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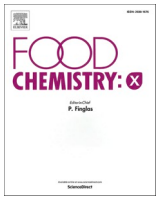

Identification of aroma active compounds in Shanxi aged vinegar and tracing the source in the entire production process

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ABSTRACT

The aroma-active compounds (AACs) of traditional vinegars are formed through complexed pathways. Moreover, their source is not clear yet. The composition of AACs throughout the entire process of Shanxi aged vinegar (SAV) production from the raw materials, alcohol fermentation (AF), acetic acid fermentation (AAF), fumigation and aging stages were analyzed. A total of 121 AACs were determined by GC × GC-O-TOF-MS, of which 88 were identified by GC–MS/MS. There were 27 and 41 AACs (FD ≥ 400) detected in before-aging and after-aging SAV, respectively. Furthermore, it can be concluded the AAF and fumigation were the main sources of AACs. Also, the AAF was the main source of the compounds with caramel, creamy, floral, fruity, sour, cheesy notes, while the compounds owned roasted, nutty, spicy and woody aromas mainly came from fumigation. Finally, the potential transformation pathway of AACs was mapped and discussed. Overall, GC × GC-O-TOF-MS was a useful technique to enrich the AACs of SAV.

1. Introduction

Vinegar is the most widely used acidic condiment in the world, with a history of more than 5000 years. In China, most vinegars are produced with cereals as raw materials, while many European and American countries use fruits as raw materials, highlighting grapes and apples, to produce vinegar. The flavors of Chinese vinegars are different from each other due to varied fermentation arts and the raw materials. There are several typical Chinese cereal vinegars, including Shanxi aged vinegar (SAV), Zhenjiang aromatic vinegar (ZAV), Fujian Monascus vinegar, and Sichuan Baoning vinegar. Among them, SAV is one of the most influential and marketable vinegar. Also, SAV is the only vinegar that has been shortlisted as a certified product with geographical indication in both China and Europe.

The unique flavor of each type vinegar type is closely related to its production processes. To obtain a genuine SAV, it is necessary to go through four important production stages (Fig. 1 and Fig. S1): alcohol fermentation (AF), acetic acid fermentation (AAF), fumigation, and aging (Han et al., 2023; Wang et al., 2012; Wu et al., 2021). Previous studies have shown that various microorganisms appear and dynamically change during AAF, and at the same time, generating a variety of aroma compounds (Zhu et al., 2018). Fumigation is one of the essential stages of the SAV brewing, and is a unique craft of SAV, compared with the other traditional Chinese vinegars. Moreover, fumigation proffers a unique burnt, smoky, caramel odor, and special color (Han et al., 2023). Subsequently, the abundant chemical reactions and physical changes make the flavor of SAV more soft, round, and rich during aging (Liang et al., 2016). A comprehensive analysis of the flavor compounds changes

Abbreviations: SAV, Shanxi aged vinegar; AACs, aroma active compounds; ZAV, Zhenjiang aromatic vinegar; AF, alcohol fermentation; AAF, acetic acid fermentation; GC-O-MS, gas chromatography-olfactory-mass spectrometry; SPME, solid phase microextraction; AEDA, aroma extract dilution analysis; SAFE, solvent-assisted flavor evaporation; FD, flavor dilution; GC × GC-O-MS, comprehensive two-dimensional gas chromatography-olfactometry-mass spectrometry; TOF-MS, time-of-flight mass spectrometry; AAB, acetic acid bacteria.

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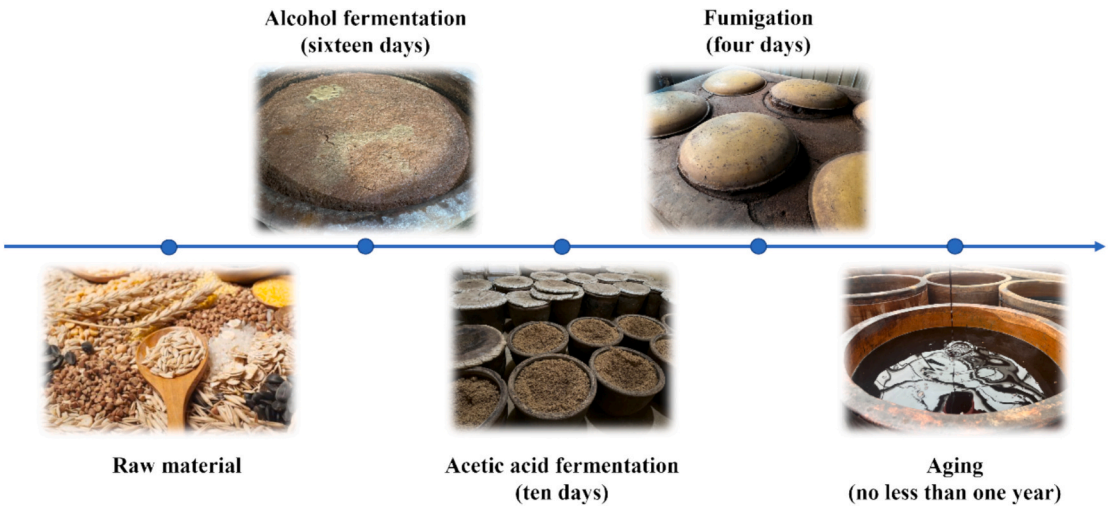

Fig. 1. The main production stages of SAV. The process includes such four steps as alcohol fermentation, acetic acid fermentation, fumigation and aging.

and sources in the entire production process is worth understanding the SAV brewing.

Flavor is a key factor in determining products quality and consumers choice. Many studies have focused on the flavor of vinegar (Callejón et al., 2010; Cirlini et al., 2011; Corsini et al., 2019; Liang et al., 2016; Wang et al., 2012; Zhou et al., 2020). Nowadays, gas chromatography-olfactory-mass spectrometry (GC-O-MS) has proved as the most reliable technique for qualitative and quantitative of aroma active compounds (AACs), and has been popularly adopted owing to its convenience, simplicity, and efficiency (Fischer et al., 2021). 2,3-Butanedione, 2-methylpropanal, acetic acid, 2,4,5-trimethyloxazole, sotolon, 3-methyl-butanoic acid, and tetramethyl pyrazine, identified as the key AACs in ZAV, were analyzed by solid phase microextraction (SPME)-GC-O-MS (Zhou et al., 2020). To understand the contribution of AACs on the odor quality of SAV, Liang et al. (2016) appraised the aroma profiles of both before-aging and after-aging SAV by GC-O-MS and aroma extract dilution analysis (AEDA). The results found that 87 odor-active regions were recognized, and 80 AACs in SAV were identified by the GC-O-MS and solvent-assisted flavor evaporation (SAFE). Methional, 2,3-butanedione, vanillin, tetramethyl pyrazine, γ-nonalactone, 3-methylbutanoic acid, 3-(methylthio)propyl acetate, guaiacol, dimethyl trisulfide, among others, were found to be some important AACs considering their high flavor dilution (FD) factors (Liang et al., 2016). However, owing to the complexity and diversity of aroma profiles, and the lower separation capacity, sensitivity, and peak capacity of GC-O-MS, which makes it laborious to fully characterize AACs of SAV, especially some trace compounds. Consequently, some innovative instrumental analytical techniques have been developed flavor analysis in food (Li et al., 2023). Comprehensive two-dimensional gas chromatography-olfactometry-mass spectrometry (GC × GC-O-MS) has been applied on aroma analysis of *Zanthoxylum bungeanum* (Zhao et al., 2022), oyster sauce (Yu et al., 2022), *rougui* tea leaves (Yang, Song, et al., 2021), and milk (Guo et al., 2023) as a novel technique in recent years. The co-elution analytes from one-dimensional column are collected and determined by two-dimensional column and time-of-flight mass spectrometry (TOF-MS) after passing through the modulation process in Deans Switch, which extremely enhances the sensitivity and resolution of the GC × GC-O-TOF-MS. Compared to GC-O, more odor active regions were determined more accurately by combining sniffed data with two-dimensional data (Zhao et al., 2022). In a previous study, Li et al. (2023) found a total of 274 volatile compounds in tomato pastes by GC × GC-O-TOF-MS, far more than previously reported, and exceeding 6 times that of GC-O-MS.

