Analysis of Flavor Components in HS-GC-IMS and Antioxidant Properties of Black *Lycium barbarum* Rice Wine

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**Abstract**

Aim: Black *Lycium barbarum* rice wine is a kind of rice wine produced by the co-fermentation of black Chinese wolfberry and glutinous rice. In this study was to evaluate and compared the antioxidant activity and flavor substances of this type of rice wine with others. Methods: Through headspace gas chromatography-ion mobility spectroscopy (HS-GC-IMS), compared the flavor substances of black wolfberry rice wine, black rice wine, and common rice wine. Many components such as anthocyanins, total sugar, total acid, amino acid nitrogen, etc. in black Chinese wolfberry wine was observed. Results: It was found that the content of anthocyanins in black Chinese wolfberry rice wine increased continuously, approached the maximum value at 24 h, and decreased gradually after 24 h. The scavenging rates of \( \bullet \text{OH} \), DPPH, and ABTS in black wolfberry rice wine increased continuously after 24 h. The contents of total sugar, total acid, and amino acid nitrogen showed an increasing trend within 24 h and were close to the maximum. the flavor substances of black wolfberry rice wine and black rice wine were similar, but those of black wolfberry rice wine and common rice wine were different. Conclusion: From the perspective of more than 20 flavor substances, except for the fact that individual components such as butyl acetate, ethyl propionate, ethyl 3-methyl butyrate, and hexanol are much higher in black wolfberry rice wine than in black rice wine, the types and concentrations of other flavor substances are relatively similar, while some esters are absent or very low in common rice wine.

**Keywords:** *Lycium barbarum*, rice wine, HS-GC-IMS, flavor substances, hydroxyl radical, 1-diphenyl-2-trinitrophenylhydrazine, 2min-2-azo-bis-3-ethylbenzothiazoline-6-sulfonic acid

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1. Introduction

Black wolfberry is a kind of traditional Chinese medicine that can be used as both food and medicine. Compared with common wolfberry, black wolfberry has a higher nutritional value. It is rich in carotenoids, vitamins B1, B2, C, and E [1], and also Fe, Zn, Se, and other inorganic elements [2,3]. Moreover, black wolfberry is also rich in pigments. Black wolfberry pigment is a flavonoid whose main component is anthocyanin. It has the ability to scavenge superoxide anion (\( \text{O}_2^- \)), hydroxyl radical (\( \bullet \text{OH} \)), and 1,1-diphenyl-2-trinitrophenyl hydrazine (DPPH) radicals [4]. Black wolfberry pigment is easily soluble in water and ethanol, and its content is higher than that of fruit juice pigment. For example, the pigment content of blueberry, black currant, and mulberry has strong acid resistance, reduction resistance, and heat resistance [5]. Sucrose and preservative sodium benzoate have no effect on the stability of the pigment, and there is no significant effect of the reductant on the pigment [6]. Anthocyanin is extracted by microbial fermentation, which can be destroyed the cell wall, cell membrane, and other structures of the plant tissue. At the same time, it can be decomposed organic acids, polysaccharides, and other impurities in plants, and accelerate the dissolution of anthocyanin [7,8,9,10].

In recent years, people have started paying more and more attention to their health. Many experiments showed that oxidative stress could be induced a variety of chronic diseases (cancer, inflammation, cardiovascular disease, etc.) [11,12]. However, anthocyanins can be prevented oxidative stress [13]. Epidemiological studies have confirmed that regular intake of foods rich in antioxidants can be effectively reduced the risk of chronic diseases [14]. Rich anthocyanins in *Lycium barbarum* can be reduced the blood lipid level of mice to a certain extent, significantly inhibited the hemolysis of mice red blood cells, enhanced the antioxidant capacity of mice serum [15,16], effectively inhibited the damage of oxidized LDL on vascular endothelial cells, and played a protective role [17]. Headspace gas chromatography-ion mobility spectroscopy (HS-GC-IMS) has emerged as a new separation and
detection technology in recent years [18,19]. It has the advantages of high separation performance of GC and quick response and high sensitivity of IMS, especially suitable for trace detection of some volatile organic compounds. It has been widely used in food safety, disease detection, environmental protection, and other safety fields [20], especially in food flavor analysis [21,22,23,24].

