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Effects of different koji on aroma components of Rice Wine

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Abstract

Aroma composition is critical to the quality of rice wine. In order to clarify the effect of different koji on the aroma components of rice wine. The Yuanyang rice was selected as raw material, and three kinds of rice wine koji were added during rice wine fermentation. The aroma components of three wine samples fermented at 31 °C for nine days were qualitatively and quantitatively analyzed by headspace solid-phase microextraction and gas chromatography-mass spectrometry (HS-SPME-GC-MS). The results showed that a total of 81 aroma components were detected in the three groups of wine samples, including 30 esters, 18 alcohols, 4 acids and 29 other organic substances. The ester with the most content was ethyl palmitic acid, and the alcohol with the most content was ethanol. Therefore, the amount and type of esters, the ratio and interaction among esters, alcohols and acids in wine play an important role in the sensory quality of rice wine, and the joint action of various aroma components affects the quality of rice wine.

Keywords: rice wine; brewing koji; headspace solid-phase microextraction; Gas chromatography-mass spectrometry; aroma components.

Practical Application: In this paper, the effects of three kinds of koji on the volatile aroma components of rice wine were studied by HS-SPME-GC-MS. The data presented for production of rice wine can be used to develop similar products in food industries.

1 Introduction

Rice wine originated during the Yangshao culture period and is one of the oldest low-alcohol beverages in the world. Chinese rice wine is a low-alcohol beverage obtained by the natural fermentation of glutinous rice, rice, red rice, black rice, and other grains with koji and water (Jiao et al., 2017; Wei et al., 2017). It is reported that rice wine contains many beneficial ingredients such as protein, amino acid, polypeptide, and lactic acid, as well as various minerals such as calcium, zinc, and phosphorus (Wang et al., 2018). In addition, rice wine has anti-cancer, anti-inflammatory and anti-oxidant effects, so many domestic and foreign tourists and wine lovers have always loved it (Lee et al., 2018).

With the continuous improvement of people's living standards, the commercial value of rice wine is also continuously improved, so it is necessary to study rice wine. Koji is one of the important auxiliary materials for wine-making, including microorganisms needed for wine-making, saccharifying agent, starter and aroma-producing agent of wine, and an important parameter in the fermentation process of rice wine (Cheng et al., 2020). There are many kinds of rice wine koji on the market, and the taste and flavor of fermented rice wine are also different. In the food industry, it is undoubtedly necessary to strengthen the quality control of rice wine and the development of rice wine products. It is also necessary to make consumers quickly perceive the importance of new products. The development of mascarpone cheese using sheep milk resulted in an innovative and differentiated product with enhanced functional properties, texture and flavors (Munieweg et al., 2021). Sensory evaluation is an important reference index in food research and development, and commonly used sensory evaluation methods are Quantitative Descriptive Analysis (QDA) and Check-All-That-Apply (CATA) (Lima et al., 2022). The types and relative contents of flavor substances in rice wine are related to many factors, such as raw materials, koji (Jiuqu) and brewing conditions. The study found differences in the quality of rice wine prepared by different koji (Zhao et al., 2019). Chen et al. (2021) studied the interaction of microorganisms in rice wine and the changes in physicochemical and aroma components of rice wine through different inoculation strategies. The results showed that inoculation methods and non-yeast had effects on the volatile acid, total acid and alcohol content of rice wine. There are many volatile aroma components in rice wine, including acids, alcohols, esters, phenols, etc. The type, content, sensory threshold, and interaction between these components determine the flavor of rice wine. Some researchers have determined the volatile components in beverage wine by headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) (Cai et al., 2020; Pati et al., 2021; Philipp et al., 2019; Tufariello et al., 2022).However, there are few studies on the effects of different koji on the aroma components of rice wine. This paper aims to identify the aroma components of rice wine brewed by three kinds of koji by headspace solid-phase microextraction and gas chromatography-mass spectrometry (HS-SPME-GC-MS), so as to provide a scientific basis for

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further research on the quality control of rice wine. The data presented for production of rice wine can be used to develop similar products in food industries.

