Quality Evaluation of Fermented Mulberry Wine

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Abstract [**Objectives**] This study to conducted to clarify various nutritional components and the composition and contents of aromatic substances in mulberry wine prepared by fermentation, and promote the quality improvement and product development of mulberry wine. [**Methods**] Mulberry wine was prepared with mulberry Shengguo 1 as the material using *Saccharomyces cerevisiae*, and its contents of total sugars, total phenols, total acids, resveratrol, protein, dry extract, ethanol content, Fe, methanol, sulfur dioxide, volatile acids, total flavonoids and proanthocyanidins were determined. Aromatic substances were extracted by n-hexane, and their components and relative contents were determined by gas chromatography-mass spectrometry (GC-MS). [**Results**] The physical and chemical indexes of the mulberry wine prepared by fermentation were in accordance with the national standards, and it was rich in resveratrol (10.5 ± 0.6 mg/L), protein (381 ± 11 mg/L), total flavonoids (406 ± 18 mg/L) and proanthocyanidins (855 ± 22 mg/L), which were 8.95, 19.49, 3.76 and 1.63 times higher than those of grape wine, respectively. The aromatic substances were mainly composed of ethyl formate (9.9%), ethyl acetate (7.6%), acetal (6.2%), propanol (5.7%), isobutanol (5.6%) and isoamyl acetate (5.3%). [**Conclusions**] The results of this study demonstrated that mulberry wine was rich in active substances, which suggested that mulberry wine has great potential in the fruit wine market.

Key words Mulberry wine; Resveratrol; Proanthocyanidin; Total flavonoid; Aromatic substance

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It is easy to grow mulberry, and its yield can reach 22 500 - 37 500 kg/hm^2, but its short picking period and high water content make it inconvenient to transport. Brewing mulberries into wine can not only avoid the economic loss caused by untimely treatment, but also effectively retain the nutritional active components such as anthocyanins, flavonoids and resveratrol in mulberries. Mulberries are cold in nature, and making wine with mulberries can neutralize the coldness of mulberries and increase their consumers. At present, it has been difficult for the fruit wine market dominated by grape wine to meet people's demand for fruit wine with high nutritional value and health care function. Therefore, the mulberry wine-making market with the homology of medicine and food has a broad prospect[1], and it will also play a leading role in poverty alleviation and rural revitalization, and has important research significance and practical value.

Mulberry wine is known as "the wine of longevity", and *Compendium of Materia Medica* records: Mashing mulberries to juice and drinking it can relieve alcohol poisoning; making them into wine and drinking the wine can promote uresis to alleviate water retention and achieve the effects of resisting aging and keeping beauty", affirming its health care function. Mulberry wine is rich in a variety of active substances, among which resveratrol, protein and antioxidant substances are much higher than grape wine, and it thus has the effects of resisting aging, reducing blood fat, lowering blood pressure and softening blood vessels[2]. Low-temperature treatment on mulberries can make the volatile acid content in the prepared wine lower and improve the taste of fruit wine[3]. Optimizing the fermentation process can improve the quality of mulberry wine[4]. Immobilization of traditional *Saccharomyces cerevisiae* cells[5] can reduce the loss of anthocyanins in the process of wine making. However, there are few studies on the types and contents of active ingredients in mulberry wine in China. In this study, Shengguo 1 was selected as the raw material to prepare mulberry wine by *S. cerevisiae* fermentation, and its total sugars, total phenols, total acids, ethanol, volatile acids, Fe, methanol, resveratrol, protein, dry extract, sulfur dioxide, total flavonoids and anthocyanins were determined. Aromatic substances in mulberry wine were extracted with n-hexane, and their components and relative contents were determined by gas chromatography-mass spectrometry (GC-MS). This study aimed to clarify various nutritional components and the composition and contents of aromatic substances in mulberry wine prepared by fermentation, and promote the quality improvement and product development of mulberry wine and the healthy development of fruit wine market.

Materials and Methods

Materials and reagents

Experimental materials Mulberries: the planting base of Hubei Shengguo Jiuzhuang Co., Ltd.; yeast: *S. cerevisiae*.

