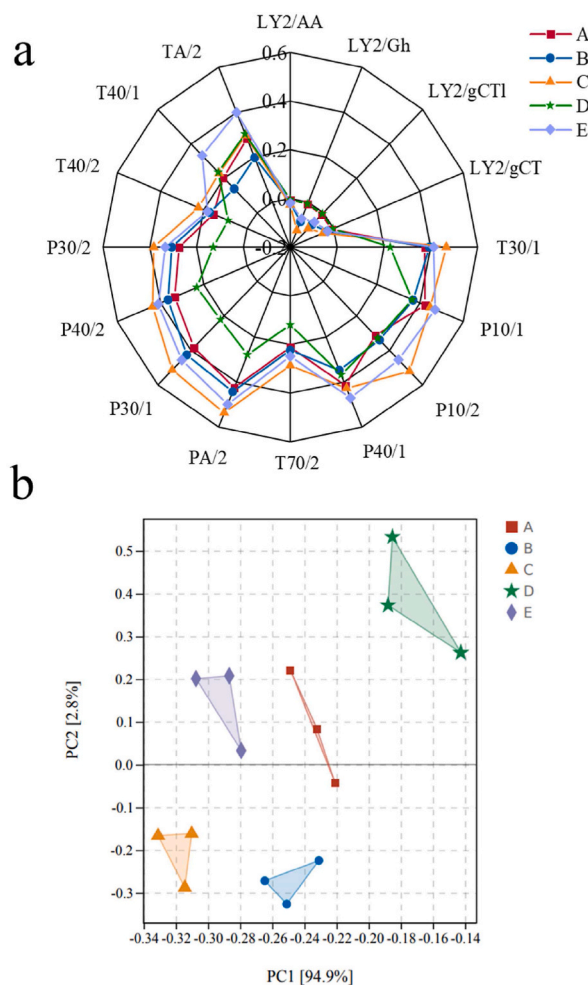


Fig. 6. E-nose radar plot and PCA 2D image of the samples of Yu-Shiang Shredded Pork. (a) E-nose Radar plot; (b) PCA graph for the E-nose analysis. PCA, principal component analysis.

total of 19 variables with VIP >1.0 were identified, namely 1-hexanol-M, 1-hexanal-M, Butanoic acid, 3-hydroxy-, ethyl ester (monomer and dimer), 2-ethyl hexanol, alpha-Pinene-D, Butanoic acid ethyl ester, 1-Propanol-D, Allyl sulfide, (+)-limonene (monomer and dimer), Acetic acid ethyl ester, Tetrahydrofuran, Acetoin, 2-Butanone, 1-Pentanol-M, (E)-2-Heptenal, n-Pentanal-D, 1-Pentanol-D, were considered the characteristic aromatic components. PCA and thermogram collection were performed using these discriminating indicator chemicals (Fig. 5b and e). The representation of the five Yu-Shiang Shredded Pork samples in Fig. 5b was better than that in Fig. 5a, and the specificity of most of the samples could be isolated by PCA. The clustering heat map results showed