To comprehensively understand the composition of AACs and their changes and sources in the entire production process (raw materials, AF, AAF, and fumigation) of SAV, samples from different production processes were analyzed by the methods of GC × GC-O-TOF-MS and SPME-GC–MS/MS in this research. In particular, the source, contribution, and possible transformation pathways of main AACs in different production stages were elaborated.

2. Materials and methods

2.1. Materials

All samples were collected from Shanxi Zilin Vinegar Industry Co., Ltd., Taiyuan, China. Sample collection includes raw materials (cereal husk, rice hull, barley, sorghum, peas, and wheat bran), *Daqu*, alcohol fermentation (AF, 0d, 1d, 3d, 7d, 11d, 15d), acetic acid fermentation (AAF, 1d, 3d, 5d, 7d, 9d), and fumigation (F-0d, F-1d, F-2d, F-3d, F-4d). The AF samples were taken out after stirring the fermenter. The AAF samples, named *Cupei*, were collected in the upper, middle, and lower layers of the fermenter, and five positions (east, south, west, north and center) of each layer were collected, all position samples were mixed. Similarly, fumigation samples were collected and mixed. The liquid sample before and after aging were collected on the aging day 0 and year 1. The detailed production process of SAV is illustrated in Fig. S1.

2.2. Chemicals

All the chemicals (chromatographic grade), including ethyl acetate (≥99.7 %), 2,3-butanedione (≥99.0 %), acetic acid (≥99.8 %), benzaldehyde (≥99.5 %), 2-methyl-propanoic acid (≥99.5 %), phenylethyl alcohol (≥99.5 %), phenol (≥99.5 %), heptanoic acid (≥99.0 %), nonanoic acid (≥99.0 %), 2-octanol (≥99.5 %), and n-decanoic acid (≥99.5 %) were purchased by Macklin Biochemical Co. Ltd. (Shanghai, China). C_7-C_{40} normal alkanes used for calculating the retention indices (RIs) were purchased from O2si Smart Solutions (South Carolina, USA).

2.3. SPME of volatile components

Two grams sample (except 1 g vinegar) was transferred into a 20 mL glass vial with a silicon septum, and then 3 mL saturated sodium chloride solution were added. The sample was placed into a thermostatic water bath and equilibrated at 60 °C for 20 min. A SPME fiber (1 cm, 50/30 μm, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), Agilent Technologies, USA) was exposed to the sample headspace for 40 min. After extraction, the SPME fiber was desorbed for 5 min at 250 °C in the injector port. The injector temperature was 250 °C (Zhou et al., 2020).

Table 1
RI, Odor Descriptors, and FD Factors Determined by GC-O Analysis and the Compounds Identified in the SAV before-aging and after-aging.

RI	Compound	Odor descriptors[1]	FD[2]		Identification method[3]
			B-aging	A-aging	
876	2-Methyl-furan	smoky	9	3	MS, RI, O
889	Ethyl Acetate	fruity	27	9	MS, RI, O, S
889	2-Butanone	fruity	3	0	MS, RI, O
916	3-Methyl-butanal	liquorice	81	243	MS, RI, O
970	2,3-Butanedione	cream, yogurt	400	400	MS, RI, O, S
977	n-Propyl acetate	fruity, sweet	1	0	MS, RI, O
1016	Methyl thiolacetate	onion	1	0	MS, RI, O
1060	2-Methyl-2-butenal	smelly	27	9	MS, RI, O
1062	2-Methyl-1-propanol	alcohol	27	9	MS, RI, O
1100	3-Penten-2-one	fruity	0	9	MS, RI, O
1122	Acetyl valeryl	cheesy	0	1	MS, RI, O
1159	Heptanal	fruity	0	3	MS, RI, O
1208	2-Pentyl-furan	earthy, green	1	0	MS, RI, O
1253	Acetoin	buttery	400	400	MS, RI, O
1264	Octanal	fruity	1	0	MS, RI, O
1275	1-Octen-3-one	mushroom	3	3	MS, RI, O
1296	2,5-Dimethyl-pyrazine	nutty, fatty	9	400	MS, RI, O
1301	2,6-Dimethyl-pyrazine	roasted peanuts	27	81	MS, RI, O
1307	Ethyl-pyrazine	nutty	27	9	MS, RI, O
1345	3-Ethoxy-1-propanol	fruity	243	400	MS, RI, O
1350	2-Acetoxy-3-butanone	sweet	3	3	MS, RI, O
1380	Trimethyl-pyrazine	musty, roasted peanuts	9	400	MS, RI, O
1401	5-Methyl-2(3H)-furanone	chocolate	243	81	MS, RI, O
1412	Acetic acid	sour	9	400	MS, RI, O, S
1420	Linalool oxide	floral	400	400	MS, RI, O
1421	3-Ethyl-2,5-dimethyl-pyrazine	French fry	81	81	MS, RI, O
1422	1-Octen-3-ol	mushroom	81	0	MS, RI, O
1431	Furfural	smelly, woody	400	400	MS, RI, O
1439	2,3-Dimethyl-5-ethylpyrazine	cocoa	243	400	MS, RI, O
1447	trans-Linalool oxide	floral, caramel	9	3	MS, RI, O
1453	Tetramethyl-pyrazine	nutty, fatty	1	3	MS, RI, O
1462	2-Ethyl-1-hexanol	sweet	243	81	MS, RI, O
1472	2-Acetylfuran	caramel	243	400	MS, RI, O
1490	2,3,5-Trimethyl-6-ethylpyrazine	musty	0	3	MS, RI, O
1491	Benzaldehyde	bitter almond	243	81	MS, RI, O, S
1503	2-Furanmethanol, acetate	pungent	3	9	MS, RI, O
1507	2,3-Butanediol	fruity	81	400	MS, RI, O
1519	5-Methyl-2-furancarboxaldehyde	sweet	0	1	MS, RI, O
1529	1-Octanol	fatty, fruity	3	0	MS, RI, O
1580	2-Acetyl-5-methylfuran	sweet, fruity	3	9	MS, RI, O
1593	Butyrolactone	cream	243	400	MS, RI, O
1597	3-(Methylthio)propyl acetate	fruity, fatty	400	400	MS, RI, O
1607	Benzeneacetaldehyde	pungent floral	400	400	MS, RI, O
1622	2-Furanmethanol	burnt	400	400	MS, RI, O
1629	2-Methyl-propanoic acid	fruity, milky	1	1	MS, RI, O, S
1642	Ethyl succinate	mild-sweet	243	400	MS, RI, O
1649	2-Hydroxy-benzaldehyde	pungent almond	3	0	MS, RI, O
1668	4-Hexanolide	herbal	3	9	MS, RI, O
1668	α-Terpineol	clove	81	0	MS, RI, O
1673	2,5-Dimethyl-3-isoamylpyrazine	musty	0	1	MS, RI, O
1681	3-Methyl-butanoic acid	rancid, cheesy	400	400	MS, RI, O
1683	Methionol	sulfur, brewis	9	400	MS, RI, O
1694	Benzyl acetate	sweet, fruity	400	400	MS, RI, O
1708	2,3-Dimethyl-5-(Z-1-propenyl)-pyrazine	smelly	400	400	MS, RI, O
1714	2(5H)-Furanone	winey	3	400	MS, RI, O
1721	unknown	rancid	0	243	RI, O
1728	3,4-Dihydroxy-benzaldehyde	green	3	9	MS, RI, O
1743	Methyl salicylate	green	0	9	MS, RI, O
1752	Ethyl phenacetate	sweet, honey	400	81	MS, RI, O
1768	γ-Heptalactone	caramel	243	243	MS, RI, O
1772	1-Phenyl-1,2-propanedione	fatty	3	1	MS, RI, O
1774	α-Methyl-benzenemethanol	nutty	9	9	MS, RI, O
1781	Phenethyl acetate	honey, green	400	400	MS, RI, O
1790	β-damascenone	rose	400	9	MS, RI, O
1790	1-(2-Furanylmethyl)-1H-pyrrole	coffee	3	0	MS, RI, O
1808	unknown	herbal	0	400	RI, O
1820	2-Methoxy-phenol	sweet, woody	243	400	MS, RI, O
1836	Benzyl alcohol	sweet	243	400	MS, RI, O
1849	Phenethyl propionate	fruity, floral	400	243	MS, RI, O
1858	4-Methyl-pentanoic acid	herbal	1	3	MS, RI, O
1865	4-(2-Furanyl)-3-buten-2-one	herbal, floral	9	3	MS, RI, O
1872	Phenylethyl alcohol	floral	400	400	MS, RI, O, S
1880	γ-Octalactone	coconut	400	400	MS, RI, O

