Analysis of variation of main components during aging process of Shanxi Aged Vinegar

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Abstract

Shanxi aged vinegar (SAV) is the most famous traditional vinegar in northern China. It is produced from several kinds of cereal by spontaneous solid-state fermentation techniques. The distinctive processing techniques such as *smoking of the Pei*, aging by *insolating in summer* and *taking out ice in winter* formed during the long-term production practice have been named as the national intangible cultural heritage. Some research reports about the nutritional composition, flavor compounds of SAV have been published, but there is no report on the changes of main components during aging process in SAV. In this study, the volatile flavor compounds, amino acids, organic acids, trace elements and other conventional ingredients in SAV were examined by headspace solid-phase microextraction gas chromatography-mass spectrometry, automatic amino acid analyzer, high performance liquid chromatography, plasma emission spectroscopy and other modern analytical techniques. The results showed that most conventional ingredients (organic acids, free amino acids, carbohydrates) were increased during aging process. There were 20 different amino acids in SAV, the concentration of total amino acids reached 19.73 mg×mL^{-1} in eight-year-old vinegar. There were 8 different organic acids in SAV, and acetic acid and lactic acid were main organic acids. A total of 58 different flavor compounds were detected in SAV. The results of this study can help us to understand the class and concentration of main components in SAV, and provide data for manufacturers to improve production process and product quality.

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Introduction

Vinegar has been made and used dating from around 3000 BC and is an important seasoning in Asian, European and other traditional cuisines of the world. Vinegar has physiological effects, such as aiding digestion, stimulating the appetite, and promoting recovery from exhaustion.[1] Traditional Chinese vinegars, also called cereal vinegars, are important seasonings in Chinese daily life. Among these, the four famous China-style vinegars are Shanxi aged vinegar (SAV), Zhenjiang aromatic vinegar, Sichuan bran vinegar and Fujian *Monascus* vinegar, which are used sticky rice, sorghum, wheat bran and red yeast rice as the main raw materials, respectively.[2]

SAV which is special for the organoleptic attribute of *sweet, soft and acid taste, good aftertaste linger*, is the famous traditional Chinese vinegar made from several kinds of cereal by spontaneous solid-state fermentation techniques.[3] The SAV distinctive processing techniques formed during the long-term production practice such as *smoking of the mash*, aging by *insolating in summer* and *taking out ice in winter* have been named as the national intangible cultural heritage. The general scheme in SAV production mainly includes following five stages.[2] Starter (called *Daqu* in Chinese) preparation, during which the dominant microorganisms are enriched by choosing the raw materials and controlling the cultural conditions. Saccharification and fermentation (alcohol and acetic acid); alcohol fermentation, during which the dominant microorganisms, such as moulds and yeasts from the starter, grow in fermentation substrates to hydrolyze starch into sugar and convert sugars to ethanol; acetic acid fermentation, during which ethanol is fermented into acetic acid by acetic acid bacteria. Smoking the mash, during which the mash after acetic acid fermentation is smoked and turns black. Leaching vinegar, during which the final products are dissolved from mash. Aging process, during which the vinegar aged by *insolating in summer* and *taking out ice in winter*; namely, the vinegars were exposed to the sun in opened container in summer, the water in vinegars freeze and the ice was taken out of the container in winter; the flavor components are formed during aging period, acetic acid, furfural, 3-hydroxy-2-butanone, 2,3-butanedione and tetramethyl pyrazine were the main volatile components in SAV.[4] The production process of SAV was showed in Figure 1.