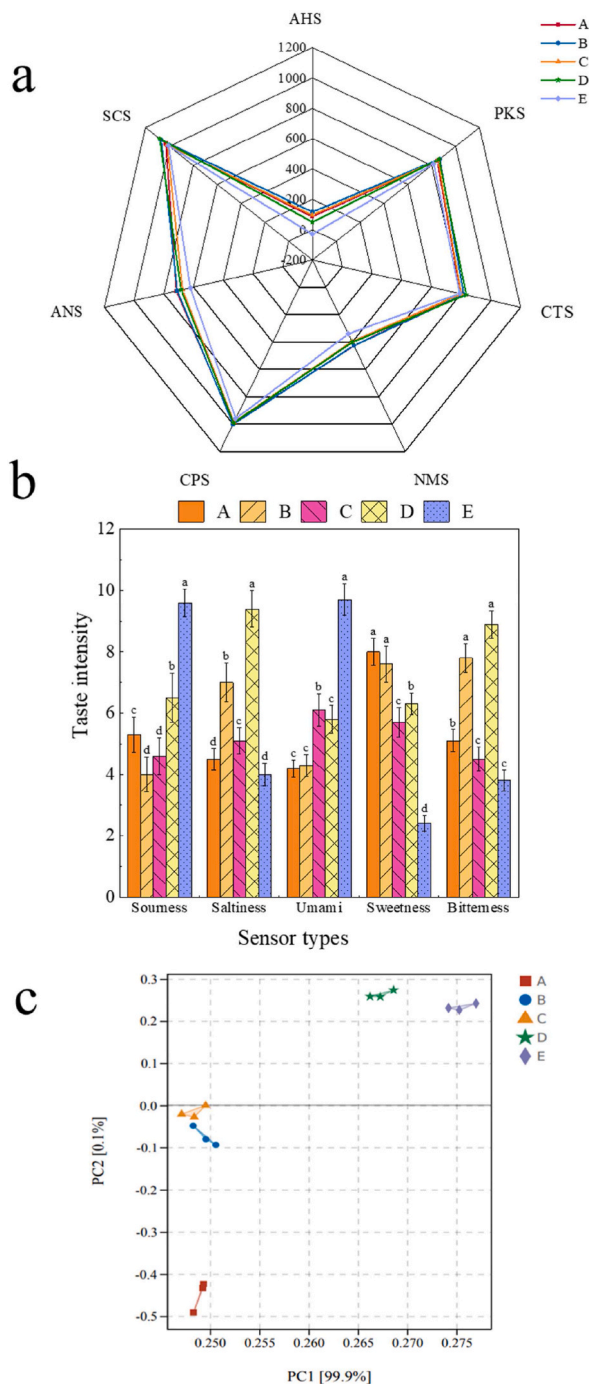


Fig. 7. (a) Radar graph for the E-tongue analysis. (b) Taste intensity histogram. (c) PCA graph for the E-tongue analysis. Values marked with different letters in the five columns of different colours ($p < 0.05$, $n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

that the variability among these 19 chemicals was more obvious. The results of related studies showed that the differentially labelled odour components screened by OPLS-DA plus those with VIP values above 1.0 could be used for classification between different samples [38]. Therefore, it is feasible to screen differential volatile flavor compounds in Yu-Shiang Shredded Pork based on the OPLS-DA model and VIP values [39]. Meanwhile, the results of this study also confirmed that combining the 19 indicative odour chemicals with PCA and aggregation plots could better distinguish the differences among the five types of Yu-Shiang Shredded Pork. The low threshold value of some volatile compounds with VIP <1.0 also has an essential impact on odorous smell, which requires further analysis.

3.4. Intelligent senses analysis

3.4.1. E-nose analysis

Various E-nose sensors exhibit distinct characteristics, and a single sensor can concurrently assess the overall concentration of diverse categories and similar substances [40,41]. As shown in Fig. 6a, the response values of the five types of Yu-Shiang Shredded Pork on LY2/AA, LY2/Gh, LY2/gCTI, and LY2/gCT sensors were almost zero, possibly due to the low content of ethanol, acetone, ammonia, propane, butane and sulfur compounds in samples, which was consistent with the results of chromatographic analysis. Based on the data shown in Fig. 6b, it can be concluded that the first and second principal components account for over 85 % of the PCA analysis. This indicates that the main flavor profile of the sample is accurately represented and suggests that the samples are reliable and consistent. When it comes to aroma, the B and C samples are quite similar, as their horizontal coordinate distribution distance is closer, indicating that there is no significant difference in the aroma characteristics of Yu-Shiang Shredded Pork without adding lettuce and fungus. However, there is a noticeable variation in the odour characteristics of the A, D, and E samples, as evidenced by the discrepancy in their cross-coordinates. Therefore, further analysis is necessary to explore the impact of specific flavor compounds in conjunction with the GC-IMS findings.