(*continued on next page*)

Table 1 (*continued*)

RI	Compound	Odor descriptors[1]	FD[2]		Identification method[3]
			B-aging	A-aging	
1891	α-Ethylidene-benzeneacetaldehyde	green	3	243	MS, RI, O
1904	α-(2-Methylpropylidene)-benzeneacetaldehyde	cocoa	1	9	MS, RI, O
1909	trans-β-Ionone	smoky	3	0	MS, RI, O
1913	Creosol	spicy	400	400	MS, RI, O
1928	1-(1H-pyrrol-2-yl)-ethanone	nutty	3	27	MS, RI, O
1931	1-Dodecanol	fatty	400	27	MS, RI, O
1958	2-Methyl-phenol	green	1	27	MS, RI, O
1961	Phenol	herbal	3	243	MS, RI, O, S
1981	1H-Pyrrole-2-carboxaldehyde	fatty, roast	9	1	MS, RI, O
1988	4-Ethyl-2-methoxy-phenol	floral, herbal	400	400	MS, RI, O
1991	γ-Nonalactone	coconut	243	400	MS, RI, O
2012	3-Phenylpropanol	floral	243	81	MS, RI, O
2016	Heptanoic acid	sour, fatty	243	9	MS, RI, O, S
2031	2,5-Dimethyl-phenol	herbal	0	9	MS, RI, O
2037	p-Cresol	Sour Bamboo Shoot	400	400	MS, RI, O
2037	5-Methyl-2-phenyl-2-hexenal	fruity	3	0	MS, RI, O
2065	2-Methoxy-4-propyl-phenol	spicy	400	400	MS, RI, O
2083	1-(4-Methoxyphenyl)ethanone	rose	400	400	MS, RI, O
2089	Cedrol	smoky, woody	0	9	MS, RI, O
2098	3,5-Dimethyl-phenol	green	0	9	MS, RI, O
2120	Eugenol	nutty, clove	3	27	MS, RI, O
2125	4-Ethyl-phenol	sweet, brewis	243	81	MS, RI, O
2134	1-Tetradecanol	wax	3	9	MS, RI, O
2149	2-Methoxy-4-vinylphenol	fatty, spicy, clove	400	243	MS, RI, O
2166	2-Methyl-5-(1-methylethyl)-phenol	spicy	0	27	MS, RI, O
2171	3,4-Dimethyl-phenol	woody	0	9	MS, RI, O
2177	unknown	soap	400	400	RI, O
2193	5,6-Dihydro-6-pentyl-2H-pyran-2-one	fatty, floral	9	400	MS, RI, O
2218	γ-Undecalactone	fruity	0	243	MS, RI, O
2218	2,6-Dimethoxy-phenol	woody, green	0	9	MS, RI, O
2227	Ethyl hexadecanoate	sweet, floral	400	400	MS, RI, O
2264	Nonanoic acid	spicy, cheesy	81	400	MS, RI, O, S
2295	trans-Isoeugenol	spicy	0	9	MS, RI, O
2304	Dihydroactinidiolide	musky	9	400	MS, RI, O
2315	n-Decanoic acid	smoky, rancid	0	9	MS, RI, O, S
2331	γ-Dodecalactone	fruity, floral	9	0	MS, RI, O
2336	2,3-Dihydro-benzofuran	nutty	9	9	MS, RI, O
2365	Undecanoic acid	fatty	0	9	MS, RI, O
2380	unknown	smoky	0	400	RI, O
2407	unknown	herbal, jasmine	0	9	RI, O
2443	5-Hydroxymethylfurfural	green, nutty	27	9	MS, RI, O
2461	α-Methyl-benzeneethanol	floral	0	9	MS, RI, O
2566	Vanillin	caramel, creamy	400	400	MS, RI, O
2614	Ethyl vanillate	roast, coffee	1	3	MS, RI, O
2636	Benzyl Benzoate	mild-sweet	400	400	MS, RI, O
2654	Apocynin	vanilla	1	3	MS, RI, O
2664	2-Phenyl-3-(2-furyl)-propenal	herbal, rancid	3	1	MS, RI, O
2676	unknown	roasted	0	1	RI, O

[1] Odor quality as perceived at the sniffing port during GC-O.
[2] FD = flavor dilution; B-aging = Before-aging; A-aging = After-aging.
[3] MS, mass spectra; O, odor; RI, retention index; S, standard compounds.

2.4. GC × GC-O-TOFMS and AEDA analysis

The AACs from the before-aging and after-aging SAV samples were analyzed by GC × GC-O-TOF-MS following the method of Yang et al. with minor modifications (adjusting heating procedure and scan range) (Yang, Ye, et al., 2021). An Agilent 8890 series GC (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with a sniffing system (ODP3 Gerstel, Germany) and a TOF-MS (He Xin Mass Spectrometry Co., ltd., Guangzhou, China) was used to position AACs. The first-dimension columns (DB-wax polor; 60 m × 0.25 mm, 0.25 μm; Agilent, Palo Alto, CA, USA) and the second-dimension columns (DB-17: mid-polar; 1.85 m × 0.18 mm, 0.18 μm; Agilent, Palo Alto, CA, USA) were used in GC. In order to control the heating and cooling of volatiles, an SSM1800 solid-state modulator (J&X Technology Co., ltd, Shanghai, China) with a modulation period at 4 s was placed between the two columns. The cold zone temperature was maintained (−51 °C). The initial column temperature was set at 40 °C, followed holding 5 min, and a subsequent increase to 229 °C at the rate of 3 °C/min, which was held for 5 min, finally increased to 232 °C at a rate of 3 °C/min and kept 9 min. Electron-impact mass spectra at 70 eV ionization energy within 35–350 *m/z* range were generated. The temperature of MS source, MS quadrupole, transfer line and olfactory port were set at 230 °C, 150 °C, 250 °C and 230 °C, respectively.

To avoid the loss of potential AACs, GC-O and AEDA were carried out by 3 well-trained panelists. Each panelist evaluated the sample once in two times segments of 83 min to avoid fatigue. The panelists recorded the aroma notes and intensity as well as the time when the aroma compound occurred during the GC-O. If two or more panelists perceived the AACs, an aroma active location was identified. The FD factor of each compound was determined by AEDA. The isolate was extracted by SPME, the dilution was conducted by adjusting the split ratio (1:3, 1:9, 1:27, 1:81, 1:243, 1:400) (due to pressure reasons, the maximum split ratio of the instrument can only be set to 1:400). Sniffing at all dilution levels was repeated three times. The FD factor of each compound represents the maximum dilution at which the odorant can be recognized. Identification of each AACs was carried out by comparing their RI,