2 Materials and methods

2.1 Materials

Rice (Henan Province Xinsheng Rice Industry Co., Ltd.); Suzhou bee sweet wine koji, (Suzhou Cereals, Oils and Foodstuffs Co., Ltd.); Septuagenarian mash lees sweet wine koji (Sichuan Dazhu Dongliubai traditional handmade plant sweet wine koji), Wine cakes (Ziguixiang Food limited company)

2.2 Instruments and equipment

Agilent 7890A-5977A gas chromatography-mass spectrometer (Agilent Technologies Co., Ltd.); KQ-250B ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); PC-400D Magnetic heating stirrer (Supelco, USA); 65 μm PDMS/DVB solid-phase microextraction head (Supelco, USA); Agilent WAX type strong polar chromatography column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Inc., USA)

2.3 Experimental method

Rice wine processing process

Rice→bubble rice→Steamed rice→drenched in cold→mixed with koji→pour into the tank→build a nest→fermentation→rice wine.

Pretreatment and extraction of aroma components

The aroma components of three rice wine samples were obtained by HS-SPME. About 6 mL rice wine was introduced into a 20 mL HS vial. The fiber was coated with 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), which is usually used for the absorption of volatile aroma components. The sample was maintained at 60 °C for 20 min. During the sampling time, the sample was stirred at a constant speed of 200 rpm. Following HS extraction, SPME fibers were injected into the GC apparatus and then maintained in the GC inlet for 3 min.

GC-MS analysis

Chromatographic conditions: chromatographic column, Agilent WAX type strong polar chromatographic column (30 m × 0.25 mm × 0.25 um). The injector temperature was 240 °C, and the split ratio was 1:1. High-purity helium (99.999%) was used as the carrier gas at a flow rate of 1.5 mL/min. The GC oven temperature was then programmed as follows: initial temperature 35 °C for 2 min, 4 °C/min Raise to 220 °C for 2 min, 4 °C/min to 240 °C for 8min. And the injection volume is 0.5 μL. The mass spectrometer was fitted with an EI+ source operated at 70 eV with a source temperature of 230 °C, and mass spectra were recorded in the range of m/z 40-500 amu in full-scan acquisition mode.

Aroma components were identified based on their retention indices and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored in the MS library.

3 Results and discussion

3.1 GC-MS analysis of volatile aroma components of rice wine brewed from different koji

Koji and rice are combined in a ratio of 1:100 and fermented according to the method described in Section 2.3 "Rice wine processing process". The rice wine fermented by three kinds of koji was detected and analyzed by GC-MS. The GC-MS total ion chromatograms of the aroma components are shown in Figures 1-3, and the peak area content of each aroma component is shown in Table 1 according to NIST11 search.