The aim of this study was to understand the nutritional components, flavor compounds and antioxidant activity of black wolfberry rice wine, and to provide theoretical guidance for the further development and utilization of black wolfberry rice wine.

2. Materials and Methods

2.1. Materials and Reagents

Glutinous rice (purchased from Baiyi supermarket), Sweet Wine Koji (purchased from Putian, Fujian), common rice wine (purchased from Baiyi supermarket), black rice wine and black Lycium barbarum rice wine (brewed in Kongfujia distillery), phenanthrene, DPPH, ABTS.

2.2. Instruments and Equipment

Headspace gas chromatography-ion mobility spectroscopy (HS-GC-IMS) for performing flavorspec was used.

2.3. Methods

Sweet wine was prepared according to Yan Huawen’s method [25] and the national standard for yellow rice wine of China 6.2.1(GB/T 13662-2008) [26]. Samples were taken at 24, 48, 72, 96, and 120 h and refrigerated at <20°C for sealing and storage.

The component analysis of black wolfberry rice wine, we determined the anthocyanin content according to the method of Yan Yamei et al. [27] (pH Chromatic aberration method). The total sugar content was referred to the national standard for yellow rice wine of China 6.2.1 (GB/T 13662-2008) [26]. For determining the total acid content, used to the national standard for yellow rice wine of China 6.6(GB/T 13662-2008) [27]. For analyzing and comparing flavor components of black wolfberry rice wine with those of market rice wine, used the HS-GC-IMS. A sample of 1 ml was put in a 20 ml Headspace bottle, incubated at 45°C for 10 min, and then injected. The analysis time was 30 min. The column type was FS-SE-54-CB-1, and 15 m ID was 0.53 mm. The column temperature was 60°C and the carrier gas/drift gas was N2 at 45°C. The column temperature was 50°C and incubation speed was 500 rpm. Table 1 showed for gas chromatography conditions.

| Time   | E1  | E2  | R     |
|--------|-----|-----|-------|
| 00:00:000 | 150mL/min | 2ml/min | Rec   |
| 02:00:000 | 150mL/min | 2ml/min | -     |
| 20:00:000 | 150mL/min | 100ml/min | -    |
| 30:00:000 | 150mL/min | 150ml/min | stop  |

For determining the antioxidant capacity, firstly performed the DPPH radical scavenging according to Liu Chao’s method [28] for dissolving and diluting DPPH free radical with 95% ethanol to 0.1 mmol/l. We took 1 mL of rice wine for 24, 48, 72, 96, and 120 h, respectively. DPPH solution of the equal volume was added and mixed evenly. It was then kept away from light for 30 min at room temperature. We measured the absorbance A at 517 nm, took 95% ethanol as the blank control, and measured the absorbance A0. At the same time, BTH was used as a positive control, and the experiment was carried out under the same conditions.

Secondly, the ABTS - radical scavenging was used according to Liu Chao’s method [28], a mixed solution of 7 mmol/l ABTS and 2.45 mmol/l potassium persulfate was prepared with ultrapure water as the solvent. The mixture was kept away from light for 12-16 h at room temperature and then diluted with ultrapure water. The absorbance of 734 nm was 0.7 ± 0.005 as the working solution. Mixed 0.1 ml 24, 48, 72, 96, and 120 h rice wine with 3.9 ml of the above working solution evenly, then left it at room temperature for 6 min. Ultrapure water was taken as the blank control, and the absorbance at 734 nm was measured. At the same time, BTH was used as a positive control, and the experiment was carried out under the same conditions.

Thirdly, the OH radical scavenging was used according to Smirnoff et al. method [29] with some changes. Before the experiment, 4 mmol/l salicylic acid ethanol solution, 4 mmol/l ferrous sulfate solution, and 4 mmol/l hydrogen peroxide solution were prepared as a standby. 1 ml of black fruit Lycium anthocyanin solution of different concentrations was taken, then added 2 ml of salicylic acid ethanol solution, 2 ml of ferrous sulfate solution, and 2 ml of hydrogen peroxide solution, in turn, keeping it away from light, mixed it well and let it stand for 30 min. The absorbance at 510 nm was then measured. At the same time, we took BTH as the positive control and carried out the experiment under the same conditions.