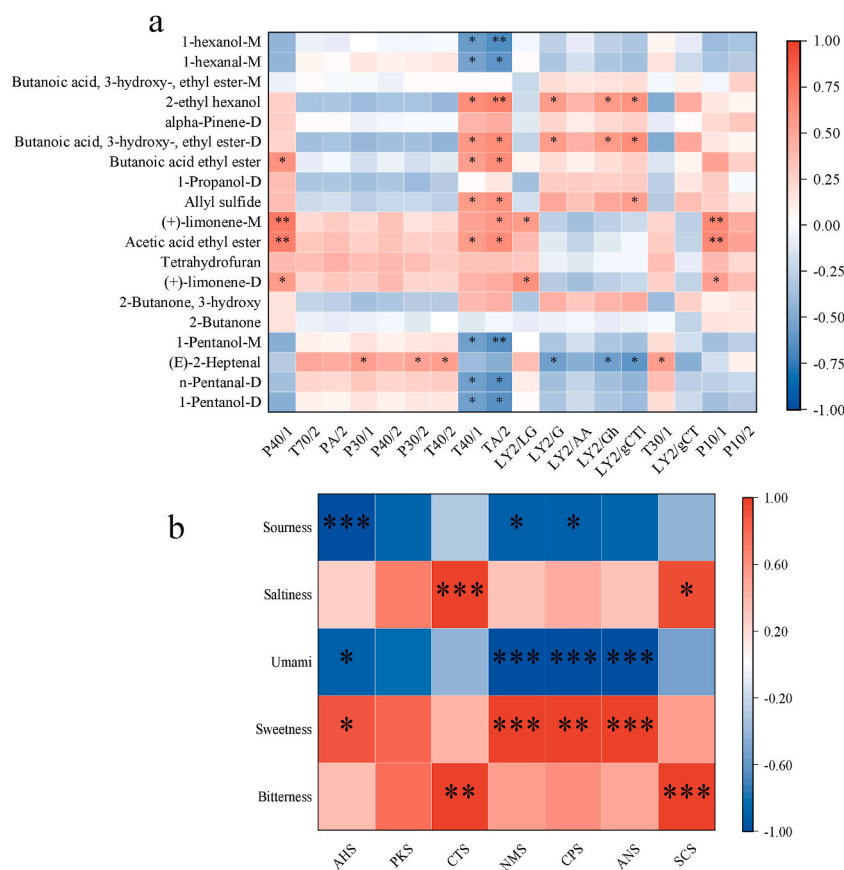


Fig. 8. (a) The heat map of the correlation between the response values of the E-nose sensors and the levels of differential volatile compounds. (b) The heat map of the correlation between the response values of the E-tongue sensors and the contents of FAAs. The symbols "*" and "***" signify significant ($p < 0.05$) and extremely significant ($p < 0.01$) correlations, respectively. Positive ($0 < r < 1$) and negative ($-1 < r < 0$) correlations are displayed in red and blue, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4.2. E-tongue analysis

From the electronic tongue analysis of the flavor radar diagram Fig. 7a of Yu-Shiang Shredded Pork, it can be seen that there were differences in the corresponding intensity values of the AHS, ANS, and NMS sensors for the five samples. Moreover, as shown in Fig. 7b, there was a significant difference in the four taste intensities between E and the other four Yu-Shiang Shredded Pork. Compared to E, the sourness intensity of other samples sharply decreased, and the saltiness, sweetness, and bitterness were each increased by a certain degree. Based on the analysis of the PCA results, as depicted in Fig. 7c, it is evident that sample E stands out from the rest of the samples (A, B, C, and D) due to its clear distinction and significant independence. Conversely, samples A and C were relatively closer, suggesting minor variances. The first and second principal components contributed 99.9 % and 0.1 % respectively, with a combined total exceeding 85 %, indicative of distinct taste profile differences overall.

3.5. Correlation analysis

3.5.1. Correlation between E-nose and GC-IMS

To improve the overall effectiveness of both E-nose and GC-IMS, an investigation was conducted to explore the potential correlation between E-nose sensor responses and volatile compound levels detected by GC-IMS. As depicted in Fig. 8a, the potential correlation between the response values of the E-nose sensor and the levels of 19 differential volatile compounds with VIP value > 1.0 detected by GC-IMS was analyzed. Several sensors including LY2/G, LY2/AA, LY2/Gh, LY2/gCT, T40/1, and TA/2 showed a positive correlation with major compounds such as 2-ethyl hexanol, Butanoic acid, 3-hydroxy-, ethyl ester-D, Butanoic acid ethyl ester, Allyl sulfide, which were identified at high levels in A compared to E through GC-IMS analysis. Conversely, sensors LY2/G, LY2/AA, LY2/Gh, LY2/gCT, T40/1, and TA/2 were negatively correlated with compounds such as 1-Pentanol (monomer and dimer), (E)-2-Heptenal, and n-Pentanol-D, which were found to be higher in D compared to E. This may be the key potential flavor precursors for the enhancement of key aroma compounds of dry-fried shredded beef samples. The remaining volatile compounds that did not show a significant correlation with the electronic nose sensor, such as alpha-Pinene-D and Tetrahydrofuran were expressed at higher levels in B and C, respectively. Therefore, the combination of E-nose and GC-IMS can distinguish the five Yu-Shiang Shredded Pork based on their olfactory sense.