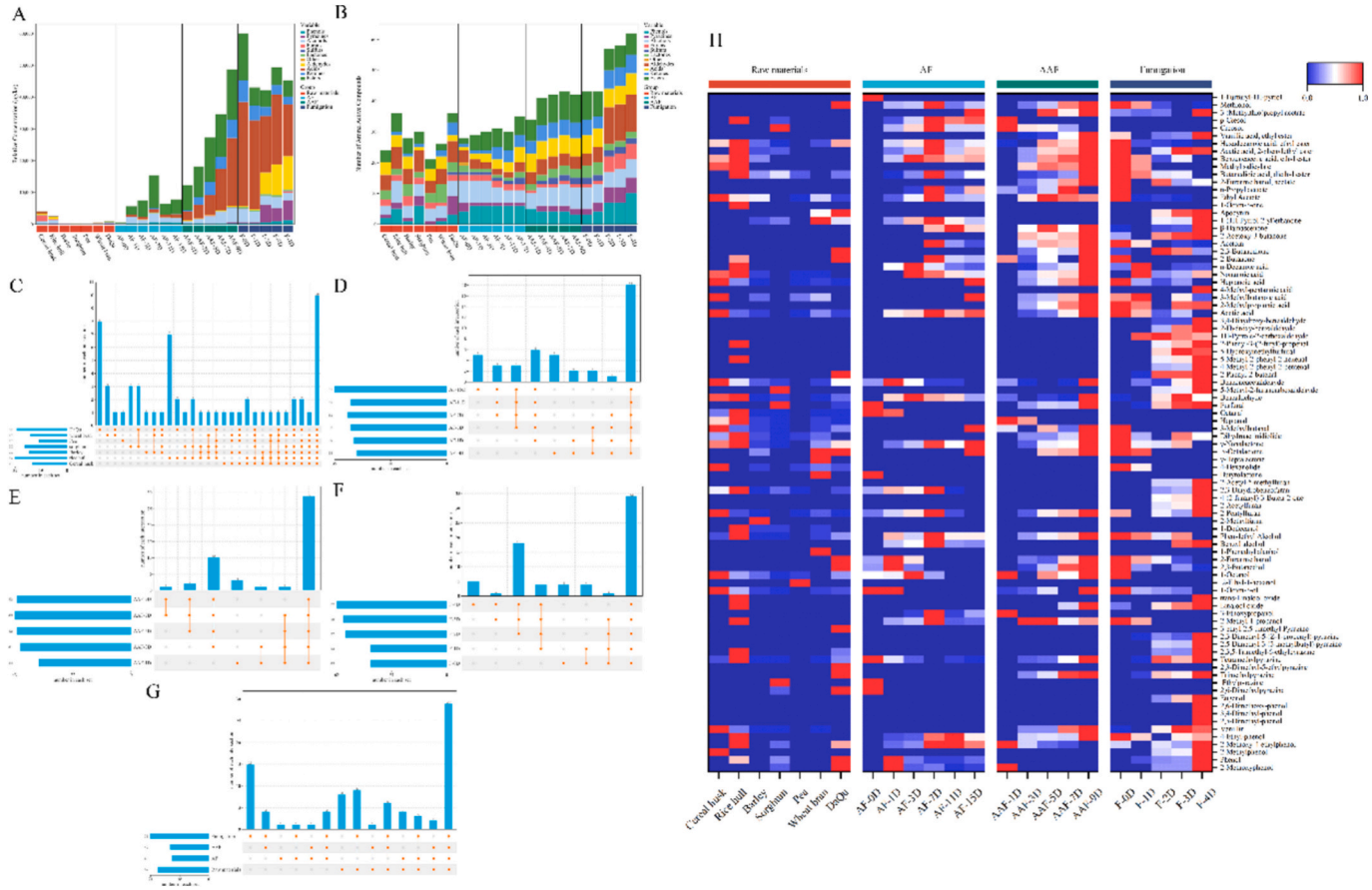

Fig. 2. The concentration (A) and numbers (B) changes of AACs in SAV at different production stages. The numbers changes of AACs in SAV at different production stages (C-G). The concentration changes of AACs in SAV at different production stages (H). Alcohol fermentation (AF); Acetic acid fermentation (AAF).

odors, and MS with standard compounds. High-purity nitrogen (99.999 %) was applied as the carrier gas of GC-O.

2.5. GC–MS/MS analysis

GC–MS/MS analyses were carried out with an Agilent 8890 GC equipped with an Agilent 7000D/TQ (Agilent Technologies Inc., Santa Clara, CA, USA). The extraction fiber was inserted into the injector port, and split mode (5:1) was set. The separation of volatiles was performed on DB-wax column and HP-5 column (30 m × 0.25 mm i.d × 0.25 μm film, Agilent Technologies). The gas flow rate was 0.8 mL/min at constant flow mode. The oven temperature was held at 40 °C for 2 min, and then increased to 170 °C at a rate of 3 °C/min held for 2 min, finally increased to 230 °C at a rate of 5 °C/min. The mass spectrometer in the electron impact (EI) mode with a 70 eV ionization energy was operated, and the source temperature and injector port were set at 230 °C and 250 °C, respectively. Full-scan acquisition (35–350 *m/z* range) was used (Zhou et al., 2020).

2.6. Qualitative and quantitative analysis of volatile compounds

The volatile compounds were determined and confirmed by MS, retention index (RI), odor descriptor (O), and standard compounds (S). The results were compared to the data from the NIST 2017. Subsequently, the identified compounds were reconfirmed by calculating RI. Finally, the standard compounds were used for verification (Al-Dalali et al., 2020).

Besides, the internal standard (2-octanol) was used to calculate the semi-quantitative concentration of volatile compounds on the DB-wax and HP-5 column. The concentrations of AACs were identified by the ratio of the aroma compound's peak area relative to the internal standard's peak area against the ratio of their concentrations (Yang, Ye, et al., 2021).

2.7. Statistical analysis

The changing trend of volatile compounds in samples at different production stages was analyzed by Venn diagram, bar chart, and heatmap via the genescloud tools, a free online platform for data analysis (https://www.genescloud.cn). All experiments have been carried out in triplicates (expressed as the mean ± standard deviation).

3. Results and discussion

3.1. Aroma active compounds of SAV identified by GC × GC-O-TOFMS

A total of 121 aroma active regions (including 6 unknown compounds) were detected by GC × GC-O-TOFMS (Table 1) from the before and after aging samples of SAV. The typical aroma characteristics perceived were sour, nutty, caramel, fruity, cheesy, sweet, floral, fatty, roasted, etc. Among a total of 115 compounds were determined, including alcohols (18), aldehydes (17), ketones (16), phenols (16), esters (14), pyrazines (10), lactones (9), acids (8), furans (5), sulfur compound (1), and other (1). It was previously reported that 80 AACs were detected in SAV by GC-O (Liang et al., 2016), of which 43 were consistent with this experiment, possibly due to differences in chromatographic column and extract method (HP-5 column and SAFE in previous study; DB-wax and SPME in this study). Among all of 114 volatiles, 58 compounds were found to have the highest FD (≥243). These compounds contributed aroma notes described as caramel (2,3-

butanedione), brewis (methionol), cheesy (3-methyl-butanoic acid), fruity (3-(methylthio)propyl acetate), honey (phenylethyl alcohol), coconut (γ-nonalactone), creamy (vanillin), sour bamboo shoot (p-cresol), and so on. It provided a variety of unique flavors, and played a key role on SAV aroma.

With the FD ranging between 9 and 243, the 13 aroma compounds (Table 1) made some contribution to the overall flavor of SAV, especially to the background aromas including clove, green, French fry, fruity, mushroom, and smelly. Fifty aroma compounds ($9 \geq FD \geq 1$) were found in the SAV samples. Although these ingredients had low FD, they probably had a relatively little impact on the aroma of SAV and may contribute especially to the background aromas including coffee, herbal, cocoa, onion, roasted, woody, and smoky.

Alcohols bring fruity, floral, sweet, burnt, and fatty odor to SAV. Seven compounds with high FD (≥400) were detected, including 3-ethoxy-1-propanol, linalool oxide, 2,3-butanediol, benzyl alcohol, 2-furanmethanol, 1-dodecanol, and phenylethyl alcohol, following by 2-ethyl-1-hexanol and 3-phenylpropanol. They played an extremely important role in enhancing the floral and green aroma of SAV. They are originated from raw materials (linalool oxide, 1-octen-3-ol, 1-dodecanol and 1-octanol), AF (2-methyl-1-propanol, phenylethyl alcohol and 3-ethoxypropanol), AAF (2,3-butanediol and 2-furanmethanol) and Fumigation (trans-linalool oxide and benzyl alcohol). In addition, phenylethyl alcohol with honey and floral notes has been hypothesized to originate from the raw materials or come from the reduction of phenylacetaldehyde during AF (Liang et al., 2016). Our study has just proved that phenylethyl alcohol can be derived from raw materials (rice hull) or by the reduction of benzeneacetaldehyde (Fig. 2).