Chemical reagents Fehling reagent; hydrochloric acid solution; sodium hydroxide standard solution; glucose standard solution (2.5 g/L); gallic acid; foline-phenol reagent; sodium carbonate solution; resveratrol standard; Coomassie brilliant blue G250 dye; anhydrous ethanol; 85% (W/V) phosphoric acid; bovine serum protein; iodine standard titration solution; concentrated sulfuric

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acid; iron standard use liquid; 1,10-phenanthroline solution; hydrogen peroxide solution; potassium permanganate-phosphoric acid solution; oxalic acid-sulfuric acid solution; magenta-sulfurous acid; phenolphthalein indicator; starch indicator; potassium iodide; sodium borate; rutin solution; sodium nitrite solution; aluminum nitrate solution.

Instruments and equipment

Thermo Trace DSQ GC-MS: Thermo Company, USA; 722S visible light spectrophotometer: Shanghai Jinghua Science and Technology Instruments Co., Ltd.; F-7000 spectrophotometer: Hitachi, Japan; RE-52AA rotary evaporator: Shanghai Yarong Biochemical Instrument Factory; HH-6 constant temperature water bath pot: Changzhou Guohua Co., Ltd.; model CP413 electronic balance (precision 0.001, range 0 – 400 g): Orhaus Instruments (Changzhou) Co., Ltd.; BCD-190F/A refrigerator: Hisense electric; GL-21M high-speed centrifuge: Changsha Pingfan Instrument & Meter Co., Ltd.

Experimental methods

Technical process of mulberry wine brewing Mulberry wine was brewed through steps of raw material picking, squeezing and pulping, enzymolysis of fruit juice, ingredient adjustment, fermentation and clarification.

Key operation points:

① Raw material picking: After picking, the stalks, poor-quality mulberries and sundries in the raw material were removed, and all tools were washed with sulfur dioxide water for disinfection before fermentation, so as to prevent the taste from being affected by impurities during subsequent fermentation.

② Squeezing and pulping: Mulberries were squeezed with a juicer to get juice.

③ Enzymolysis: A certain amount of pectinase was added to the fruit juice for hydrolysis in a water bath at constant temperature.

④ Ingredient adjustment: pH and sugar content was regulated with citric acid and white sugar.

⑤ Fermentation: Yeast and glucose aqueous solution were added, and the fermentation was continued after heating in a water bath at 36 ℃ for 10 min.

⑥ Clarification: Filtration was performed to remove residue, and the filtrate was clarified to obtain mulberry wine.

Determination of active substances in mulberry wine prepared by fermentation

Determination of total sugars Total sugars in wine were determined referring to Yang Jufen's determination method[6]. In specific, 10 ml (V_1) of mulberry wine was added into a 100 ml volumetric flask, and then added with 10 ml (1 + 1) of hydrochloric acid solution and 30 ml of water in sequence, followed by shaking well. The volumetric flask was put in a water bath at 69 ℃, and taken out after about 15 min and cooled to 20 ℃. Next, the wine was adjusted to neutral with sodium hydroxide solution, and diluted to constant volume. Subsequently, 10 ml of treated sample hydrolysate was added into a 100 ml round conical flask, and added with 5 ml of solution A and solution B of Fehling solution respectively, and the result was recorded as F, corresponding to the grams of glucose. Finally, titration was performed with glucose standard solution with a concentration of 2.5 g/L, and the consumed volume was recorded as V, which was substituted into formula (1) to calculate the total sugar.

$$\text{Total sugar (g/L)} = \frac{F - 2.5 \times V}{\frac{V_1}{100} \times 10} \times 1\,000 \quad (1)$$

