International Food Research Journal 27(5): 814 - 824 (October 2020)

Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of the different baking processes on the aroma profiles of Shanxi aged vinegar mashes

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Article history

Received: 24 March 2020
Received in revised form:
10 June 2020
Accepted:
22 June 2020

Keywords

Shanxi aged vinegar,
baking process,
aroma profile,
HS-SPME-GC-MS,
PCA analysis

Abstract

As one of the typical appreciated seasonings, Shanxi aged vinegar is famous all over the world for its unique aroma formed during the baking stage. To evaluate the influence of two baking methods on the aroma profiles, headspace solid-phase microextraction (HS-SPME) in combination with gas chromatography-mass spectrometry (GC-MS) were carried out, and the odour intensity was evaluated by odour activity values. Results showed that there were 72 volatile compounds that could be identified and quantified in the analysed samples. Moreover, among them, the odour activity values (OAV) of 35 compounds were ≥ 1, and the contents of acetic acid, ethyl acetate, and acetoin were significantly higher than the other volatile compounds. There were 17 odour-active compounds newly produced after baking stage. Based on the principle components analysis (PCA), 11 newly produced volatile compounds as well as benzeneacetaldehyde and furfural, of which the OAVs had remarkable differences in two baking methods, were closely associated with the baking process. Additionally, acetophenone was formed specifically in the traditional methods, and five odour-active compounds (3-methylbutyl-acetate, hexanal, 2,3-dimethyl-5-ethylpyrazine, trimethyloxazole, and dimethyl disulphide) were detected only in the modern method. In conclusion, baking process has important influence on the formation and composition ratio of aroma profiles in Shanxi aged vinegar. The results obtained from the present work might provide guidance for improvement of the production process and quality optimisation of Shanxi aged vinegar.

Introduction

Vinegar is one of the common seasonings around the world, and has a long history in brewery and consumption (Giudici *et al.,* 2017). In China, there are numerous vinegar manufacturers, of which Shanxi aged vinegar, Sichuan bran vinegar, Fujian *Monascus* vinegar, and Zhenjiang aromatic vinegar, are regarded as the most famous (Chen *et al.,* 2013). Using specific raw materials or applying specific processes yield different tastes and flavour characteristics (Xiao *et al.,* 2011). For examples, baking and long aging time yield specific aroma in Shanxi aged vinegar (Zhu *et al.,* 2016); the specific flavour of Sichuan bran vinegar is due to the 60 different herbs used in the fermentation (Chen *et al.,* 2009); Fujian *Monascus* vinegar is bright in colour due to the addition of *Monascus* as a fermentation starter (Jiang *et al.,* 2019); and Zhenjiang aromatic vinegar is famous for its elegant and complex aroma (Al-Dalali *et al.,* 2019).

Shanxi aged vinegar is produced from sorghum as the main raw material. The sorghum is crashed and steamed into mashes. After cooling, the mashes are mixed with starter (called Daqu in Chinese) and water. Accompanied by the spontaneous growth of microorganisms from the starter, ethanol starts to produce. Acetic acid fermentation begins after alcohol fermentation for three days, and chaff and wheat bran are added into the mashes, and ethanol is fermented into acetic acid (Wu *et al.,* 2012). Upon acetic-acid fermentation, the vinegar mashes are baked at an internal temperature of 98°C for three to five days, and transferred from one jar or trough to the next after every 24 hours until the baking process is finished (Figure 1).

For baking, traditional method of baking (TB) means heating the vinegar mashes in big ceramic jar and transferring vinegar mashes from one jar to the next by labour. It is inefficient and labour-intensive. In contrast, modern method of baking (MB) is replacing ceramic jar with a big rectangular stainless-steel trough, with auto-transferring of vinegar mashes from one trough to the next. Besides, MB also tries to reproduce the excellent flavour of TB, and not just only increases the volume of production.

Baking is a necessary heating process which leads to significant change in chemical profile of the

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aroma compounds in the production of Shanxi aged vinegar (Zhu *et al.,* 2016). In this stage, the aroma compounds are formed of the reducing sugars and amino acids as a result of the Maillard reaction. They are obtained from the hydrolysis of the residual starches, hemicelluloses, proteins, and metabolites of microorganisms in a weakly acidic environment (Semmelroch and Grosch, 1995). Pyrazines, aldehydes, and ketones are thought to be the main products of such reactions (Zhu *et al.,* 2016). The sorghum underwent through a long fermentation process to produce over 87 non-volatile metabolites, which belong to amino acids, alcohols, sugars and sugar derivatives, organic acids, fatty acids, and alkanes before baking stages (Nie *et al.,* 2017; Zhu *et al.,* 2018b), which provided an important material base for the Maillard reaction during the baking stages.