A large number of substances present in SAV, including organic acids, free amino acids, carbohydrates, alcohols, esters, and various microconstituents. Some components (*e.g.*, carbohydrates) were already present in raw materials, whereas others emerge during the production process, either (bio-) chemically or microbially.[5]

There are many researches about determination and analysis of components in vinegars. Simultaneous determination of sugars and organic acids in Aceto Balsamico Tradizionale of Modena (ABTM) by gas chromatograph (GC) was developed.[6] The main organic components of vinegar were identified and quantified by high resolution 1H NMR spectroscopy.[7] A solid-phase extraction method for the determination of volatile compounds in traditional balsamic vinegar has been developed.[8] A static headspace GC coupled to mass spectrometry (HS-SPME-GC-MS) method was validated to determine several major volatile components during the production process of fruit vinegars.[9] Some research reports about the nutrition, flavor components of SAV have been published in China.[10-14] However, there is no reports on the changes of main components of SAV in the aging process.

Thus the present work was aimed at using modern analytical methods to study the variation of main components (nutrition, flavor components, etc.) during the aging process of SAV production. This study may contribute to reveal the rule of form and change of main components during aging process in SAV.

Materials and Methods

Vinegar samples and materials

Vinegar samples at different aging times were obtained from Shanxi aged vinegar Group Co., Ltd. (Shanxi, China). Briefly, vinegar samples from storage times of 0, 1, 2, 3, 5 and 8 years were collected, and one sample of different years was used. All the chemicals used were analytical-reagent grade and purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China).

Determination of soluble saltless solid

The content of soluble saltless solid was measured by the gravimetric method. Briefly, 2 mL vinegar sample was dried in an oven at 103-105°C until constant and weighed as the content of total soluble solid (ρ_1). The content of sodium chloride in vinegar (ρ_2) was determined by titration with $AgNO_3$ standard solution. The content of soluble saltless solid (ρ_3) was calculated using the following: $\rho_3=\rho_1-\rho_2$.

Determination of total sugars by the anthrone colorimetry method

Content of total sugars in vinegar was determined by the anthrone-sulfuric acid colorimetry method.[15] Vinegar samples were decolorized by activated carbon and diatomaceous earth firstly, then diluted 10 times with distilled water. Dilution (2 mL) was taken to test tube, and

Figure 1. Flow sheet for Shanxi aged vinegar production. The primary materials are jowar and *Daqu*; the process may be divided into five mainly processes, namely starter preparation, fermentation, smoking, sprinkling and aging. The whole production period lasts one year or longer.

added anthrone reagent (5 mL) to the tube. Tube bathed in boiling water for 1 min and naturally cooled to room temperature. The absorbance was then measured at 620 nm. The concentration of total sugars in vinegar was calculated from a standard curve of glucose.

Determination of amino nitrogen by formaldehyde titration

The content of amino nitrogen in vinegar samples was determined by formaldehyde titration according to the GB/T 5009.39-2003 method.[16] Vinegar samples were diluted 10 times with distilled water. The diluted vinegar (20 mL) was taken in 200 mL beaker, 60 mL distilled water was added to the beaker. The pH was adjusted to 8.2 with 0.1 mg×mL^{-1} sodium hydroxide standard solution by pH meter. Formaldehyde solution (10 mL) was added, titrated with 0.1 mg×mL^{-1} sodium hydroxide standard solution until the pH reach to 9.2, and the volume of sodium hydroxide titrant was recorded. 80 mL distilled water used as a blank, using the titration procedure described above, recorded the volume of sodium hydroxide titrant necessary to reach the end point. The content of amino nitrogen was calculated as follow.

$$X = \frac{(V_0 - V) \times C \times 0.014}{L} \times 100$$

where X represents the content of amino nitrogen, V_0 is volume of standard sodium hydroxide used in titration of control, V is volume of standard sodium hydroxide used in titration of sample, C is the content of standard sodium hydroxide solution, 0.014 is content of nitrogen equivalent to 1 mL sodium hydroxide solution, and L is the volume of samples.