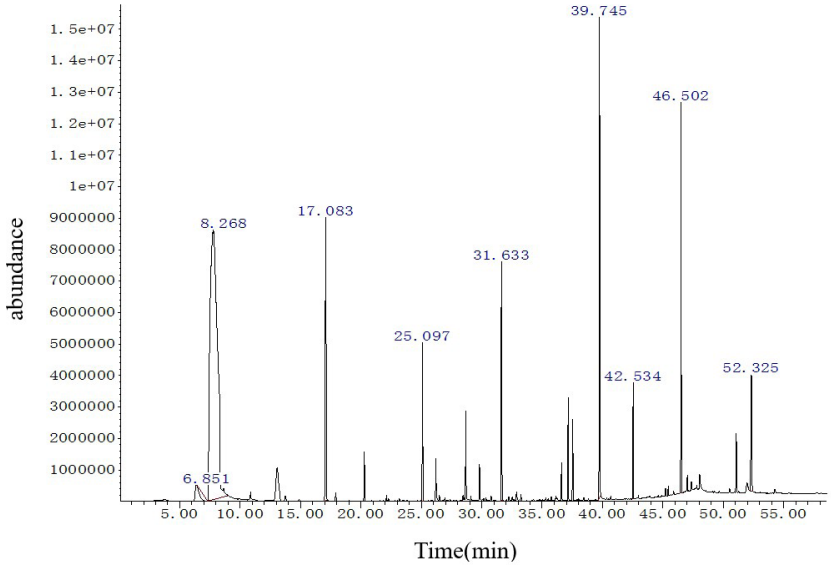

Figure 1. Total ion chromatogram of volatile components in wine cake fermented rice wine.

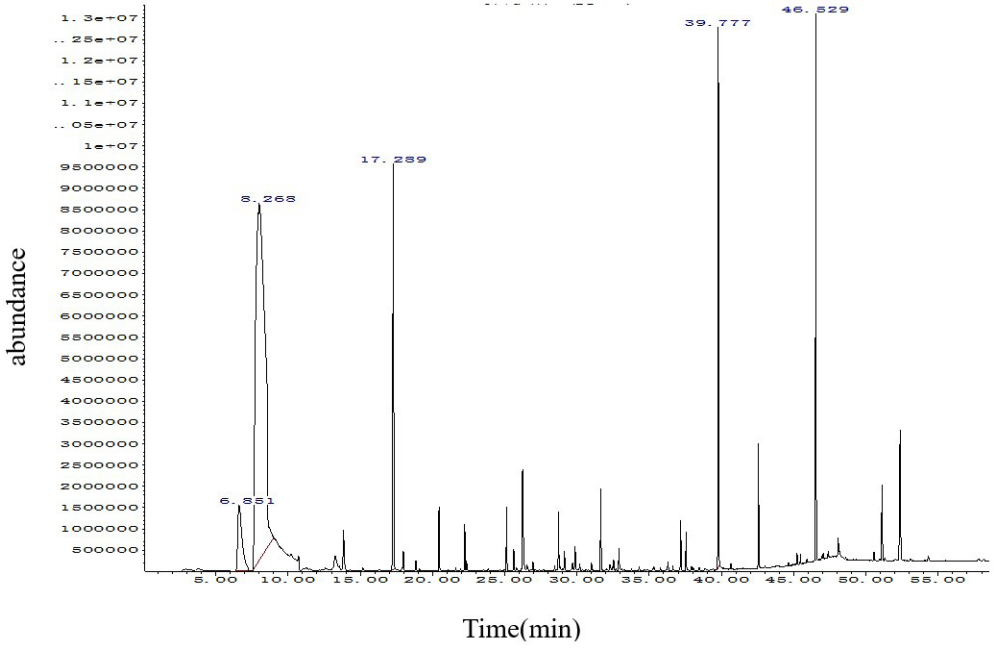

Figure 2. Total ion flow diagram of volatile components of fermented rice wine from fermented glutinous rice.