To the best of our best knowledge, no previous studies have focused on the difference in the chemical profile of the key aroma compounds that are formed during the baking processes in the mashes of Shanxi aged vinegars. Therefore, the present work aimed to investigate the presence of the key aroma compounds and their changes during the baking stage by headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). Beside the investigation of the chemical profile, the effects of the different baking methods (Figure 1) on the aroma profiles of Shanxi aged vinegar mashes was also assessed. The results of the present work would provide guidance for the improvement of the production process and quality optimisation of the Shanxi aged vinegar.

Materials and methods

Vinegar mash sampling

In the present work, the mash samples of Shanxi aged vinegar were provided by Dengshanjing Vinegar Group Co. Ltd in February, 2019. The sampling method is shown in Figure 1. For traditional method, the vinegar mashes were baked at an internal temperature of 98°C for 3 d in big ceramic jar (0.5 m^3). The baked mashes were transferred from one jar to the next at 24-h intervals, and samples were designated as TS_1, TS_2, and TS_3. Except for fourth jar, the top, middle, and bottom cross-section were selected for sampling in each jar. For each cross-section, three subsamples were collected to a total of nine subsamples. The fourth jar was divided into two parts (upper and lower); six subsamples were collected in each part. Finally, 18 subsamples (500 g each) were collected at 1st day. Fifteen subsamples (500 g each) were collected at 2nd and 3rd d, respectively. For modern method, the vinegar mashes were baked at an internal temperature of 98°C for 5 d in big rectangular stainless-steel trough (50 m^3). The baked samples were transferred from one jar to the next at 24-h intervals, and samples were designated as MS_1, MS_2, MS_3, MS_4, and MS_5. In each trough, the top, middle, and bottom cross-section were selected for sampling. Each cross-section was divided into three sections, to a total of nine subsamples. Twenty-seven subsamples (500 g each) were collected in one trough. The subsamples of one jar or trough were fully mixed to make a single representative sample. Then, 500 g sample of each jar or trough was taken by method of

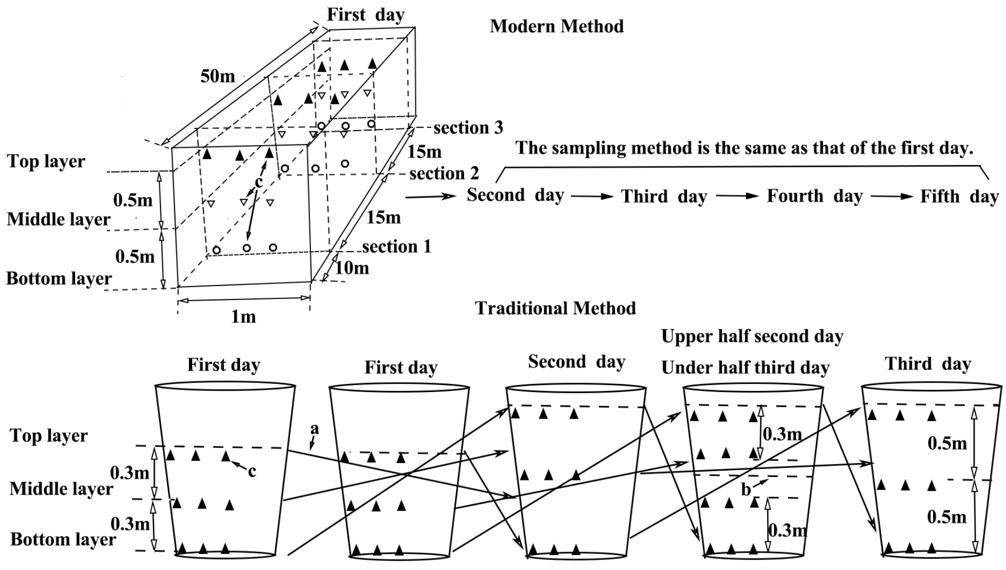

Figure 1. The flowchart of modern and traditional baking method and sampling points in each jar and trough. (a) the arrows present corresponding position of vinegar mashes that were transferred from one jar to another; (b) the dotted line represents the boundary between vinegar mashes in the middle of the second and third day (the fourth jar); and (c) the positions of subsamples in each jar.

coning and quartering. We also sampled the vinegar mashes that were ready for baking (designated as S_0). All the samples were placed in a cooler at -80°C.

Headspace Solid-Phase Microextraction (HS-SPME) method

The volatile compounds were extracted by the HS-SPME. The SPME fibre (Supelco, Bellefonte, PA, USA) was coated with 50/30 µm of divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS). It was conditioned for 30 min at 230°C in the GC injector before each extraction. A 15-mL glass vial was used to place 1 g of a sample and 10 µL of octanal (IS, 500 µg/ml in methanol), then tightly crimped by a silicon septum. The glass vial was placed in a water bath, maintained at 45°C for 30 min for the SPME extraction of the volatiles. Then, the SPME fibre was inserted into the GC injection port for thermal desorption of the absorbed volatiles at 250°C for 5 min in a splitless mode.