Determination of volatile compounds by headspace gas chromatograph coupled to mass spectrometry

A HS-SPME device was used for the isolation of volatile compounds from vinegar samples. Briefly, 4 mL vinegar sample was placed in a 15 mL vial, and 1 g sodium chloride was added. This sample was extracted at 50°C for 40 min with stirring in a multipurpose sampler with SPME capability. After extraction, analytes were desorbed from the fiber coating in the injection port of GC at 250°C for 5 min in splitless mode. GC-MS analysis was performed on a 7890A/5975C model (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with a DB-5 ms capillary column (30 m×0.25 mm×0.25 mm). Helium (flow rate, 1.0 mL×min^{-1}) was used as carrier gas. GC oven temperature was maintained initially at 40°C for 4 min, followed by increases to 120°C at a rate of 5°C min^{-1}, and from 120 to 240°C at a rate of 8°C min^{-1}, and then this temperature was held constant for 10 min. The mass spectra were acquired with a source temperature of 230°C under a 70 eV ionization potential, and data collected at a rate of 0.7 scans s^{-1} over a range of 33-450 amu.

Determination of reducing sugar by Fehling's method

Content of reducing sugar in vinegar samples was determined according to the GB/T 5009.7-2003 method.[17] Briefly, the vinegar sample (5 mL) was diluted with distilled water (95 mL), and the diluted vinegar (2 mL) was used to determine the content of reducing sugar by direct titration method of Fehling's reagent. The content of reducing sugar is calculated using the following formula:

$$X = \frac{(V_0 - V) \times 0.1\%}{\frac{5}{100} \times 2} \times 100$$

where X represents the content of reducing sugar (glucose equivalent), V is volume of standard glucose used in titration of control, V_0 is volume of standard glucose used in titration of sample, and 0.1% is the content of standard glucose solution.

Determination of amino acids

Amino acid content in vinegar samples was analyzed using Entire Automatic Amino Acid Analyzer model L-8000 (Shanghai Huxi Analytical Instrument Factory Company Ltd., Shanghai, China) with a fluorescence detector.[18] Briefly, the vinegar samples were centrifuged (2000 g, 15 min), diluted 50 times with 0.02 mg×mL^{-1} hydrochloric acid solution, and filtrated by vacuum using a cellulose membrane (pore size 0.45 μm). A Zorbax SB-C_{18} (4.6 mm ID×60 mm) column (Agilent Technologies) was used, and the flow rate was 0.75 mL×min^{-1}. The column temperature was 40°C; detector: Ex 340 nm, Em 450 nm; mobile phase A: 20 mmol sodium acetate solution, the flow rate was 0.40 mL×min^{-1}; mobile phase B: 20 mmol sodiumacetate-methanol-acetonitrile (1:2:2, (v/v/v), the flow rate was 0.35 mL×min^{-1}. Each amino acid was quantified by the calibration curve of the authentic standards.

Determination of organic acid by high performance liquid chromatography

Organic acids were determined by high performance liquid chromatography (HPLC), using equipment from Shimadzu (Kyoto, Japan). All vinegar samples were centrifuged (6000 g, 15 min), and the supernatant was filtered through a 0.45 μm membrane prior to HPLC analysis. Injection volume of the sample was 10 μL. The instrument was equipped with an automatic injector and a photodiode array detector (PDA) UV at 210 nm. Separation was achieved using Kromasil C_{18} column (5 μm, 4.6×150 mm) at 30°C. The mobile phase was 20 mmol NaH_2PO4, with a flow rate of 0.8 mL×min^{-1}. All organic acids were recorded on a computer based data system. Each organic acid was quantified by the calibration curve of the authentic standards.

Determination of total acid and pH

Total acid were determined according to the GB/T 5009.41-2003 method.[19] Briefly, added the vinegar sample (1 mL) to fresh distilled water (50 mL) in flask and blended, then added sodium hydroxide (0.1 mmol) to the flask until the pH of mixture reach to 8.2. The reading was taken of the sodium hydroxide standard solution used. The content of total acid is calculated using the following:

$$X = \frac{C \times V \times 0.06}{V_1}$$

where, X represents the content of total acid (acetic acid equivalent), V is volume of standard hydroxide solution used in titration of control, and C is the content of standard sodium hydroxide solution. 0.06 is content of acetic acid equivalent to 1mL sodium hydroxide solution, and V_1 is the volume of samples.