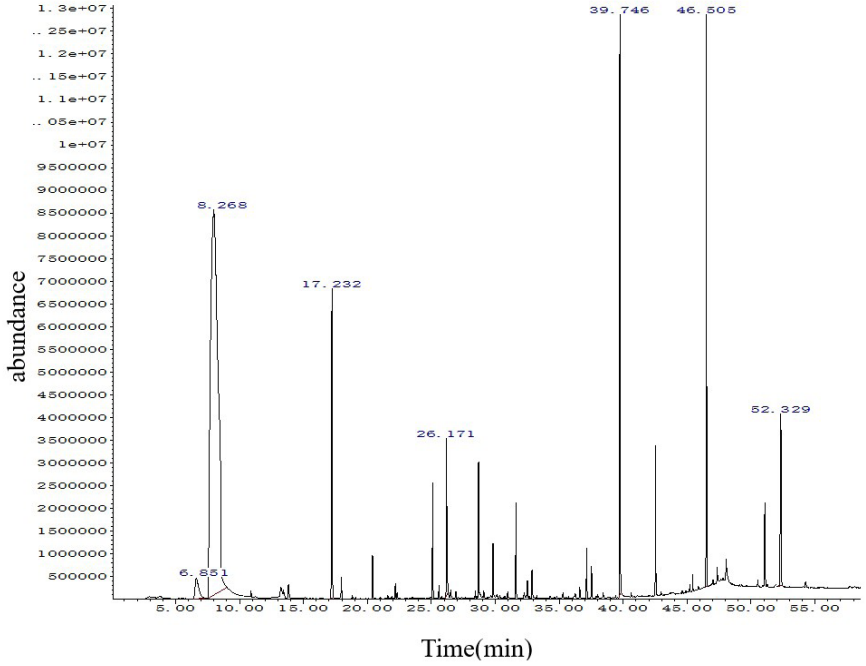

Figure 3. Total ion chromatogram of volatile components in bee fermented rice wine.

According to the experimental data, the aroma components detected by the three kinds of koji under the same fermentation conditions can be divided into esters, alcohols, acids and others. It can be seen from Table 1 that the content of esters and alcohols in the samples of the three koji is high. This shows that esters and alcohols are important components in rice wine. The ethanol content in the rice wine samples added with the koji type of wine cakes, fermented glutinous rice and bee is 52.5054%,58.0068%, and 59.0175%, respectively.

3.2 Analysis of volatile aroma components and relative content of rice wine brewed from different koji

The types and contents of the main volatile aroma components in rice wine brewed by different koji types also vary greatly. After adding wine cakes koji, 43 kinds of organic matter were detected after fermentation. Among them, there are 18 kinds of esters, including ethyl hexanoate, ethyl nonanoate, ethyl octanoate, diethyl succinate, ethyl decanoate, trimethylene acetate, ethyl laurate,

Table 1. Aroma components of rice wine fermented with different koji.

Serial number	Chemical compound	Relative content of Jiuqu(%)		
		Wine cake	fermented glutinous rice	Bee
1	Isoamyl acetate	0.1732	0.8219	0.2856
2	Ethyl hexanoate	0.1946	0.2721	0.2688
3	2-methoxy isopropyl cyanoacetate	0.7652	-	-
4	2-Hydroxypropionate ethyl ester	0.0807	-	-
5	Ethyl acetate	-	5.2512	-
6	Ethyl octanoate	2.4718	0.7102	-
7	Ethyl nonanoate	0.0843	0.0524	0.0756
8	Ethyl decanoate	4.1014	0.9093	1.0858
9	Diethyl succinate	0.2022	0.3015	0.3473
10	Trimethylene acetate	0.0269	-	-
11	phenylethyl acetate	1.4804	0.572	0.582
12	Ethyl laurate	1.2412	0.4429	0.3869
13	Ethyl myristate	1.4484	1.2175	1.5346
14	Ethyl N-ethylcarbamate	0.0156	-	-
15	Ethyl palmitate	5.0291	5.7204	6.0327
16	9-Hexadecenoicacid	0.2452	-	-
17	Methyl 17-methyl stearate	0.0854	-	-
18	Ethyl Oleate	1.0138	1.0581	1.2195
19	Ethyl linoleate	2.9906	2.3812	3.4513
20	Ethyl heptanoate	-	0.0428	0.044
21	Ethyl lactate	-	0.5286	-
22	Hexyl formate	-	0.1268	-
23	Ethyl trans-4-decenoate	-	0.1888	0.271
24	Methyl α-methyl phenylacetate	-	0.0344	-
25	Ethyl phenylacetate	-	0.1218	-
26	Ethyl pentadecanoate	-	0.0409	-
27	Ethyl octadecanoate	-	0.1137	0.1002
28	Lsoamyl formate	-	-	4.9665
29	Ethyl L(-)-lactate	-	-	0.1734
30	Ethyl undecanoate	-	-	1.3019
31	Ethanol	52.5054	58.0068	59.0175
32	Isobutanol	2.338	0.7264	0.6185
33	Lsoamyl alcohol	7.0454	6.978	-
34	2,3-Butanediol	1.9168	0.6708	1.5985
35	1,2-Propanediol	0.052	-	-
36	Furfuryl alcohol	0.0565	-	-
37	Phenylethyl alcohol	8.1809	6.8121	7.5048
38	Glycerol	0.4533	-	-
39	1-Butanol	-	0.0665	-
40	1-Octen-3-ol	-	0.226	0.1429
41	1-Heptanol	-	0.0435	-
42	Diisobutylcarbinol	-	0.0513	-
43	1-Octanol	-	0.2111	-
44	1-Nonanol	-	0.1094	0.1134
45	3-Methylthiopropanol	-	0.0597	0.0592
46	n-Hexyl alcohol	-	-	0.0783
47	(2S,3S)-(+)-2,3-Butanediol	-	-	0.7145
48	Trans-2-Octen-1-ol	-	-	0.0624
49	Acetal	1.0409	-	-
50	3-ethoxy-1-propanol	0.0327	-	-
51	Acetic acid	0.8314	1.6284	2.8759
52	2-Furaldehyde	0.1158	0.1105	0.1395
53	n-Pentadecane	0.0506	0.122	0.0846
54	Benzaldehyde	0.0497	-	-