Gas Chromatography-Mass Spectrometry (GC-MS)

Analyses were performed using an Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer (Agilent Technologies). Helium was used as the carrier gas at a rate of 1 mL/min. Separation occurred on an HP-5 MS column (30 m × 0.25 mm × 0.25 µm) and an RTX-Wax column (30 m × 0.25 mm × 0.25 µm) (Agilent Technologies), respectively. For both columns, the initial oven temperature was 40°C, which was held for 3.5 min, then ramped to 90°C at a rate of 5°C/min, and ramped again to 230°C at a rate of 6°C/min, which was held for 2 min. The mass detector operated at 150°C in electron impact mode at 70 eV. The ion source and transfer line temperatures were 230 and 250°C, respectively. The chromatograms were recorded in the full scan mode in the mass range of m/z 33-450.

Identification and quantitation of volatile compounds

The volatile compounds were identified by mass spectra (MS) and retention indices (RI). The mass spectra of the compounds were compared with the reference mass spectra (91% minimum similarity) from the National Institute of Standard and Technology library (NIST 11). The RI of unknown compounds were calculated from the retention times of *n*-alkanes (C6-C30) for both columns using the linear interpolation (Aili *et al.,* 2012). Then, the RI were compared with those previously reported in the literature and at the NIST Chemistry WebBook (DOI: https://doi.org/10.18434/T4D303) database.

The compounds were quantified using internal standard method. Octanal (IS, 500 µg/mL in methanol) was added as an internal standard to each sample. The relative concentration of each volatile compound was calculated as follows:

$$C_c = C_i \times A_c \div A_i$$

where, C_c = concentration of the volatile compound, C_i = concentration of internal standard, A_c = peak area of the volatile compound, and A_i = peak area of the internal standard. Each sample was analysed in triplicate.

Odour Activity Values (OAV)

The contribution of each volatile compound to the aroma profile was evaluated by the odour activity value, which was measured as the ratio of the concentration of each compound to its detection threshold in water. The odour descriptions and threshold values were taken from those available in the literature and two online databases, i.e. Flavornet (www.flavornet.org) and Perflavory Information System (www.perflavory.com).

Statistical analysis

All assays were conducted in triplicate, and results were expressed as mean ,± standard deviation. The data of OAVs were used to study the differences in the aroma during the baking processes by heat map (false colour image) analysis. The principal component analysis (PCA) was performed as an unsupervised method to ascertain the degree of differentiation between two baking methods. All statistical analyses were performed by using R (software version 3.6.1).

Results and discussion

Chemical profile of both final baked mashes

The characteristic volatile profiles of the samples were determined by the HS-SPME/GC-MS method. A total of 72 volatile compounds (listed in Table 1) were identified in the MS_5 and TS_3 samples. These volatile compounds were classed into nine classes: alcohols (five), acids (eight), esters (19), aldehydes (14), ketones (four), phenols (six), heterocycles (14), hydrocarbons (one), and sulphides (one). Zhu *et al.* (2018b) reported that 42 volatile compounds, which belonged to esters, alcohold, ketones, and aldehydes, were found at the stages of alcohol and acetic acid fermentation. The types of volatile compound, by contrast, had significant increase after baking. Most of 72 volatile compounds were also reported as odour-active compounds in other kinds of vinegar, including the Zhenjiang aromatic vinegar (Al-Dalali *et al.,* 2019), balsamic vinegar

Table 1. Identification and quantification of volatile compounds in MS_5 and TS_3.