The pH value was measured using a pH meter (Model: PHS-4CT, Shanghai Zhicheng Instrument Co. Ltd., Shanghai, China), with accuracy of 0.001 pH unit.

Results and Discussion

Analysis of conventional physical and chemical indicators

Results of analysis for main components were shown in Table 1. The content of total acid, total sugar, reducing sugar, NaCl, soluble solids, soluble saltless solid and amino nitrogen were increased during aging period, but the pH changed little (Table 1). This may own to the technology *insolating in summer* and *taking out ice in winter* during aging process, and a large amount of water was removed, so it was a condensation process. In the former technology, NaCl was added to *Pei* at the end of acetic acid fermentation to inhibit growth of acetic acid bacteria, but no NaCl was added in the new technology. So it was showed that the content of NaCl in the vinegars was low (Table 1), NaCl maybe originated from raw material.

Analysis of amino acids

Amino acids were determined by amino acid analyzer, the composition of amino acids was showed in Table 2. The results indicated that the total amount of amino acids increased during aging, but tryptophan was not detected in SAV. The possible reason was that free tryptophan easily decomposed when coexisted with other amino acids, sugars, aldehydes.[20] Taurine only existed in new, one-year and three-year vinegar, and the content of taurine decreased during aging. Taurine existed widely in animal tissues, but it also detected in plant with a low level.[21] So Taurine in SAV maybe came from raw material. Water was removed and the content of most of amino acids increased during aging. Amino acids (such as lysine, histidine, arginine) reacted with saccharide and produced dark pigments or melanoidines through Maillard reaction. Some kinds of amino acids (lysine, histidine) may decrease due to Maillard reaction. The total amount of amino acids was high, alanine, leucine, aspartic acid, valine, proline and glutamate were the main compositions, and it was different from other vinegar, such as Zhenjiang aromatic vinegar.[22] The amino acids endowed SAV with special flavor.

Analysis of organic acids

Organic acids were determined by HPLC. The result was showed in Figure 2, the contents of all kinds organic acids were showed a increasing trend during aging because of condensation. In particular, as regards the organic acids content, acetic acid and lactic acid were present at high concentration, they were main organic acids, following were citric acid, succinic acid, tartaric acid and malic acid. The amount of acetic acid was highest in total organic acids accounting for 30.8%-34.7%. Small amounts of acetic acid disappeared by volatilization and esterification. The concentrations of some non-volatile acids (lactic acid and citric acid) were high also, and increased more than volatile acids during aging. The amount of non-volatile acid took more than 60% the proportion of total acids in the eight-year-old vinegar.

Most of organic acids were formed in the process of making *Daqu* and transformed by microorganisms, a small part derived from the raw material. It was reported that lactic acid as a by-product of alcoholic fermentation from pyruvic acid, or by lactic bacteria from malic acid in wine.[23] The proportion of lactic acid in vinegar of total organic acids was 15.6-26.3%. It was well known that grain contained fewer malic acids, so the content of malic acid in SAV was still low.

The organic acids in SAV were different from others vinegar due to different raw material and special technology process. There was much malic acid in ABTM from Italy,[6] because ABTM was brewed from grape must which contained more malic acid,[24-26] but the concentration of malic acid was low in SAV which made from grains. Citric acid was formed during alcoholic fermentation and may occasionally be used as a substrate by some microorganisms which produced acetic acid.[27] Citric acids reacted with certain metals (Ca, Mg, etc.), forming soluble complexes, and stabilizing the vinegar.[28]