Aldehydes gave SAV a rich floral fragrance. There were some aldehydes with relatively high FD detected, including vanillin, furfural, benzeneacetaldehyde, and 3-methyl-butanal, followed by benzaldehyde and α-ethylidene-benzeneacetaldehyde. Furthermore, the level of aldehydes was shown to increase after fumigation, which is consistent with the results of (Li et al., 2015; Wang et al., 2012). Notably, vanillin and furfural were detected during AAF, and with the highest concentration F—4D sample (Fig. 2). Vanillin, with sweet and caramel odors, had also been identified as a key AACs in ZAV (Zhou et al., 2020) and wine vinegars (Callejón et al., 2010). It probably derives from the microbial metabolism or raw materials of wheat bran and wheat *Qu* in the vinegar production (Liang et al., 2016). It is generally acknowledged that furfural can be generated from pentoses (Giordano et al., 2016). The presence of furfural has been previously reported in a wide variety of vinegars, such as balsamic vinegar (Cirlini et al., 2011) and ZAV (Yu et al., 2012). Benzeneacetaldehyde, which also called hyacinthin because of its distinctive hyacinth aroma, is mainly produced by the metabolism of phenylalanine in the AAF. Due to its unstable properties, it can be oxidized to phenylacetic acid or reduced to phenylethyl alcohol. Then ethyl phenylacetate and phenylethyl acetate can be generated. These compounds are the main contributors to the floral and fruity aroma of vinegar.

The ketones mostly presented caramel, creamy, fruity, rose, and mushroom odors. Among the ketones, 2,3-butanedione, acetoin, butyrolactone, 2(5H)-furanone, β-damascenone, and 1-(4-methoxyphenyl) ethenone ($FD \geq 400$) had a high FD factor, which was consistent with previous reports (Liang et al., 2016). Acetoin and 2,3-butanedione usually were key AACs in fermented foods (Wang et al., 2021) and condiments (such as ZAV), which are considered the precursors of tetramethyl pyrazine (Xiao et al., 2018). Next, acetoin was also reported mainly produced at vinegar fermentation stage (Zhang, Wang, et al., 2019). Acetoin had a higher concentration in the AAF9d and F—0D samples, while 2,3-butanedione had a higher concentration in the F—2D sample in this study (Fig. 2), a result that is consistent with the above literature report. β-Damascenone and other aromatic ketones should derive from the raw materials used in the vinegar fermentation (Zhou et al., 2020).

2-Methoxy-phenol (sweet, woody), creosol (spicy), phenol (herbal), 4-ethyl-2-methoxy-phenol (floral, herbal), p-cresol (sour bamboo shoot), 2-methoxy-4-propyl-phenol (spicy), 4-ethyl-phenol (sweet, brewis), and 2-methoxy-4-vinylphenol (fatty, spicy, clove) were found in the samples. They can be formed by the degradation of lignin in the raw materials (Xiao et al., 2011). Compared to the aforementioned literature on phenol compounds (no more than 6) of Chinese cereal vinegars (Liang et al., 2016; Zhou et al., 2020), the largest amount of phenols (16) were determined all in this work. It is known to all those phenols are polar compounds with low volatilities and weakly soluble in water. The application of GC × GC-O facilitated the identification of phenol compounds in SAV samples. It is worth noting that p-cresol with unique sour odor is not only an important AACs in vinegar (Liang et al., 2016), but also a key AACs in the other fermented products such as sour bamboo shoots (Tian et al., 2023).

The esters with a typical floral and fruity odor were usually considered to be the most important compounds for aroma profile of vinegars, often providing smoothness and complexity. At the same time, the esters also play an important role in other fermented foods (Li et al., 2024; Olejar et al., 2015). They were mainly derived from esterification reactions between alcohols and organic acids, also come from the reaction between alcohols and acids during fumigation and aging. Many esters had been found in Spanish PDO wine vinegars (Rios-Reina et al., 2020). In this work, 14 esters with FD values ranging from 1 to 400 were identified. It must be noted that according to the FD values, the esters are indeed important for the overall flavor of SAV. Ethyl succinate, 3-(methylthio)propyl acetate, benzyl acetate, phenethyl propionate, ethyl phenacetate, phenethyl acetate, ethyl hexadecanoate, and benzyl benzoate had the highest FD (≥400), followed by ethyl acetate ($9 \leq FD \leq 27$), 2-furanmethanol, acetate and methyl salicylate($0 \leq FD \leq 9$), ethyl vanillate, n-propyl acetate, and methyl thiolacetate ($0 \leq FD \leq 3$). The concentration of ester compounds was mainly accumulated of higher in the AF and AAF (Fig. 2).

Nutty and roasted are typical odor characteristics of pyrazines. Pyrazines in SAV are mainly generated through the Maillard reaction due to an abundance of sugar and nitrogen compounds during fumigation and aging (Wang et al., 2012). Ten pyrazines were sniffed in this work, including 2,3-dimethyl-5-(Z-1-propenyl)-pyrazine, 2,3-dimethyl-5-ethylpyrazine, 2,5-dimethyl-pyrazine, trimethyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2,6-dimethyl-pyrazine, ethyl-Pyrazine, tetramethyl-pyrazine, 2,3,5-trimethyl-6-ethylpyrazine, and 2,5-dimethyl-3-isoamylpyrazine. The concentration of pyrazine compounds was mainly higher in the fumigation. The FD factors of pyrazines after aging were not lower than those before aging. Also, the increase of the pyrazines, and at the same time the decrease of some other compounds (such as acids) with pungent odors, contributed to improving the overall flavor profile of SAV.

Compared to previous reports, nine lactones (4-hexanolide, γ-heptalactone, γ-octalactone, butyrolactone, γ-nonalactone, γ-undecalactone, 5,6-dihydro-6-pentyl-2H-pyran-2-one, dihydroactinidiolide, γ-dodecalactone) was sniffed in the samples. In addition, butyrolactone (creamy), γ-heptalactone (caramel), γ-undecalactone (fruity), 5,6-dihydro-6-pentyl-2H-pyran-2-one (floral), dihydroactinidiolide (musky) and γ-dodecalactone (fruity, floral), which were propounded for the first time as odorants in SAV. γ-Undecalactone was exclusively sniffed in the after aging samples, while γ-dodecalactone was only sniffed in the before aging samples. Liang et al. (2016) found six lactone compounds (γ-pentalactone, 4-hexanolide, pantolactone, γ-octalactone, γ-nonalactone, and γ-decalactone) in SAV by SAFE-GC-O. Zhou et al. (2020) sniffed only 1 lactone compound (pantolactone) in ZAV by SPME and GC-O. These lactones compounds provided fruity and sweet notes to SAV.