No.	Compound	Retention Index		Identification	Concentration (mg/kg)		Threshold (mg/kg)	OAV	
		HP-5 MS	RTX-Wax		MS_5	TS_3		MS_5	TS_3
Alcohol									
1	ethanol	b	b	MS	2.1 ± 0.11	1.29 ± 0.81	2000	< 1	< 1
2	3-methyl-1-butanol	734.8	1213.9	RI, MS	0.58 ± 0.13	2.42 ± 0.3	0.004	112.5 - 177.5	530 - 680
3	2-methyl-1-propanol	n.d	1085.8	RI, MS	n.d	0.14 ± 0.09	6.505	-	< 1
4	2,3-butanediol	788.3	1540.9	RI, MS	4.5 ± 3.09	3.69 ± 1.45	100	< 1	< 1
5	phenylethyl alcohol	1117.3	1912.2	RI, MS	4.02 ± 0.4	9.21 ± 0.24	0.564	6.42 - 7.84	15.9 - 16.76
Acid									
6	acetic acid	657.3	1439.8	RI, MS	91.75 ± 7.99	81.41 ± 2.59	180	< 1	< 1
7	propanoic acid	n.d	1535.3	RI, MS	0.36 ± 0.07	0.28 ± 0.05	11.2	< 1	< 1
8	2-methylpropanoic acid	n.d	1566.1	RI, MS	n.d	0.55 ± 0.13	200	-	< 1
9	3-methylbutanoic acid	859.5	1666.8	RI, MS	1.25 ± 0.09	1.74 ± 0.21	1.2	< 1 - 1.12	1.28 - 1.63
10	hexanoic acid	997.4	1820.2	RI, MS	0.5 ± 0.05	1.05 ± 0.03	3	< 1	< 1
11	heptanoic acid	n.d	1947.2	RI, MS	0.17 ± 0.03	0.27 ± 0.13	3	< 1	< 1
12	octanoic acid	n.d	2054	RI, MS	0.27 ± 0.02	0.43 ± 0.1	0.5	< 1	<1 - 1.06
13	benzoic acid	n.d	2488	RI, MS	0.11 ± 0.01	0.2 ± 0.04	1220	< 1	< 1
Ester									
14	ethyl acetate	n.d	789.4	RI, MS	3.41 ± 1.27	0.42 ± 0.39	6.2	< 1	< 1
15	3-methylbutyl-acetate	n.d	1120.6	RI, MS	0.66 ± 0.25	n.d	0.002	205-455	-
16	hexanoic acid ethyl ester	1006.3	1241.2	RI, MS	n.d	0.09 ± 0.04	0.0022	-	22.73 - 59.09
17	pentanoic acid 2-hydroxy-4-methyl-ethyl ester	1063.8	n.d	RI, MS	0.16 ± 0.05	n.d	N/A	-	-
18	isoamyl lactate	1074	n.d	RI, MS	n.d	0.09 ± 0.04	N/A	-	-
19	acetic acid hexyl ester	n.d	1280	RI, MS	0.1 ± 0.03	0.1 ± 0.06	0.67	< 1	< 1
20	heptanoic acid ethyl ester	1102.9	1341.6	RI, MS	0.16 ± 0.05	0.37 ± 0.01	0.002	55 - 105	180 - 190
21	octanoic acid ethyl ester	1201.2	n.d	RI, MS	0.66 ± 0.1	0.45 ± 0.03	0.0194	28.87 - 39.18	21.65 - 24.74
22	isopentyl hexanoate	1254.7	n.d	RI, MS	0.06 ± 0	0.16 ± 0.12	0.9	-	< 1
23	acetic acid octyl ester	1216.8	1481.7	RI, MS	0.1 ± 0.01	0.17 ± 0.12	0.3	< 1	< 1

24	2-furanmethanol acetate	1003.2	1538.4	RI, MS	0.05 ± 0.02	0.1 ± 0.04	N/A	-	-
25	nonanoic acid ethyl ester	1300.7	1542.4	RI, MS	0.21 ± 0.01	0.31 ± 0.25	3.15061	< 1	< 1
26	decanoic acid ethyl ester	1399.6	1644.2	RI, MS	0.7 ± 0.03	1.05 ± 0.72	0.2	3.35 - 3.65	1.65 - 8.85
27	benzoic acid ethyl ester	1176.6	1672.6	RI, MS	0.14 ± 0.03	n.d	0.0556	1.98 - 3.06	-
28	butanedioic acid diethyl ester	1186.5	1678.3	RI, MS	0.15 ± 0.02	0.54 ± 0.41	500	< 1	< 1
29	benzeneacetic acid ethyl ester	1251.2	1789.5	RI, MS	0.35 ± 0.11	0.37 ± 0.17	0.1556	1.54 - 2.96	1.29 - 3.47
30	acetic acid 2-phenylethyl ester	1262.2	1820.2	RI, MS	2.61 ± 0.29	7.5 ± 3.24	0.25	9.28 - 11.6	29.04 - 30.96
31	dodecanoic acid ethyl ester	1599.8	1850.5	RI, MS	0.19 ± 0.1	0.32 ± 0.08	0.8	< 1	< 1
32	hexadecanoic acid ethyl ester	2000.8	2259	RI, MS	1.37 ± 0.09	1.43 ± 0.27	1	1.28 - 1.46	1.16 - 1.7
Aldehyde									
33	2-methylbutanal	n.d	825.7	RI, MS	0.4 ± 0.16	0.19 ± 0.06	0.003	80 - 186.67	43.33 - 83.33
34	3-methylbutanal	656.9	839.4	RI, MS	2.03 ± 0.27	1.04 ± 0.02	0.0012	1466.67 - 1916.67	850 - 883.33
35	hexanal	n.d	1065.1	RI, MS	0.08 ± 0.03	n.d	0.01	5 - 11	-
36	5-methyl-2-furancarboxaldehyde	967.8	1574.7	RI, MS	4.26 ± 0.69	3.36 ± 0.07	0.006	595 - 825	548.33 - 571.67
37	1H-pyrrole-2-carboxaldehyde	1019.3	2022	RI, MS	0.62 ± 0.16	0.42 ± 0.17	5	< 1	< 1
38	benzeneacetaldehyde	1048.6	1643.7	RI, MS	0.3 ± 0.08	0.45 ± 0.05	0.004	55 - 95	100 - 125
39	nonanal	1108.3	n.d	RI, MS	0.08 ± 0.01	0.06 ± 0.01	0.008	8.75 - 11.25	6.25 - 8.75
40	2-methyl-3(2-furyl)acrolein	1196.3	1876.9	RI, MS	0.2 ± 0.12	0.24 ± 0.17	N/A	-	-
41	furfural	836.5	1465.3	RI, MS	68.81 ± 2.42	78.13 ± 10.48	3	22.13 - 23.74	22.55 - 29.54
42	benzaldehyde	963.3	1526.8	RI, MS	2.42 ± 1.6	2.09 ± 0.56	0.75	1.09 - 5.36	2.04 - 3.53
43	5-methyl-2-phenyl-2-hexenal	1498.4	2078.6	RI, MS	0.2 ± 0.02	0.23 ± 0.13	N/A	-	-
44	α-ethylidene-benzeneacetaldehyde	1278.8	1933.7	RI, MS	0.28 ± 0.1	0.37 ± 0.42	1.7	< 1	< 1
45	dihydro-5-pentyl-2(3H)-Furanone	1370.1	2034.8	RI, MS	0.2 ± 0.01	0.43 ± 0.08	0.00008	2375 - 2625	4375 - 6375
46	1-methyl-1H-pyrrole-2-carboxaldehyde	n.d	1624.3	RI, MS	0.07 ± 0.01	0.07 ± 0.01	0.037	1.62 - 2.16	1.62-2.16
Ketone									
47	acetophenone	n.d	1655.2	RI, MS	n.d	0.18 ± 0.09	0.0659	-	1.37 - 4.1
48	acetoin	710.3	1288.4	RI, MS	2.08 ± 0.44	1.68 ± 0.23	8	< 1	< 1
49	benzyl methyl ketone	1133.8	n.d	RI, MS	0.15 ± 0.02	0.27 ± 0.04	N/A	-	-
50	3-(acetyloxy)-2-butanone	n.d	1385.1	RI, MS	0.25 ± 0.05	0.28 ± 0.1	N/A	-	-