Analysis of mineral elements

Among mineral elements, Pb, Cd and Cr were determined by atomic absorption spectrometry, Se, As and Hg were determined by atomic fluorescence spectrometry, the other elements were determined by plasma emission spectroscopy. The results were showed in Table 3. The amount of all kinds of trace elements almost increased during aging except 3-years vinegar, the irregular results probably caused by the sampling errors. The order of concentration was first K, next Na, then Ca, Mg and P. The concentrations of heavy metals (Cd and Pb) in the vinegar samples were very low and not considered health risks. The

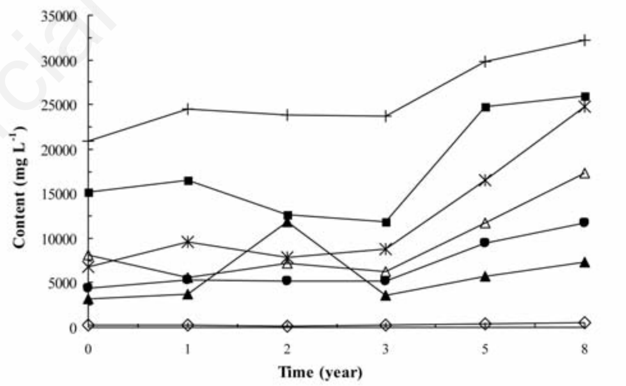

Figure 2. Contents of organic acids in Shanxi aged vinegar aged for different years. ◇, oxalic acid, ◆, tartaric acid, ▲, malic acid, ■, lactic acid, *, citric acid, △, succinic acid, +, acetic acid.

Table 1. Conventional physical and chemical indicators in Shanxi aged vinegar during aging.

Indexes ($mg \times mL^{-1}$)	New vinegar	One-year vinegar	Two-years vinegar	Three-years vinegar	Five-years vinegar	Eight-years vinegar
pH	3.67	3.79	3.72	3.68	3.84	3.88
Total acid	65.30	70.50	70.00	76.10	96.70	98.50
Total sugar	75.90	204.80	182.20	295.00	593.20	572.30
Reducing sugar	13.00	28.00	22.00	35.00	61.00	75.00
NaCl	2.90	2.90	2.00	4.10	8.20	13.10
Soluble solids content	107.10	140.90	109.00	151.20	294.70	349.40
Soluble saltless solids content	104.10	138.00	107.00	147.10	286.50	336.40
Amino nitrogen	2.50	3.00	2.80	2.80	4.70	5.80

Table 2. Composition of amino acids in Shanxi aged vinegars aged for different years.

Amino acids	Content (mg×mL^{-1})					
	New vinegar	One-year vinegar	Two-years vinegar	Three-years vinegar	Five-years vinegar	Eight-years vinegar
Essential amino acid						
Lysine	0.67	0.82	0.87	0.70	0.83	0.87
Valine	0.80	0.87	0.91	1.20	1.35	1.63
Phenylalanine	0.40	0.54	0.52	0.54	0.73	0.68
Leucine	1.09	1.24	1.24	1.12	1.96	1.90
Isoleucine	0.50	0.56	0.58	0.53	0.82	0.87
Threonine	0.42	0.50	0.49	0.44	0.61	0.61
Methionine	0.19	0.19	0.21	0.31	0.30	0.39
Essential amino acids to infant						
Histidine	0.13	0.14	0.13	0.10	0.13	0.12
Nonessential amino acid						
Tyrosine	0.09	0.12	0.08	0.11	0.40	0.53
Aspartic acid	1.01	1.03	1.09	1.12	1.64	1.75
Serine	0.56	0.62	0.64	0.58	0.79	0.75
Glutamate	1.11	1.23	1.25	1.13	1.53	1.33
Glycine	0.45	0.46	0.49	0.48	0.68	0.69
Alanine	1.59	1.72	1.81	1.81	2.98	3.51
Cysteine	0.15	0.11	0.13	0.56	0.27	0.66
Arginine	0.31	0.60	0.48	0.46	0.72	0.63
Proline	0.69	0.78	0.76	0.75	1.39	1.64
Non-protein amino acid						
Ornithine	0.58	0.54	0.73	0.50	0.47	0.61
Ammonia	0.97	0.98	1.01	1.16	1.60	1.79
Taurine	0.05	0.04	0.05	0.00	0.00	0.00
Total amount	11.06	12.39	12.81	12.71	18.08	19.73

Table 3. Contents of mineral elements in Shanxi aged vinegars aged for different years.