3.5.2. Correlation between E-tongue and the content of FAAs

The E-tongue can provide information on five taste intensities of Yu-Shiang Shredded Pork: saltiness (CTS), umami (NMS), sourness (AHS), sweetness (ANS), and bitterness (SCS). Flavor amino acids affect the texture and taste of Yu-Shiang Shredded Pork. Therefore, the electronic tongue response values of sensor were correlated with the content of five flavor types. As shown in Fig. 8b, saltiness, and bitterness were positively correlated with CTS and SCS, on the other hand, sweetness was positively correlated with AHS, NMS, CPS, and ANS. Notably, sourness and umami exhibited a negative correlation with AHS, NMS, and CPS. The results indicated that there was a certain correlation between the content of flavor amino acids in Yu-Shiang Shredded Pork and the response value of E-tongue sensors, which could be used in combination to analyze the taste characteristics of Yu-Shiang Shredded Pork.

4. Conclusion

The current study thoroughly evaluated aroma compounds and the formation of flavor precursors (FAAs) in Yu-Shiang Shredded Pork with different raw materials. The results of the study showed that pork affected the fat and energy levels of Yu-Shiang Shredded Pork, lettuce affected the moisture and protein levels of the Yu-Shiang Shredded Pork, and fungus affected the carbohydrate levels of the Yu-Shiang Shredded Pork. The levels of free amino acids in Yu-Shiang Shredded Pork showed a descending order of D, A, E, C, and B. Notably, the umami amino acid played a primary role in defining the dish's taste. Upon GC-MS analysis, samples A, B, C, D, and E displayed 43, 42, 53, 36, and 50 VOCs, respectively. The most significant difference in the volatile matter was between samples A and D, indicating that the absence of shredded pork and pickled chillies had a greater impact on the flavor of Yu-Shiang Shredded Pork. A total of 68 compounds, including 22 aldehydes, seven esters, seven ketones, 17 alcohols, one pyrazine, three furans, one pyrrole, one thioether, eight olefins, and one amine, were identified by GC-IMS, among which esters, aldehydes, and alcohols were the main chemical constituents in Yu-Shiang Shredded Pork. During the study, 19 compounds were analyzed using the OPLS-DA model, revealing that the combination of E-nose with GC-IMS and E-tongue with free amino acids was able to differentiate between five variants of Yu-Shiang Shredded Pork via their respective smells and tastes. Based on research findings, pickled chili is an essential component that imparts a distinct flavor to Yu-Shiang Shredded Pork. Its omission can significantly impact the overall taste of the dish. Thus, pickled chili ranks as the most crucial seasoning element, and it should not be disregarded when cooking Yu-Shiang Shredded Pork. However, the flavor formation of Yu-Shiang Shredded Pork was a complex system working together, and the analysis of the flavor-presenting mechanism of Yu-Shiang Shredded Pork by raw materials alone may still be relatively weak, but these results lay a foundation for further research on flavoring substances of Yu-Shiang Shredded Pork in the future.

Ethics statement

Review and/or approval by an ethics committee was not needed for this study because it did not involve animal or human clinical trials and was not unethical.

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Data availability

Data will be made available on request.

CRediT authorship contribution statement

Jia Chen: Writing – original draft, Investigation, Formal analysis. **Xuemei Cai:** Writing – review & editing, Supervision. **Junliang Liu:** Data curation. **Can Yuan:** Writing – review & editing, Supervision. **Yuwen Yi:** Formal analysis, Data curation. **Mingfeng Qiao:** Writing – review & editing, Visualization, Data curation, Conceptualization.

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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