Phenol									
51	2-methoxyphenol	1093	1858.9	RI, MS	0.81 ± 0.06	0.97 ± 0.31	0.0016	468.75 - 543.75	412.5 - 800
52	4-ethylphenol	n.d	2168.5	RI, MS	0.1 ± 0.02	0.32 ± 0.25	0.021	3.81 - 5.71	3.33 - 27.14
53	4-ethyl-2-methoxyphenol	1284.1	2029.3	RI, MS	0.34 ± 0.05	0.34 ± 0.24	0.016	18.13 - 24.38	6.25 - 36.25
54	2-methoxy-4-vinylphenol	n.d	2191.5	RI, MS	0.15 ± 0.01	0.31 ± 0.09	0.019	7.37 - 8.42	11.58 - 21.05
55	maltol	n.d	1964.7	RI, MS	0.23 ± 0.07	0.26 ± 0.12	1530	< 1	< 1
56	phenol	n.d	1998.7	RI, MS	0.28 ± 0.07	0.54 ± 0.38	5	< 1	< 1
Heterocycle									
57	1-(2-furanyl)-ethanone	916.6	1506.5	RI, MS	0.24 ± 0.03	0.1 ± 0.06	80	< 1	< 1
58	2-pentylfuran	996.5	n.d	RI, MS	0.27 ± 0.06	n.d	0.006	35 - 55	-
59	2,3-dimethylpyrazine	n.d	1353.2	RI, MS	0.37 ± 0.02	0.11 ± 0.06	2.5	< 1	-
60	2-ethyl-6-methylpyrazine	1004.2	1392.8	RI, MS	0.12 ± 0.02	0.14 ± 0.02	6	< 1	< 1
61	2-ethyl-5-methylpyrazine	1006.8	1399.3	RI, MS	0.21 ± 0.03	0.24 ± 0.05	0.1	1.8 - 2.4	1.9 - 2.9
62	trimethylpyrazine	1006.3	1412.4	RI, MS	0.7 ± 0.06	0.57 ± 0.15	0.033	19.39 - 23.03	12.73 - 21.82
63	methylpyrazine	n.d	1271	RI, MS	0.11 ± 0.04	0.08 ± 0.03	60	< 1	< 1
64	2,6-dimethylpyrazine	n.d	1335.8	RI, MS	0.08 ± 0.03	0.15 ± 0.04	1.5	< 1	< 1
65	ethylpyrazine	n.d	1341.2	RI, MS	0.1 ± 0.02	0.13 ± 0.08	6	< 1	< 1
66	2,3,5-Trimethyl-6-ethylpyrazine	1165.5	1522.1	RI, MS	0.22 ± 0.03	0.21 ± 0.07	N/A	-	-
67	2,3-dimethyl-5-ethylpyrazine	n.d	1470.2	RI, MS	2.67 ± 0.1	n.d	0.135	19.04 - 20.52	-
68	tetramethylpyrazine	n.d	1482.9	RI, MS	2.53 ± 0.39	1.52 ± 0.19	2	1.07 - 1.46	< 1
69	1-(1H-pyrrol-2-yl)-ethanone	n.d	1969.2	RI, MS	0.97 ± 0.13	0.57 ± 0.06	58.585	< 1	< 1
70	trimethyloxazole	n.d	1202.3	RI, MS	0.15 ± 0.08	n.d	0.005	14 - 46	-
Hydrocarbon									
71	tetradecane	n.d	1399.8	RI, MS	n.d	0.32 ± 0.06	100	-	< 1
Sulphide									
72	dimethyl disulphide	n.d	1050.7	RI, MS	0.25 ± 0.09	n.d	0.0011	145.45 - 309.09	-