Note: "-" is not checked out.

Table 1. Continued...

Serial number	Chemical compound	Relative content of Jiuqu(%)		
		Wine cake	fermented glutinous rice	Bee
55	n-Hexadecane	0.0372	-	0.0543
56	l-Caryophyllene	0.0947	-	-
57	Naphthalene	0.0623	0.0501	0.0803
58	2-methylthiolane	0.5481	0.0521	0.1271
59	Hexanoic acid	0.0254	0.0481	0.03
60	3,3,5-trimethylcyclohexylamine	0.0342	-	-
61	2-Methylnaphthalene	0.0605	0.0537	0.0342
62	Octanoic acid	0.0367	-	0.0352
63	4-Ethylphenol	0.1262	0.0964	0.1625
64	2-Methoxy-4-vinylphenol	0.0598	0.0438	0.0426
65	Palmitic acid	0.5234	-	-
66	Hexanal	-	0.0498	-
67	2-Pentylfuran	-	0.0415	-
68	3-Octanone	-	0.1473	0.0518
69	Styrene	-	0.035	-
70	6-Methyl-5-hepten-2-one	-	0.028	-
71	1-Nonanal	-	0.0379	-
72	Nonadecane	-	0.0928	-
73	2,5-Dithiobiurea	-	0.0851	-
74	Dicyclohexano-18-crown-6 (mixture isomeres)	-	0.0387	-
75	Diisopentyl ether	-	-	0.104
76	Geranyl acetone	-	-	0.0517
77	1-Methylnaphthalene	-	-	0.0856
78	Diisoamylamine	-	-	0.0482
79	Azidotrimethylsilane	-	-	0.0913
80	Trimethylethoxysilane	-	-	0.0741
81	Diglycerol	-	-	0.755

Note: "-" is not checked out.

2-methoxy isopropyl cyanoacetate, ethyl myristate, phenylethyl acetate, ethyl palmitate, ethyl N-ethylcarbamate, isoamyl acetate, 2-Hydroxypropionate ethyl ester, 9-Hexadecenoicacid, ethyl Oleate, methyl 17-methyl stearate, ethyl linoleate. The relative content is 21.65%. There are 8 kinds of alcohols, including ethanol, isoamyl alcohol, isobutanol, 2,3-butanediol, 1,2-Propanediol, phenylethyl alcohol, glycerol, and furfuryl alcohol, with a relative content of 72.55%. Other organic substances include n-pentadecane, n-hexadecane, acetic acid, hexanoic acid, octanoic acid, palmitic acid, caryophyllene, 3,3,5-trimethylcycloethylamine, 2-methylthiolane, naphthalene, 2-methylnaphthalene, acetal, 3-ethoxy-1-propanol, 4-ethylphenol, 2-methoxy-4-vinylphenol, benzaldehyde, 2-furaldehyde. The relative content of acid is 1.4169%.