2.4 Calculation

The anthocyanin concentration was calculated as follows:

\[ C(\text{mg}/\text{L}) = \frac{\Delta A \cdot M_W \cdot DF \cdot 1000}{\varepsilon \cdot I} \]  

where  \( \Delta A = (A_{510\text{nm}} - A_{700\text{nm}}) \) pH 0.8 - (A_{510\text{nm}} - A_{700\text{nm}}) pH 4.5,  \( A_{510} \) are the absorbance values measured before and after bleaching with sodium sulfite,  \( M_W = 449.38 \text{ g/mol} \) (cyanidin-3-O-glucoside),  \( DF = \text{dilution multiple} \),  \( \varepsilon = \text{molar extinction coefficient at 26900 1 mol}^{-1} \text{ cm}^{-1} \) (cyanidin-3-O-glucoside), and  \( I = \text{cuvette optical path of 1 cm} \).

Then, the total sugar concentration was calculated as the reference [28].

Then, the total organic acid concentration was calculated as

\[ X_I = \frac{(V_1 - V_3) \cdot C \times 0.090}{V} \times 1000 \]
where $X_1$ is the content of amino acid nitrogen in the sample (in g/l), $V_1$ is the volume of 0.1 mol/l sodium hydroxide standard titration solution consumed in the determination of the sample (in ml), $V_3$ is the volume of 0.1 mol/l sodium hydroxide standard titration solution consumed in the blank experiment (in ml), $C$ is the concentration of sodium hydroxide standard titration solution (in mol/l), 0.090 is the value of the molar mass of lactic acid (in g/mol), and $V$ is the volume of the absorption sample (in ml).

Then, the amino acid nitrogen concentration was calculated as

$$X_2 = \frac{(V_2 - V_4) \times C \times 0.014 \times 1000}{V}$$

where $X_2$ is the content of amino acid nitrogen in the sample (in g/l), $V_2$ is the volume of 0.1 mol/l sodium hydroxide standard titration solution consumed in the determination of the sample after adding formaldehyde (in ml), $V_4$ is the volume of 0.1 mol/l sodium hydroxide standard titration solution consumed in the blank experiment (in ml), $C$ is the volume of sodium hydroxide standard titration solution consumed in mL concentration (in mol/l), 0.014 is the value of the molar mass of nitrogen (in g/mol), and $V$ is the volume of the absorption sample (in ml).

Then, the DPPH free radical scavenging was calculated as

$$DPPH \text{ radical scavenging rate} = \frac{(A_0 - A_1)}{A_0} \times 100\%,$$

where $A_0$ is the absorbance of the blank group, and $A_1$ is the absorbance of the experimental group.

The ABTS free radical scavenging was calculated as follows:

$$ABTS \text{ radical scavenging rate} = \frac{(A_0 - A_1)}{A_0} \times 100\%,$$

where $A_0$ is the absorbance of the blank group, and $A_1$ is the absorbance of the experimental group.

Under the OH radical scavenging was calculated as follows:

$$OH \text{ radical scavenging rate} = \frac{(A_3 - A_1 + A_2)}{A_3} \times 100\%,$$

where $A_1$ is the absorbance measured by adding black wolfberry rice wine, $A_2$ is the absorbance measured by using pure water instead of salicylic acid, and $A_3$ is the absorbance measured by using pure water instead of black wolfberry rice wine.

### 3. Results and Composition Analysis

#### 3.1. Anthocyanins

Figure 1 showed that with the increase in fermentation time, the content of anthocyanin (mg/l) in black wolfberry rice wine decreased at first (72 h) and then increased, reaching 61.258 in 24 h and 39.91 in 72 h. The content of anthocyanin is the most at 24 h, which is mainly caused by the dissolution of anthocyanin in black wolfberry rice wine. As the fermentation progressed, some anthocyanins decomposed, resulting in a decrease of anthocyanin content. Due to longer immersion, more anthocyanins in wolfberry dissolved in rice wine, resulting in a slight increase of anthocyanin content detected later.

![Figure 1. Effect of fermentation time on anthocyanin content](image)

#### 3.2 Total Sugar Content

Figure 2 showed that the total sugar content as a whole had a decreasing trend first and then increasing. The total sugar content is maximum in 24 h, reaching 0.63 g/l, which gradually decreased with the progress of fermentation, the minimum in 96 h at 0.223 g/l, and then increased. This process is mainly due to the fact that during the fermentation of rice wine, a large number of maltose and other sugars are produced by the starch saccharification of rice. The total sugar content in rice wine rised to a certain height, and then these sugars are mellowed into ethanol, so the total sugar content decreased.