The organic acids which were the main components in vinegars, mainly contributed to their sour flavors, which were formed during AAF. Eight acids were found in this work. Among them, 3-methylbutanoic acid, acetic acid, and nonanoic acid showed the highest FD factor ($FD \geq 400$), followed by heptanoic acid ($9 \leq FD \leq 243$), n-decanoic acid (0

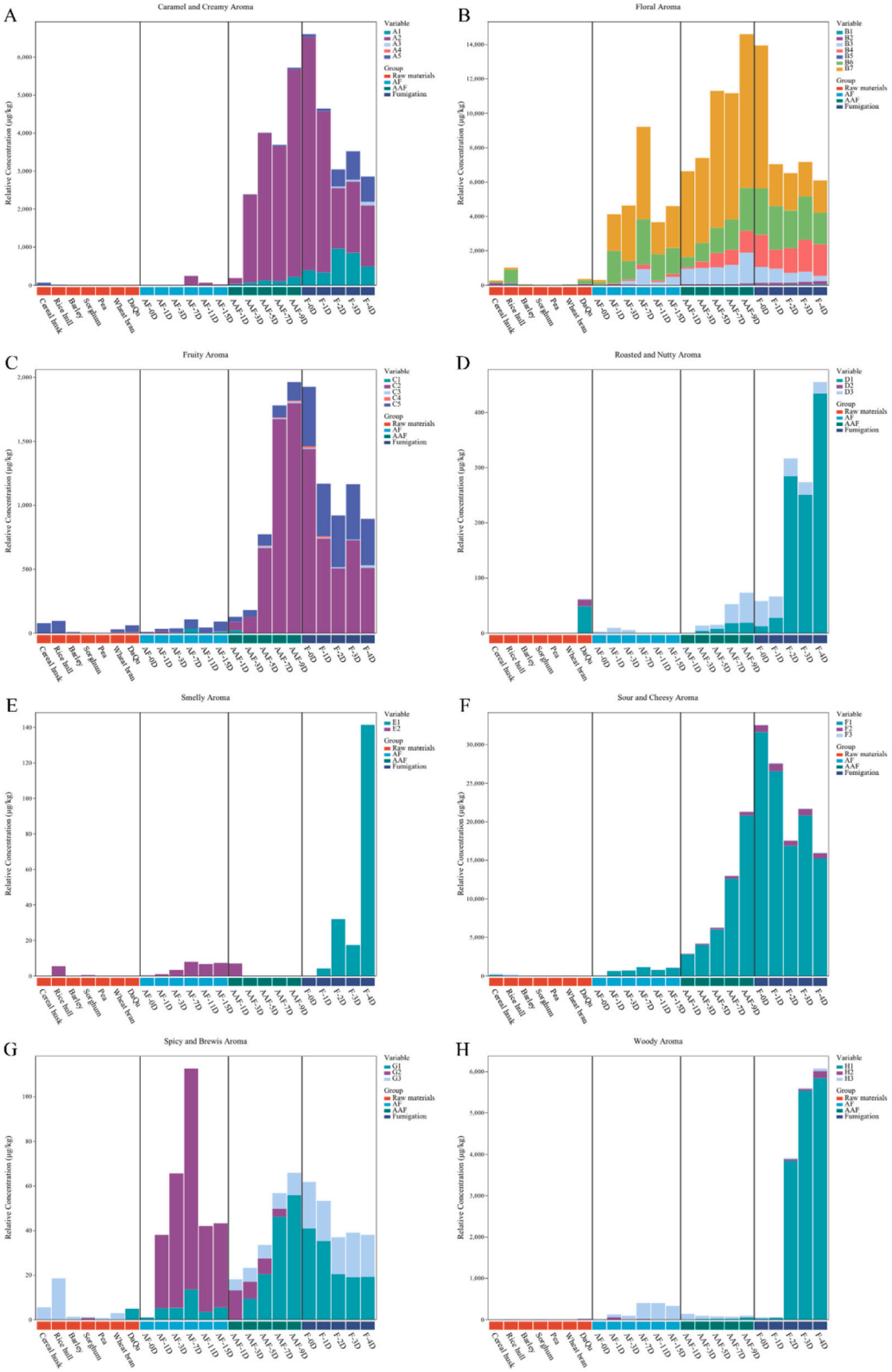

Fig. 3. The contribution changes of AACs (FD $\geq$ 400) for SAV at different production stages. Alcohol fermentation (AF); Acetic acid fermentation (AAF). A1: 2,3-Butanedione; A2: Acetoin; A3: 2-Acetylfuran; A4: Butyrolactone; A5: Vanillin; B1: Linalool oxide; B2: Benzeneacetaldehyde; B3: Ethyl succinate; B4: Phenethyl acetate; B5: Benzyl alcohol; Phenylethyl Alcohol: Ethyl hexadecanoate; C1: 3-Ethoxypropanol; C2: 2,3-Butanediol; C3: 3-(Methylthio)propyl acetate; C4: γ-Octalactone; C5: γ-Nonalactone; D1: Trimethylpyrazine; D2: 2,3-Dimethyl-5-ethylpyrazine; D3: 2-Furanmethanol; E1: 2,3-Dimethyl-5-(Z-1-propenyl)-pyrazine; E2: p-Cresol; F1: Acetic acid; F2: 3-Methylbutanoic acid; F3: Nonanoic acid; G1: Methionol; G2: Creosol; G3: Dihydroactinidiolide; H1: Furfural; H2: 2-Methoxyphenol; H3: 2-Methoxy-4-ethylphenol.

≤ FD ≤ 9), undecanoic acid (0 ≤ FD ≤ 9), 2-methyl-propanoic acid (0 ≤ FD ≤ 3), and 4-methyl-pentanoic acid (0 ≤ FD ≤ 3). Five furans with caramel, smoky, green, and nutty odor (2-methyl-furan, 2-pentyl-furan, 2-acetyl-5-methylfuran, 2-acetylfuran, and 2,3-dihydro-benzofuran) were also sniffed in the samples. Besides, 2-acetylfuran (sweet) had also been found in Italian balsamic vinegars by GC-O (Corsini et al., 2019). With the exception of 2-acetylfuran (243 ≤ FD ≤ 400), the FD factor of other furan compounds was lower (0 ≤ FD ≤ 9) than that of other compounds, they can modify the overall flavor of SAV. Also, the furan derivatives mainly came from Maillard reaction in the fumigation process (Fig. 2). Furan compounds played a key role in increasing the smoky and nutty odor of SAV.

The sulfur compounds with their low threshold and are important contribution to the flavor of many fermented foods, including Cheddar cheese (Wang et al., 2021) and Baijiu (Li et al., 2019). Volatile sulfur compounds, could be generated by microbial metabolism or Strecker degradation of the sulfur-containing amino acids, are important AACs in vinegars (Landaud et al., 2008). The sulfur compounds, including dimethyl disulfide, dimethyl trisulfide, methionol, 3-phenyl-thiophene, 2-(methylmercapto) benzothiazole, and methional (sulfur, brewis) were detected in SAV samples (Data was not showed). Moreover, only methionol was sniffed in this work. In addition to the above compounds, 1-(2-furanylmethyl)-1H-pyrrole (coffee) was only sniffed in SAV samples before aging.

In summary, there were 27 and 41 AACs (FD ≥ 400) in the SAV before and after aging, respectively. According to the analysis of GC × GC-O, the intensity difference of AACs of before-aging and after-aging SAV was quite obvious. Aging intensifies the roasted, caramel, and nutty flavors characteristic of SAV, while simultaneously diminishing certain undesirable pungent odors, such as that of bitter almonds. These observations align precisely with findings reported in the literature (Liang et al., 2016).

3.2. Changes of aroma active compounds in different production stages

In order to investigate the sources and concentration changes of AACs in different production processes samples, i.e., raw materials, AF, AAF, and fumigation were analyzed by GC–MS/MS. A total of 88 AACs, including aldehydes (15), alcohols (13), phenols (12), pyrazines (9), esters (9), ketones (8), acids (7), furans (6), lactones (6), sulfur compounds (2), and others (1), were determined (Fig. 2).

Compared to the production stage (AF, AAF, and fumigation), the concentration of AACs in the raw materials is not worth mentioning. Regarding the production stage, the types and concentrations of AACs of AF-7D sample were highest in the AF, especially the esters. There were obvious changes in the concentration and kinds of AACs during AAF and fumigation. The former is mainly caused by biological reactions in the microbial community, while the latter is mainly produced via thermally induced reactions including the Maillard reactions. The acids and esters with floral aroma produced in the AAF were the main volatile components of SAV. At the same time, the AAF was also the stage with the greatest trend of AACs concentration change during the production process. In addition, the types of AACs increased the most obviously in the fumigation, in which the new AACs were produced, such as aldehydes, phenols, and pyrazines (Fig. 2A, B).