After adding fermented glutinous rice koji, 51 substances were detected after fermentation. Among them, there are 21 kinds of esters, including ethyl acetate, ethyl heptanoate, isoamyl acetate, ethyl lactate, ethyl laurate, ethyl hexanoate, ethyl octanoate, hexyl formate, ethyl decanoate, ethyl trans-4-decenoate, ethyl nonanoate, diethyl succinate, methyl α-methyl, phenylethyl acetate, ethyl phenylacetate, ethyl myristate, ethyl palmitate, ethyl linoleate, ethyl pentadecenoate, ethyl oleate, and the relative content is 20.9085%. There are 12 kinds of alcohols, including ethanol, 1-butanol, isobutanol, 1-octen-3-ol, isoamyl alcohol, 1-heptanol, diisobutylcarbinol, 2,3-butanediol, 1- nonanol, 1-octanol, phenylethyl alcohol, 3-methylthiopropanol, the relative content is 73.9616%; other organic substances include n-hexadecane, nonadecane, acetic acid, hexanoic acid, naphthalene, 2-methylnaphthalene, 2-pentylfuran, styrene, methyltetrahydrothiophene, 2-methoxy-4-vinylphenol, 4-ethylphenol, 6-methyl-5-hepten-2-one, 3-octanone, 2,5-dithiobiurea, 2-furaldehyde, 1-nonanal, hexanal, dicyclohexano-18-crown-6 (mixture isomeres), the relative content is 2.7612%. Among them, the relative content of acid is 1.6765%.

After adding bee wine koji, 46 kinds of substances were detected in rice wine after fermentation. There are 17 kinds of esters, including isoamyl acetate, ethyl hexanoate, ethyl undecanoate, ethyl decanoate, ethyl trans-4-decenoate, ethyl heptanoate, diethyl succinate, ethyl laurate, phenylethyl acetate, ethyl myristate, Ethyl L(-)-lactate, ethyl oleate, ethyl palmitate, isopentyl formate, ethyl linoleate, ethyl nonanoate, ethyl linoleate, the total relative content is 22.1271% ;10 alcohols, including ethanol, n-hexyl alcohol, 2,3-butanediol, 1-octen-3-ol, (2S,3S)-(+)-2,3-butanediol, isobutyl alcohol, trans-2-octen-1-ol, 3-methylthiopropanol, 1-nonanol, phenylethyl alcohol, the total relative content is 69.91%. Others include n-pentadecane, n-hexadecane, acetic acid, hexanoic acid,

octanoic acid, 2-furaldehyde, naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, diisopentyl ether, diglycerol, diisoamylamine, methyltetrahydrothiophene, 3-octanone, geranylacetone, 4-ethylphenol, 2-methoxy-4-vinylphenol, azidotrimethylsilane, trimethylethoxysilane, the total relative content is 4.9279%. The relative content of acid is 2.9411%.