Identification methods: MS = mass spectrometry; RI = retention indices; and b = RI < RI of the first alkane (C6). Data are means ± SD of at least three assays; n.d = not detected; N/A = lack of threshold value. A dash (-) indicates that the OAV of compound was not calculated because the compound was not detected, or the threshold value of the compound was not crossed.

(Marín *et al.,* 2002), and wine vinegar (Perestrelo *et al.,* 2018). To gain a deeper insight into the chemical profile and content of the volatile compounds of the analysed samples, the 72 volatile compounds were quantified in the MS_5 and TS_3 (Table 1). Presented results (Table 1) indicated that the volatile profiles of the analysed samples were complex after the baking processes. The major volatile compounds were acetic acid (91.75 and 81.41 mg/kg in MS_5 and TS_3, respectively), and furfural (68.81 and 78.13 mg/kg in MS_5 and TS_3, respectively), which accounted for about 75.43 and 71.02% of total volatile compounds in MS_5 and TS_3, respectively. The acetic acid is a well-known major product in the fermentation process. It is formed by acetic acid bacteria (Zhu *et al.,* 2018b), but the bacteria cannot survive in the high-temperature environment (Ohmori *et al.,* 1980). Hence, the baking process, which was maintained the vinegar mashes at 98°C, should be irrelevant to the production of acetic acid. The furfural is widespread in foods (Ortu and Caboni, 2017; Srivastava *et al.,* 2018), juices (Zhu *et al.,* 2018a), wine (Ortu and Caboni, 2017), and vinegars (Marín *et al.,* 2002; Zhu *et al.,* 2016; Al-Dalali *et al.,* 2019) as an important aroma component, whose formation is promoted by heat treatment.

However, the contribution of volatile compounds in aroma depends not only on the amount of the compound, but also on the OAVs (Yi *et al.,* 2016; Talaverano *et al.,* 2017). Hence, the concentration of these compounds was converted into odour activity values (OAVs) with their odour threshold (Table 1). On the basis of the OAVs, 35 compounds (OAV $\geq$ 1) were considered as the odour-active compounds in the MS_5 or TS_3. In addition, OAV value for eight compounds cannot be calculated due to a lack of threshold values. Previous studies reported that compounds with OAV $\geq$ 1 were considered as major contributors to aroma (Gómez-Míguez *et al.,* 2007; Talaverano *et al.,* 2017; Styger *et al.,* 2011). Although the content of acetic acid was 46 and 40% of the total amount of identified compounds in MS_5 and TS_3, respectively, it was not considered as the odour-active compound because of the high threshold (OAV < 1). On the contrary, some of the compounds presented in trace amounts were considered as contributors to the aroma because of their lower threshold. Results in Table 1 showed that dihydro-5-pentyl-2(3H)-furanone (2375 - 2625 in MS_5 and 4375 - 6375 in TS_3), 3-methylbutanal (1466.67 - 1916.67 in MS_5 and 850 - 883.33 in TS_3), 5-methyl-2-furancarboxaldehyde (595 - 825 in MS_5 and 548.33 - 571.67 in TS_3), 2-methoxyphenol (468.75 - 543.75 in MS_5 and 412.5 - 800 in TS_3), 3-methyl-1-butanol (112.5 - 177.5 in MS_5 and 530 - 680 in TS_3), 3-methylbutyl-acetate (205 - 455 in MS_5 and 245 - 325 in TS_3), 2-pentylfuran (35 - 55 in MS_5 and 165 - 205 in TS_3), dimethyl disulphide (145.45 - 309.09 in MS_5 and 45.45 - 118.18 in TS_3), and heptanoic acid ethyl ester (55 - 105 in MS_5 and 180 - 190 in TS_3) were the characteristic compounds which contributed to the aroma profiles of the baked samples.