![Figure 2. Total sugar content at different fermentation times](image)

#### 3.3. Total Organic Acid Content

Figure 3 showed that the total organic acid content fluctuated around 0.5 g/l all the fermentation time. When the lowest point is 96 h, it is as low as 0.3891 g/l; when the highest point is 120 h, it is 0.5337 g/l. There are 2 main sources of acid. Firstly, some of the black wolfberry's own acids are dissolved in rice wine, whereas secondly, some acids are produced in the fermentation process. This fluctuating process is the dynamic balance process of the decomposition of original organic acids in wolfberry and the production of new acids by fermentation.

![Figure 3. Total organic acid content](image)
3.4. Amino acid nitrogen

Figure 4 showed an upward trend in the content of amino acid nitrogen in general. The lowest value is near 24 h, reaching 0.0263 g/l. The highest is 0.0385 g/l, near 96 h. Amino acid nitrogen has been on the rise, which is due to the continuous dissolution of amino acids in Lycium barbarum and the decomposition of proteins into amino acids in the fermentation process.

3.5. Flavor Profiles

To further understand the differences of flavor substances between black wolfberry rice wine and other rice wine, 3 different components of rice wine were compared and analyzed: (1) ordinary rice wine (as control), (2) black medlar rice wine, and (3) black rice wine. It can be seen from Figure 5 that flavor profile in different samples are different. The components with high volatile substances in the control group are low in black wolfberry rice wine and black rice wine, whereas the components in black wolfberry rice wine are low in ordinary rice wine.

Figure 6 showed that from the accurate qualitative analysis of more than 20 flavor substances, black wolfberry rice wine and black rice wine are relatively similar, except that individual components such as butyl acetate, ethyl propionate, ethyl 3-methyl butyrate, and hexanol are far higher in black wolfberry rice wine. The rest of the flavor substance types and concentrations are relatively close, while the difference between the flavor substance of ordinary wine and the 2 is greater in some. As shown in the box around the yellow line, the flavor substances are common in the 3 groups of samples, and the concentration difference is relatively small. However, the substances shown in the box around the green line are primarily found in ordinary brewing, which are absent or very low in the other 2 rice wines, mainly some aldehydes and ketones such as propionaldehyde, 2-butanone, benzaldehyde, and 3-hydroxy-2-butanone. However, the substances in the box around the red line are mainly found in black wolfberry rice wine and black rice wine, which are absent or in very low concentration in ordinary wine brewing, mainly some esters such as ethyl propionate, ethyl hexanoate, isoamyl acetate, ethyl butyrate, etc. For example, the concentration of ethyl acetate and isoamyl acetate is higher in black rice wine.
Figure 6. Gallery plot fingerprint of volatile substances in samples

Figure 7. Qualitative analysis of volatile organic compounds in samples

Table 2. Names of compounds corresponding to some characteristic peaks

| Serial number | Chemical Compound       | retention time/s (Rt [sec]) | Migration time/ms (Dt [a.u.]) | Peak volume [+1] | Peak volume [+2] | Peak volume [+3] |
|---------------|-------------------------|-----------------------------|-------------------------------|-----------------|-----------------|-----------------|
| 1             | acetic acid             | 971.685                     | 1.05172                      | 2454.385        | 8783.496        | 10518.822       |
| 2             | 3-Methyl-1-butanol-D    | 415.468                     | 1.49204                      | 13629.847       | 24970.855       | 23853.682       |
| 3             | 3-Methyl-1-butanol-M    | 416.011                     | 1.23969                      | 2013.32283      | 897.27057       | 633.8395        |
| 5             | Isoamyl acetate-M       | 332.468                     | 1.30613                      | 213.32283       | 897.27057       | 633.8395        |
| 6             | Isoamyl acetate-D       | 331.502                     | 1.26185                      | 78.98871        | 2431.1484       | 1258.3092       |
| 7             | butyl acetate           | 262.855                     | 1.61877                      | 25.462004       | 708.80035       | 229.11041       |
| 11            | 2-pentanone             | 226.404                     | 1.37656                      | 100.79741       | 141.84068       | 159.86913       |
| 12            | Ethyl acetate-D         | 220.719                     | 1.34195                      | 4606.839        | 5948.828        | 7399.2227       |
| 13            | Ethyl acetate-M         | 223.44                      | 1.09976                      | 1087.9595       | 737.54425       | 790.80756       |
To further understand the components of volatile substances in the sample, mark the characteristic peak, as shown in Figure 7, and analyze some compounds corresponding to the characteristic peak. The results are shown in Table 2. From the peak volume in Table 2, it can be seen that there are more esters in the black wolfberry rice wine, which gave it a unique flavor.