Besides, in terms of the compound number, there were 25, 37, 27, 30, 20, 26, and 36 AACs from cereal husk, rice hull, pea, barley, sorghum, pea, wheat bran, and Daqu, respectively. Daqu contains 8 unique AACs itself. Similarly, 7 AACs are detected from rice hull only. The number of AACs increased from 28 to 35, and the newly produced compounds were esters and ketones during AF. Compared to the AAF-1D, 10 AACs were newly added to the AAF. Nineteen AACs were produced in the fumigation, which was the stage where most compounds were generated. Raw materials (62 compounds) and fumigation (71 compounds) were the main sources of AACs.

The concentration changes of 88 AACs at different stages were analyzed and compared (Fig. 2H). It is worth mentioning that there are many kinds of aldehydes (3-methylbutanal, heptanal, octanal, benzaldehyde, and benzeneacetaldehyde) in the raw materials, which provides the fresh odor of green herbs for SAV. In addition, aldehydes can easily form alcohols under acidic conditions. The concentration of acids (acetic acid and n-decanoic acid), esters (ethyl acetate, ethyl succinate, and ethyl hexadecanoate), and alcohols (2-methyl-1-propanol, 3-ethoxypropanol, and phenylethyl alcohol) show a rising and then decreasing trend during AF, and the concentration of AACs of AF-7D sample was the highest. It has been reported that the AAF is the period when the microbial community is in the most abundance. The various biochemical reactions occur the most intensely, and the concentration changes of compounds present the highest. (Wang et al., 2016; Wu et al., 2021; Zhu et al., 2018). The results of this study are consistent with the reported literature. A large number of esters (ethyl acetate, phenethyl acetate, ethyl succinate, and ethyl hexadecanoate), acids (acetic acid, 2-methylpropionic acid, 3-methylbutanoic acid, and nonanoic acid), and alcohols (2,3-butanediol, 2-furanmethanol, and phenylethyl alcohol) are produced in the AAF. Especially, acids are the main components of vinegar, and esters with pleasant odor play a crucial role in enriching the aroma profile and softening the effect of acids of SAV. Moreover, the fumigation was also the main source of AACs in SAV, which produced a great deal of pyrazines (trimethyl-pyrazine, tetramethyl-pyrazine and 2,3-dimethyl-5-(Z-1-propenyl)-pyrazine), phenols (2-methoxyphenol, 2-methoxy-4-ethylphenol, 4-ethyl-phenol, and eugenol), aldehydes (furfural and vanillin) and furans (2-acetylfuran), giving unique roasted, nutty, clove, and woody aroma characteristics (Liang et al., 2016; Ranjha et al., 2021; Zhou et al., 2020) for SAV.

3.3. Contribution of different production stages to the aroma of SAV

Vinegar fermentation and flavor formation are not only the result of complex biochemical reactions driven by the activity of multiple microorganisms, but also attributed to abundant thermal reactions at high temperature. In order to clear the dynamic changes of the main AACs (FD ≥ 400) at different stages and their contributions to the sensory profiles of SAV, the dynamic changes of the representative AACs of eight aroma sensory profiles were plotted (Fig. 3), i.e., caramel and cream aroma (2,3-butanedione, acetoin, 2-acetylfuran, butyrolactone, and vanillin), floral aroma (linalool oxide, benzeneacetaldehyde, ethyl succinate, phenethyl acetate, benzyl alcohol, phenylethyl alcohol, and ethyl hexadecanoate), fruity aroma (3-ethoxypropanol, 2,3-butanediol, 3-(methylthio)propyl acetate, γ-octalactone, and γ-nonalactone), roasted and nutty aroma (trimethylpyrazine, 2,3-dimethyl-5-ethylpyrazine, and 2-furanmethanol), smelly aroma (2,3-dimethyl-5-(Z-1-propenyl)-pyrazine and p-cresol), sour and cheesy aroma (acetic acid, 3-methylbutanoic acid, and nonanoic acid), spicy and brewis aroma (methionol, creosol, and dihydroactinidiolide), and woody aroma (furfural, 2-methoxyphenol, and 2-methoxy-4-ethylphenol). Although the raw materials contributes little to the type and concentration of aroma compounds, it is the original source of all aroma compounds and provides the rich substrates for various reactions during the brewing process of SAV (Zhang, Wu, et al., 2019). The compounds with floral (phenylethyl alcohol and phenethyl acetate) and spicy (methionol) mainly come from the AF stage. While the AAF provides caramel (vanillin), creamy (2,3-butanedione and acetoin), fruity (2,3-butanediol), sour (acetic acid), and cheesy (3-methylbutanoic acid) notes for SAV. The odor contribution of roasted (trimethyl-pyrazine), nutty (2,3-dimethyl-5-ethyl-pyrazine), smelly (p-cresol), and woody (2-Methoxyphenol) in SAV were mainly originated from the fumigation stage. It is evident that AAF and fumigation represent the primary sources of AACs in SAV. Moreover, these AACs are predominantly generated through microbial activity during AAF. The correlations between 26 AACs and species (top 15 of abundance by metatranscriptomics) in AAF were analyzed (Fig. S2). The microorganisms that exhibited a significant positive correlation ($p \leq 0.05$) with AACs included *Saccharomyces cerevisiae*, *Acetobacter*