Elements (mg×kg^{-1})	New vinegar	One-year vinegar	Two-years vinegar	Three-years vinegar	Five-years vinegar	Eight-years vinegar
Beneficial elements						
K	4000.00	4975.00	4350.00	5250.00	10625.00	13825.00
P	1340.80	2789.10	2903.70	2153.50	5816.00	6252.10
Mg	1240.00	1520.00	1320.00	1570.00	3100.00	2775.00
Na	625.00	635.00	615.00	528.00	753.00	650.00
Ca	603.00	768.00	700.00	753.00	1070.00	1158.00
Fe	48.50	54.80	44.00	62.30	102.30	120.30
Mn	11.80	31.00	28.30	32.00	62.30	53.80
Zn	8.30	20.90	18.50	20.80	38.80	42.80
Cu	0.23	0.48	0.35	0.30	0.33	0.45
Cr	0.09	0.14	0.16	0.10	0.29	0.32
Se	0.008	0.009	0.006	0.016	0.009	0.021
Harmful elements						
Al	9.60	14.40	16.40	9.90	29.10	29.70
Pb	0.16	0.32	0.34	0.20	0.28	0.29
Cd	0.02	0.05	0.03	0.04	0.08	0.09
As	0.03	0.05	0.04	0.03	0.06	0.09
Hg	1.00	0.89	0.82	1.06	1.12	1.25
Other						
B	0.36	0.31	0.49	0.43	0.71	0.79

Table 4. Volatile components in Shanxi aged vinegars during aging.