3.3 Analysis of volatile aroma components in rice wine brewed from different koji

Esters

Ester aroma compounds are mainly generated by the esterification of alcohols and organic acids during fermentation and distillation (Lee et al., 2015). Esters are one of rice wine's most abundant and important aromatic compounds (Chen et al., 2013, 2018). Three different wine samples detected a total of 30 esters, of which ethyl palmitate had the highest relative content. The relative content of ethyl palmitate bee wine samples was 6.0327%. The relative content in the fermented glutinous rice sample was 5.7204%, and the wine cakes were the lowest, with a relative content of 5.0291%. Ethyl lactate and ethyl acetate are the main aroma components in rice wine. Ethyl lactate is mainly consumed by lactic acid bacteria to consume a large number of carbohydrates through the fermentation of the hexose phosphate pathway to produce lactic acid; lactic acid in yeast esterase catalyzed in yeast cells acyl-CoA combined with ethanol produced by yeast and formed can increase the fruity and creamy aroma of the wine. In the middle and late stages of rice wine fermentation, acetic acid and high concentration ethanol esterification reaction under the action of yeast endoesterase to generate ethyl acetate, which can increase the fruity aroma of the wine, give the wine a pleasant aroma, and help the formation of rice wine flavor (Zhou, 2016). Most esters have floral and fruity aromas, mainly produced by yeast metabolism and esterification reactions (Cao et al., 2020). For example, ethyl octanoate exhibits typical fruity and brandy aromas, and ethyl decanoate has a strong coconut aroma, furthermore, ethyl palmitate has a creamy aroma and a faint waxy aroma (Lu et al., 2022).

Alcohol compounds

Alcohols, also known as fusel alcohols, are metabolites synthesized by yeast during alcoholic fermentation (Dzialo et al., 2017). Microbial fermentation, conversion of amino acids and oxidation of linolenic acid degradation products are the main sources of volatile alcohols in the fermentation broth (Sun et al., 2018). A total of 18 kinds of alcohols were detected in three different wine samples. Studies have shown that a small amount of higher alcohol can make fruit wine have a delicate smell and phenylethyl alcohol may produce a rose aroma (Englezos et al., 2018). Because the threshold of aromatic alcohols is generally shallow, the aroma value of aromatic alcohols is very high, which plays a vital role in the formation of overall aroma (Yang et al., 2012).

Acid compounds

Acid is another important factor affecting the flavor characteristics of rice wine, mainly from volatile saturated fatty acids represented by acetic acid (Siebert et al., 2018). Acetic acid is the acid substance with the highest relative content in all samples, not only an aroma substance but also an important sour substance (Niu et al., 2017; Yu et al., 2019).Four kinds of acids were detected in three different wine samples. Acids have a nonnegligible effect on the taste of rice wine, such as caprylic acid, which can produce a marshmallow aroma (Wei et al., 2018).

Other substances

Other substances detected in the three wine samples include olefins, alkanes, ethers, furans, thiophenes, aldehydes and ketones. Acetal is a unique component in wine cake wine samples, and its relative content is 1.0409%. According to the data, acetal usually has a pleasant aroma. Hexanal is a unique and high-content aroma component in mashed lees, with raw oil, grassy and apple aromas. Diisopentyl ether is a unique and relatively high-content aroma component in the mead sample, which has a pleasant fruity aroma. Nonanal is a unique ingredient in fermented glutinous rice, with grassy, citrus, and soapy flavors (Fan et al., 2015; Zhang et al., 2012). Each aroma component detected in rice wine has its unique smell, which has a certain impact on the quality of rice wine.

4 Conclusions

The aroma components of rice wine fermented by different koji were analyzed by HS-SPME combined with GC-MS combined technology, and a total of 81 aroma components were detected in the three koji-fermented wine samples. After analysis, it can be seen that the difference in the quality of the three wine samples is mainly reflected in the difference in aroma composition and its relative content. The relatively high aroma components detected in the three wine samples were ethyl decanoate, ethyl palmitate, ethyl Oleate, ethanol, phenylethyl alcohol and acetic acid. The analysis results showed that ethyl decanoate, ethanol, phenylethyl alcohol and acetic acid were significantly different in the three wine samples. Therefore, the content and types of esters and the ratio and interaction of esters, alcohols and acids in rice wine play an important role in the quality of rice wine, and the joint action of aroma components affects the quality of rice wine.

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