### 3.5. Oxidation Resistance

Figure 8 and Figure 9 showed that the scavenging rate of the DPPH free radical of black wolfberry rice wine during 24 h fermentation is about 23%, which is equivalent to the scavenging rate of 0.05 mg/ml BTH to DPPH free radical; the scavenging rate of rice wine after 48 h fermentation is about 46%, which is equivalent to the scavenging rate of 0.1 mg/mL BTH. In 24–48 h, the best drinking time, the scavenging rate of the DPPH free radical of black wolfberry rice wine significantly improved, which indicated that black wolfberry rice wine had a strong antioxidant capacity in this period. With the progress of fermentation, the antioxidant capacity increased.

![Figure 8. DPPH radical scavenging rate of black wolfberry rice wine at different fermentation times](image)

![Figure 9. Effect of BTH on the scavenging rate of DPPH radicals](image)

![Figure 10. ABTS radical scavenging rate of black wolfberry rice wine at different fermentation times](image)

![Figure 11. Effect of BTH on the scavenging rate of ABTS radicals](image)
Figure 10 and Figure 11 showed that the ABTS free radical scavenging rate of black wolfberry rice wine during 24 h fermentation is about 38%, which is equivalent to that of 0.15 mg/ml BTH; the ABTS free radical scavenging rate of rice wine after 48 h fermentation is about 46%, which is equivalent to that of 0.2 mg/ml BTH. From 24 to 48 h, the scavenging rate of the ABTS free radical of black wolfberry rice wine significantly increased, which indicated that the antioxidant activity of black wolfberry rice wine was strong and had been enhanced in this period. It is similar to the change in the DPPH radical scavenging rate.

![Graph](image)

**Figure 12.** OH radical scavenging rate of black wolfberry rice wine at different fermentation times

Figure 12 and Figure 13 show that the ABTS free radical scavenging rate of black wolfberry rice wine is about 28% at 24 h of fermentation, which is close to the scavenging rate of 0.2 mg/mL BTH to the OH free radical. The scavenging rate of rice wine at 48 h of fermentation is about 46%, which is equivalent to that of 0.15 mg/ml BTH; the ABTS free radical scavenging rate of black wolfberry rice wine is significantly higher than that of common rice wine, while some aldehyde and ketone substances, such as propionaldehyde, 2-butanone, benzaldehyde, and 3-hydroxy-2-butanone, were found in lower or not in black wolfberry rice wine. It has a different flavor from that of common rice wine, which provides theoretical support for further development and utilization. Thus, further research will be done on the nutritional value of black wolfberry rice wine, the change of substance, and the microbial group during storage.

### 4. Discussion

From the above results showed that anthocyanin, the antioxidant substance in black wolfberry rice wine, has a certain effect of scavenging free radicals, and rice wine fermented within 24-48 h had a better function of scavenging free radicals. Rice wine also contained sugar, amino acid nitrogen, organic acid, and a large number of esters. Therefore, black wolfberry rice wine is a kind of nutrition and health drink. We found that The scavenging ability of DPPH and ABTS increased gradually, and that of OH decreased with the time of fermentation. The flavor substances in rice wine, the contents of butyl acetate, ethyl propionate, ethyl 3-methyl butyrate, hexanol, ethyl propionate, ethyl hexanoate, isoamyl acetate, and ethyl butyrate in black wolfberry rice wine were higher than those in common rice wine, while some aldehyde and ketone substances, such as propionaldehyde, benzaldehyde, and 3-hydroxy-2-butanone, were found in lower or not in black wolfberry rice wine. It has a different flavor from that of common rice wine.

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