Changes of odour-active compounds during the baking processes

To investigate the influence of the different baking methods on the volatile compounds of various baked samples, the aforementioned volatile compounds were quantified in one unbaked and eight baked samples. The results are shown in the form of the heat map of the OAVs (Figure 2). A total of 38 compounds were selected to be the principal aroma compounds of the baked samples in order to evaluate their contributions during the baking processes. These included 35 odour-active compounds, acetic acid, ethyl acetate, and acetoin. Besides, three volatile compounds were selected mainly based on the concentration of volatiles (greater than 1 mg/kg) and the OAV value (close to 1). Because the compounds with the OAV < 1 may also contribute to the aroma profile through the additive effects of the compounds with similar structure or odour (Francis and Newton, 2005). Eventually, acetic acid, ethyl acetate, and acetoin met the above-mentioned conditions.

The contributions and odour description of the principal aroma compounds in various samples are shown in Figure 2, including alcohols (two), acids (three), esters (ten), aldehydes (ten), ketones (two), phenols (four), heterocycles (six), and sulphides (one). Overall, 21 compounds, which mainly belong to alcohols, acids, esters, and aldehydes were quantified in S_0; they were usual metabolites of microorganisms (Cirlini *et al.,* 2011; Giudici *et al.,* 2017; Al-Dalali *et al.,* 2019). After baking, the hexanoic acid ethyl ester was disappeared in the MB; while the benzoic acid ethyl ester and 2-pentylfuran were disappeared in the TB. Although the OAVs of these compounds, except benzaldehyde, furfural, and 3-methylbutanoic acid decreased with the increase in baking time, they were still an important part of odour-active compounds in TB or MB after baking. This is in agreement with the previously published research on the Tartary buckwheat Vinegar (Aili *et al.,* 2012). 17 new compounds were gradually formed with the increase in the baking time, which caused a significantly different odour in both MB and TB samples before and after baking. In addition, the new compounds were not exactly the same in MB and TB. The result (Figure 2) showed that 11 new compounds (2-methylbutanal,

Figure 2. Heat map analysis of 38 principal aroma compounds in the samples of modern and traditional baking method. Each number in the atlas represents one compound listed in Table 1.

3-methylbutanal, 5-methyl-2-furancarboxaldehyde, 1-methyl-1H-pyrrole-2-carboxaldehyde, 2-methoxyphenol, 4-ethylphenol, 4-ethyl-2-methoxyphenol, 2-methoxy-4-vinylphenol, 2-ethyl-5-methylpyrazine, trimethylpyrazine, and tetramethylpyrazine) were formed in both MB and TB; five new compounds, including 3-methylbutyl-acetate, hexanal, 2,3-dimethyl-5-ethylpyrazine, trimethyloxazole, and dimethyl disulphide were detected only in MB; and the acetophenone was formed only in TB. Furthermore, the different effect of two baking methods on the same compound has been shown in the case of MB and TB. The OAVs of volatile compounds such as aldehydes, heterocycles, and sulphides gradually increased from MS_1 to MS_5. Finally, they became the main contributor to the aroma composition of the MB baked samples. On the contrary, the volatile compounds that belong to alcohols, acids, and phenols contributed more to the aroma of the TB baked samples. These odour-active compounds made up the aroma profile in the final product of TB or MB because the aging process only altered the aroma levels rather than the aroma molecules (Liang *et al.,* 2016).

The result of OAVs for these compounds between MB and TB showed a significant diversity (Figure 2). Four aldehydes (the 2-methylbutanal, 3-methylbutanal, hexanal, and 5-methyl-2-furancarboxaldehyde), four heterocycles (trimethylpyrazine, 2,3-dimethyl-5-ethylpyrazine, tetramethylpyrazine, and trimethyloxazole) and one sulphide (dimethyl disulphide) showed outstanding influence on the aroma profile of the MB baked samples. The outstanding contributors in TB were more complex. Those contributors included one alcohol (phenylethyl alcohol), two acids (3-methylbutanoic acid and octanoic acid), two aldehydes (furfural and dihydro-5-pentyl-2(3H)-furanone), four phenols (2-methoxyphenol, 4-ethylphenol, 4-ethyl-2-methoxyphenol, and 2-methoxy-4-vinylphenol), and two heterocycles (2-ethyl-5-methylpyrazine and trimethylpyrazine). As a result, the final baked sample of the TB yielded sour, woody, and baked food or smoky bacon odour (Figure 2). Contrary to TB, the final baked sample of MB exhibited a lower sour odour that was mixed with the baked or roasted food sensory (Figure 2). Regardless the difference in the odour intensity, these compounds were finally retained in products of Shanxi aged vinegar (Chen *et al.,* 2013; Zhu *et al.,* 2016), and their certain types remained stable, while only their levels were slightly altered during the aging process (Liang *et al.,* 2016). They were also formed in small amounts in the other kind of vinegars that were not baked (Marín *et al.,* 2002; Al-Dalali *et al.,* 2019) because the Maillard reaction continues slowly during the aging process.