	Relative amount %					
	New vinegar	One-year vinegar	Two-years vinegar	Three-years vinegar	Five-years vinegar	Eight-years vinegar
Alcohols (4 kinds)						
Ethanol	1.0413	0.1111	---	1.0919	0.4967	0.8581
2,3-Butanediol	1.7987	0.2557	0.8073	0.873	2.1471	3.6202
Phenylethyl Alcohol	4.5949	3.1635	3.1635	1.3842	1.5215	1.2922
Beta-thujaplicin	---	---	---	---	0.271	0.1833
Acids (9 kinds)						
Acetic acid	62.5146	68.359	56.8763	77.6511	66.6856	67.5437
3-methyl-Butanoic acid,	2.6788	2.2441	0.8824	1.2025	0.3022	0.1504
2-methyl-Pentanoic acid,	---	0.303	---	---	---	---
2-methyl-Butanoic acid,	0.7248	0.2554	---	---	---	---
4-Ketopimelic	---	0.354	---	0.072	---	0.3337
Hexanoic acid	0.9337	0.6527	0.7943	0.4499	0.519	0.3933
Heptanoic acid	0.1857	0.0556	0.1295	---	---	---
Octanoic Acid	0.2692	0.2249	0.4843	0.0985	0.048	0.0542
Benzoic acid	0.0779	---	0.2909	---	---	---
Aldehydes (8 kinds)						
Furfural	1.2298	2.9037	1.7796	3.362	3.3304	0.2332
Benzaldehyde	0.5996	0.3398	0.0499	0.1185	0.0314	0.0499
e,5-methyl-2-Furancarboxaldehyd	---	---	---	0.0181	0.054	0.0248
α-ethylidene-Benzeneacetaldehyde	0.1358	0.1376	0.1041	0.0492	---	0.0208
Salicylaldehyde	---	---	---	---	0.1838	0.0898
Vanillin	---	0.1626	0.2551	---	---	---
5-Methyl-2-phenyl-2-hexenal	---	0.0832	---	---	0.0275	0.0296
2-Phenyl-3-(2-furyl)-propenal	0.1942	0.2966	0.5487	0.0548	0.0648	0.0347
Phenols (10 kinds)						
3-Pyridinol	3.2654	---	0.1241	---	---	---
2-methyl- Phenol	---	0.0829	---	0.0275	0.086	0.0751
l,4-ethyl-1,3-Benzenedio	---	---	---	0.0154	0.0573	0.0297
2-methoxy-4-methyl- Phenol,	0.0992	0.1076	---	0.043	0.0391	0.0288
4-ethyl-2-methoxy- Phenol	0.6935	0.1308	0.2354	0.0741	0.051	0.0608
Butylated Hydroxytoluene	0.1839	---	---	0.0487	0.0885	0.1379
2,4-bis(1,1-dimethylethyl)- Phenol	---	---	---	---	0.2013	---
2,5-bis(1,1-dimethylethyl)- Phenol	1.1044	1.9858	3.8712	0.4376	---	0.1902
4-(1,1,3,3-tetramethylbutyl)- Phenol	0.1094	0.3294	0.5757	---	---	---
Propofol	0.0455	0.0867	0.2366	---	---	---
Ketones (4 kinds)						
3-hydroxy-2-Butanone	---	---	---	3.499	3.2581	1.8729
Benzyl methyl ketone	0.1163	0.104	---	---	---	0.0569
1-(2,5-dimethoxyphenyl)- Ethanone	---	0.1261	---	---	---	---
3',5'-Dimethoxyacetophenone	0.1242	---	---	---	0.0276	0.0355
Esters (10 kinds)						
1-Methoxy-2-propyl acetate	---	0.2703	---	0.385	0.2955	0.4554
1,3-Propanediol, diacetate	---	---	---	---	0.2692	0.3582
Butanedioic acid, diethyl ester	0.2053	0.1623	0.1517	0.0724	0.056	0.0581
2-Propenoic acid, 2-phenylethyl ester	---	1.7392	---	0.8137	1.1572	---
Acetic acid, 2-phenylethyl ester	1.9308	---	2.2534	---	---	0.8922
2,4-diisocyanato-1-methyl- Benzene,	1.1112	2.147	3.1044	0.0555	0.1612	0.1075
2(3H)-Furanon, dihydro-5-pentyl	0.6546	0.6582	0.8942	0.2275	0.1231	0.2021
Benzeneacetic acid, 2-propenyl ester	---	0.3162	0.4462	---	---	---
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	0.1103	0.2531	0.4962	---	0.0818	---
Linoleic acid ethyl ester	0.0992	---	---	---	---	0.0219
Heterocyclic (13 kinds)						
Trimethyl- Oxazole	1.2458	0.861	1.0489	0.9177	0.87	0.5944
2,3-dimethyl- Pyrazine	---	---	---	0.0442	---	0.0224
2-ethyl-2,4,5-trimethyl-1,3-Dioxolane	0.3246	0.371	0.756	0.3574	0.4773	0.756
trimethyl- Pyrazine	0.4686	0.6585	0.6343	0.3032	1.0431	0.6515
2-[2-(2-ethoxyethoxy)ethoxy]- Ethanol	---	0.2599	0.6705	---	---	0.0484
1-(1H-pyrrol-2-yl)- Ethanone	0.0864	0.0891	---	0.0821	0.1247	0.1472
Tetramethyl- Pyrazine	5.0967	4.1333	4.7882	3.1626	11.6474	10.0805
2,3,5-Trimethyl-6-ethylpyrazine	0.1362	0.1109	---	0.0816	0.1411	0.1205
2,3-Dimethyl-5-n-propylpyrazine	---	---	---	0.0154	---	0.0303
2,3-dimethyl-5-(1-propenyl)-, (Z)- Pyrazine	0.0313	---	---	0.0169	0.0199	0.0287
4-methoxy-N-methyl- Benzenamine	0.1837	0.3266	0.599	0.1447	0.1616	---
Tetrahydro-2-Furanmethanamine	0.2653	0.2511	---	---	---	---
2,3,5-Trimethyl-6-isopentylpyrazine	---	---	---	---	0.0451	0.048