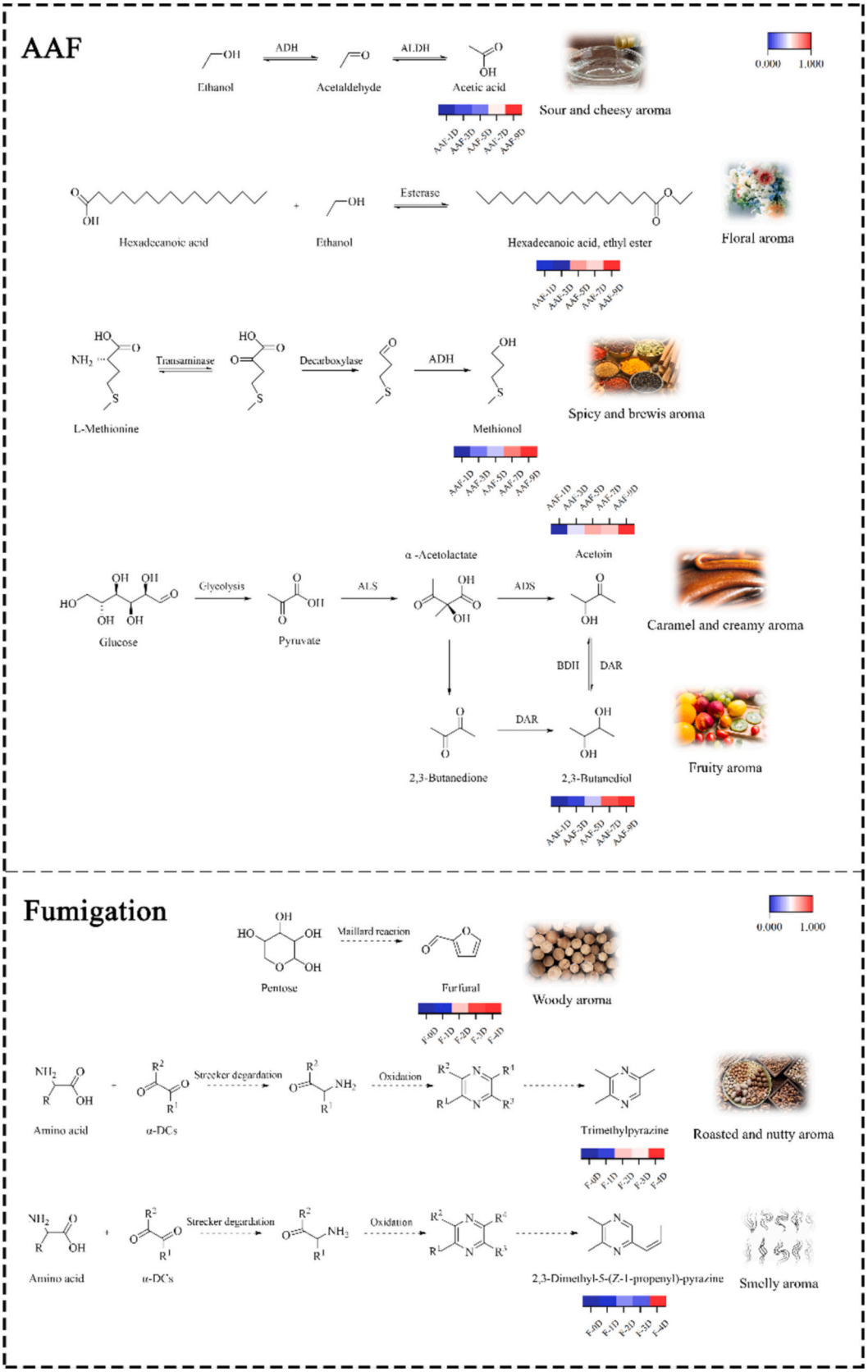

Fig. 4. Possible transformation pathway of AACs in SAV. ADH: alcohol dehydrogenase; ALDH: acetaldehyde dehydrogenase; ALS: α-acetolactate synthetase; ADS: α-acetolactate decarboxylase; DAR: diacetone acetone reductase; BDH: 2,3-butanediol dehydrogenase.

orientalis, *Acetobacter senegalensis*, *uncultured bacterium*, *Acetobacter oryzoeni*, *Acetilactobacillus jinshanensis*, *Acetobacter pasteurianus*, and *Lactobacillus acetotolerans*. Vanillin, methionol, 3-methylbutanoic acid, 2,3-butanediol, and trimethyl-pyrazine exhibited a significant positive correlation with *Saccharomyces cerevisiae*. Similarly, vanillin, methionol, 2,3-butanediol, and trimethylpyrazine displayed a significant positive correlation with *Acetobacter orientalis*. Additionally, acetoin, nonanoic acid, phenethyl acetate, methionol, 3-methylbutanoic acid, 2,3-butanediol, and trimethyl-pyrazine were significantly positively correlated with *Acetobacter senegalensis*. Furthermore, p-cresol, 4-ethylguaiacol, 2-methoxyphenol, and 3-ethoxypropanol showed significant positive correlations with *Acetobacter oryzoeni*, *Acetobacter pasteurianus*, and *Lactobacillus acetotolerans*. γ-octalactone, methionol, 2,3-butanediol, and trimethyl-pyrazine exhibited a significant positive correlation with *Acetilactobacillus jinshanensis*. Previous studies have identified that the primary contributors to the variation of volatile flavor compounds in AAF of ZAV are *Acetobacter* and *Lactobacillus*, which aligns with our findings. Acetic acid bacteria (AAB) deserve special mention, as described in Fig. S2 four species of *Acetobacter* were positively correlated with key AACs. AAB are involved in several metabolic pathways that produce volatile compounds through fermentation and oxidative processes. For instance, the production of acetoin, nonanoic acid, phenethyl acetate, and other flavor compounds are significantly linked to *Acetobacter* species (Wang et al., 2016). Those results suggest that AAB, alongside other microorganisms like *S. cerevisiae*, are integral in shaping the unique flavor characteristics of SAV. The results provided an explicit direction for strengthening the specific aroma of SAV.

3.4. Possible transformation pathways of AACs in SAV production

Most of the flavor compounds in SAV derive from raw materials (Zhou et al., 2020), degradation of amino acids and fatty acids (Liang et al., 2016), and Maillard reaction (Giordano et al., 2016; Lu et al., 2011). From the above analysis, it can be seen that the AAF and the fumigation stage are the main sources of AACs in SAV. The AACs with the highest relative concentration in each sensory profile were taken as the representative AACs. The possible transformation pathway of the compound in the AAF or the fumigation was mapped in Fig. 4.

As we all know, most of the acetic acid in AAF is from the ethanol produced by AF. Acids, alcohols, and esters are important AACs in the SAV. Esters are the main contributors to the flower aroma in the SAV, especially ethyl hexadecanoate, which is from the esterification of hexadecanoic acid and ethanol in acidic conditions. Ethyl hexadecanoate has been present in the entire production process (raw materials, AF, AAF, and fumigation) of SAV. The concentration of most esters (ethyl acetate, n-propyl acetate, diethyl succinate, methyl salicylate, ethyl benzeneacetate, and ethyl hexadecanoate) has been increasing in AF and AAF process due to the presence of a large number of alcohol and acid compounds, and reaching the maximum value at the end of AAF. The observed change can be attributed to the substantial production of esterase by the microorganisms. Then, the decreasing of it via the decomposition reaction could be caused by high temperatures during fumigation stage. Furthermore, methionol with brewis notes, has been determined as a key AAC in many fermented foods (Castillo-Lozano et al., 2007; Du et al., 2021), which can be produced from the L-methionine metabolism through the Ehrlich pathway (Etschmann et al., 2008). Acetoin and 2,3-butanediol usually can be generated by the action of microorganisms (Charles et al., 2000). Moreover, acetoin is the precursor of tetramethyl-pyrazine in the Maillard reaction of SAV (Lu et al., 2011).

In addition to the biological reactions (AAF) mentioned above, there are also some non-biological reactions (fumigation). For instance, furfural was produced from pentoses by the Maillard reaction ($pH \leq 7$) during fumigation process (Liang et al., 2016). Furthermore, the concentration of trimethyl-pyrazine and 2,3-dimethyl-5-(Z-1-propenyl)-pyrazine were increased obviously in the fumigation. It is speculated that these pyrazine compounds are derived from the Maillard reaction that occurs at high temperatures (Ara et al., 2017; Wang et al., 2012). Moreover, trimethyl-pyrazine was initially identified in Daqu, which can be formed through microbial fermentation. Although trimethyl-pyrazine was not detected in AF, its concentration exhibited an increasing trend during AAF, indicating that it could potentially be produced via microbial activity. In fact, as discussed in the previous section, trimethyl-pyrazine has been positively correlated with key microorganisms of AAF including several AAB species and also *S. cerevisiae*. Nevertheless, the thermal reactions occurring during fumigation remain the primary stage for the production of trimethyl-pyrazine (Wang et al., 2012).

4. Conclusion

SAV, with a rich and complex odor, is one of the famous traditional cereal vinegars in China. Firstly, this study targeted the AACs in the before and after aging stage of SAV by GC × GC-O-TOF-MS. The sources and changes of these AACs were further studied by GC–MS/MS. The intensity difference of AACs of SAV before-aging and after-aging was quite obvious. This study indicates that the aging process substantially contributes to the overall aroma of SAV. The raw materials primarily provide lactones and aldehydes to SAV, while the AF process generates considerable quantities of alcohols, esters, and phenols through microbial activity. Additionally, significant amounts of acids, alcohols, ketones, and esters are produced via microbial activity during AAF. Furthermore, substantial quantities of pyrazines, phenols, and aldehydes are generated through thermal reactions at elevated temperatures during fumigation. Consequently, it can be concluded that the AAF and fumigation stage were the main sources of AACs in SAV. Finally, the possible transformation pathway of eight representative AACs in different production stages was mapped and discussed. Overall, GC × GC-O-TOF-MS is an effective method to enrich the aroma profile of SAV and further reveal the flavor differences between before-aging and after-aging SAV. The results of this study are interesting to comprehensively understand and supplement the AACs in SAV, providing a theoretical foundation and direction for developing practical policies to ameliorate the sensory quality of SAV in the future.

CRediT authorship contribution statement

Ao Zhang: Validation, Supervision. **Yaao Zhou:** Validation, Supervision, Software. **Wenqing Zhang:** Validation, Supervision. **Kai Liang:** Software, Investigation. **Juan-J. Román-Camacho:** Writing – review & editing. **Jingli Zhou:** Methodology, Investigation. **Jia Song:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Yu Zheng:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition. **Min Wang:** Writing – review & editing, Supervision, Software, Resources.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101918.

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