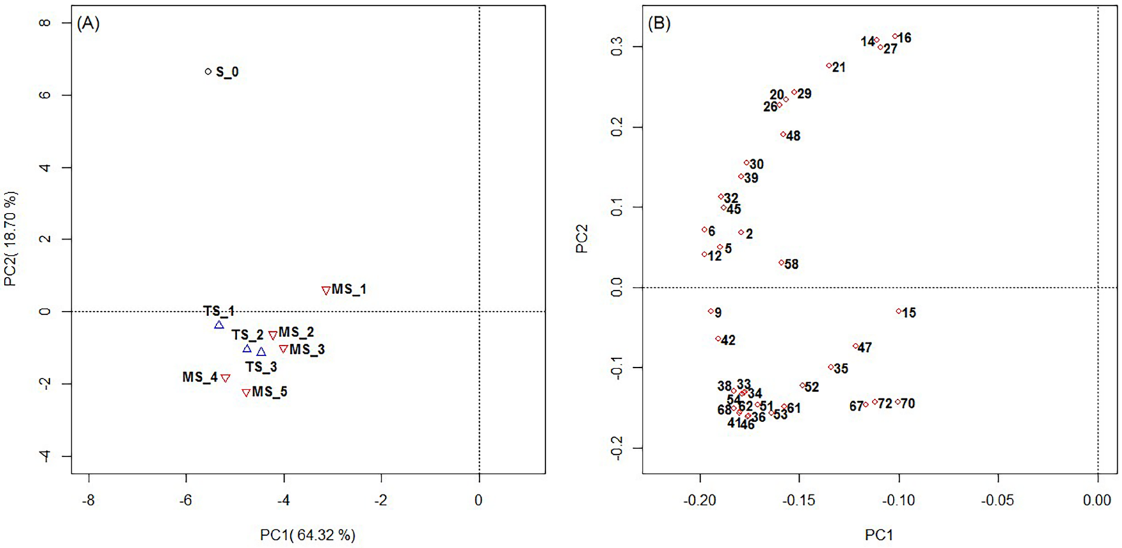

Figure 3. Score (A) and loading (B) plot of PCA according to the principal aroma compounds in the samples of modern and traditional baking method. Each number in the atlas represents one compound listed in Table 1.

Principal Component Analysis (PCA)

In order to analyse the trend in the aroma profiles of Shanxi aged vinegars more deeply, principal component analysis (PCA) was performed to check the intrinsic variation in the data matrix, and to determine which volatile compounds contributed the most to the difference. Figure 3A shows the score plot of the first principal component (PC 1, 64.32% of total variance) *versus* the second principal component (PC 2, 18.70% of total variance). The nine vinegar samples were clearly divided into the two regions. A large gap was observed between the unbaked and the baked samples (Figure 3A). S_0 is located on the top-left of plot. Except for MS_1, all the baked samples which are located in the negative region of the PC1 and PC2, were clearly isolated from the S_0. The esters including ethyl acetate, hexanoic acid ethyl ester, heptanoic acid ethyl ester, octanoic acid ethyl ester, decanoic acid ethyl ester, benzoic acid ethyl ester, and benzeneacetic acid ethyl ester were responsible for the gap (Figure 3B). The gap between the MS_1 and TS_1 was the largest in the baked samples. The most probable cause was that heating rate had difference between MB and TB. Then the gaps narrowed during the baking processes. The main reason was that newly formed volatile compounds accumulated. Meanwhile, the loading of PCA (Figure 3B) showed that 13 odour-active compounds, of which the OAVs had differences in two baking methods, were tightly associated with the baking process. They were the 2-methylbutanal, 3-methylbutanal, 5-methyl-2-furancarboxaldehyde, furfural, 1-methyl-1H-pyrrole-2-carboxaldehyde, 2-methoxyphenol, benzeneacetaldehyde, 4-ethylphenol, 4-ethyl-2-methoxyphenol, 2-methoxy-4-vinylphenol, 2-ethyl-5-methylpyrazine, trimethylpyrazine, and tetramethylpyrazine, which belong to the aldehydes, phenols, and heterocycles.

Conclusions

In total, 72 volatile compounds were identified and quantified in the baked samples of MB and TB. Among them, 38 were considered as the principal odour compounds, including the 35 odour-active compounds (OAV ≥ 1), acetic acid, ethyl acetate, and acetoin. Most of the new compounds were formed from the first day of baking. The heat map and PCA analysis indicated that the influence of different methods was stronger on the accumulation of the principal odour compounds and less on their types. In addition, the proportion of each aroma compounds between two baking methods in general had difference, which means the modern method still could not fully reproduce the aroma of the traditional method. Therefore, the modern method still requires improvement.

Acknowledgement

The present work was financially supported by the Key R&D Program of Shanxi Province (201803D221013-1) and the Commercialization of Scientific and Research Findings of Shanxi Province (201904D111004)

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