---, not detected.

results were in agreement with the former report.[26] Some harmful elements (Al, As and Hg) were determined, but their content met the limitation standard (GB 2719-2003).[19] SAV contained richbeneficial mineral elements (Table 3). Mineral elements play an important role for flavor, nutrition and quality of vinegar. The amount of mineral elements in Chinese vinegar was higher than other vinegars; it may be associated with raw material, water, container and tool.[29]

Analysis of aromatic compounds

Aroma is one of the most important determinants of food quality and acceptance. The particular aroma of vinegar is the result of high quantities of volatile compounds. These compounds may come from the raw material or may be formed during the production process.[9]

The aromatic compounds in SAV were determined by HS-SPME-GC-MS method. A total of 58 compounds were detected, including 4 kinds of alcohols, 9 kinds of acids, 8 kinds of aldehydes, 10 species of phenols, 4 kinds of ketones, 10 species of esters and 13 kinds of heterocyclic (Table 4). It was showed that acetic acid, ethanol, 2, 3-butanediol, phenylethyl alcohol, 3-hydroxy-2-butanone (acetoin), tetramethyl pyrazine, 2, 4-diisocyanato-1-methyl benzene, furfural and 2, 4-bis (1, 1-dimethylethyl) phenol were the main components of aroma (Table 4).

In the aging process, volatile compounds were evaporated and the amount almost decreased. But the content of ethanol, 2, 3-butanediol, acetic acid was almost constant. However, tetramethyl pyrazine increased at some extent, this may be attribute to the conversion of precursors to tetramethyl pyrazine through the Maillard reaction during the storage process.[18] The compositions of volatile components were different during aging. Most acids decreased during aging because of evaporation, but aldehydes, phenols and heterocyclic compounds increased. So the flavor of different aged vinegar was various. Acetic acid was the most important component in vinegar. Although acetic acid was evaporated during aging, the content changed little due to condensation ($P>0.05$ by ANOVA).

Alcohols were produced by alcoholic fermentation with soft flavor. 2, 3-butanediol tasted sweet, phenylethyl alcohol brought similar roses aroma to the vinegar and had better persistence.[30]

Aldehydes were hydrophilic, combined with water easily, formed acetal with alcohol, and producing a soft aroma for vinegar. Vinegar with the process of *smoking of the mash* contained more aldehydes.[31]

Acetoin (ACT, acyloin, 3-hydroxy-2-butanone) is an important physiological metabolite excreted by many microorganisms. The main ACT producing bacteria are *Klebisella*, *Enterobacter*, *Bacillus*, *Serratia*, and *Lactococcus* species. In vinegar, ACT is formed from 2, 3-butanediol during the acetic fermentation.[32] ACT is an important flavour in vinegar, serves as a quality index of fermented products. ACT was detected in SAV.

There were fewer esters in grain vinegar. Esters were by-products of yeast fermentation, or formed during aging in SAV, giving vinegar nice smell. But there were more esters in vinegar which was made by fruit must, such as Italian vinegar.[33] The aroma of vinegar was related with raw material.

Conclusions

In this study, the main components in SAV of different aging time were researched. It was concluded that SAV contained rich nutriment (organic acids, free amino acids, carbohydrates). The contents of most components increased due to concentration in SAV with increasing aging time. This study may contribute to reveal the rule of form and change of main components during aging process in SAV, and provide data for production high value-added SAV.

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