Determination of total acids The total acid was determined by potentiometric titration. First, 60 ml of sample was added into a 100 ml beaker, and heated in a water bath with shaking at 40 ℃ for 30 min. Next, it was taken out and cooled to room temperature, and the carbon dioxide in the sample was discharged. Next, 10 ml of sample was added at 20 ℃ into a 100 ml beaker, and added with 50 ml of water and titrated with 0.1 mol/L sodium hydroxide standard solution. At the beginning, the speed could be a little faster. After the pH value of the sample was 8.0, the titration rate was slowed down, and half a drop of standard solution was dropped every time, and then the solution hanging on the beaker wall was rinsed with distilled water until the pH value was 8.2. The amount of sodium hydroxide standard solution was recorded as V_1. In order to reduce the experimental error, the operation was repeated for multiple times to take an average value. The same volume of distilled water was used to replace the sample for blank test, and the amount of sodium hydroxide standard solution was recorded as V_0. The content of total acid in the sample was calculated according to formula (2).

$$\text{Total acids (calculated by acetic acid) (g/L)} = \frac{0.1 \times (V_1 - V_0) \times 75}{V_2} \quad (2)$$

In the formula, V_2 is the sampling amount, unit: ml; 75 is the molar mass of tartaric acid, unit: g/mol.

Determination of ethanol content An alcohol meter was used for determination[7]. The nonvolatile substances in mulberry wine were removed completely by distillation, and then its volume fraction was determined by the alcohol meter. Temperature correction was performed according to Appendix B of GB/T 15038-2006 *Alcohol Meter Concentration and Temperature Conversion Table at a Temperature of* 20℃, and the volume fraction of ethanol at a room temperature of 20 ℃ was obtained, serving as the required ethanol content.

Determination of total phenols The content of polyphenols in mulberry was determined by the Folin-phenol reagent method with gallic acid as the standard substance[8]. Gallic acid standard solutions with five gradient concentrations of 10 – 50 mg/L were prepared. Next, 1.0 ml of the prepared solutions were added into five test tubes, respectively, and 1.0 ml of Folin-phenol reagent was added. After shaking well and standing for about 3 min, 3.5 ml of 7.5% Na_2CO_3 solution was added into each test tube to obtain a mixed solution, which was shaken well and diluted to 25 ml. The solutions reacted in the dark for 1 h. Finally, the absorbance was measured at 765 nm with the test tube without the addition of any sample solutions as a blank control. A standard curve was plotted with the concentration of standard solution as abscissa and absorbance as ordinate. After the mulberry wine was diluted 100 times, 1.0 ml of the diluted wine sample was added in a test tube, which

was then added with 1.0 ml of Folin-phenol reagent and 3.5 ml of 7.5% sodium carbonate solution in sequence. After diluted to constant volume, the obtained solution reacted in the dark for 1 h. The absorbance was measured at 765 nm with the test tube without the addition of any sample solutions as a control group. The obtained absorbance was substituted into the regression equation to calculate the concentration of total phenols in 1 ml of sample, and then substituted into formula (3) to obtain the content of total phenols in mulberry wine.

$$\text{Total phenols (mg/L)} = \frac{C \times 1.0 \times N \times 1\,000}{V} \quad (3)$$

In the formula, C is the gallic acid concentration of the sample (mg/ml); 1.0 is the sampling quantity (ml); N is the dilution ratio; and V is the volume of mulberry wine (ml).

Determination of resveratrol content The content of resveratrol was determined referring to the method of Deng *et al.*[9] First, 11.8 mg of resveratrol standard substance was accurately weighed and added with a small amount of anhydrous ethanol to completely dissolve it by ultrasonic oscillation. Next, the solution was diluted with anhydrous ethanol to a constant volume of 50 ml, giving a standard solution, which should be use right after it was ready. Because resveratrol will be transformed from trans to cis[10] its activity will be greatly reduced under light conditions, so it is necessary to use a brown volumetric flask to avoid light reaction. Next, the absorbance was measured at 306 nm to make a standard curve. Subsequently, 5.0 ml of mulberry wine was diluted 100 times, and added into a brown volumetric flask and diluted to a constant volume of 50 ml with anhydrous ethanol. The absorbance was determined at 306 nm, and the content of resveratrol in the sample was calculated. The content of resveratrol in mulberry wine was calculated according to formula (4).

$$\text{Resveratrol content (g/L)} = c \times F \quad (4)$$

In the formula, c is the content of resveratrol (g/L) in the sample solution obtained from the standard curve; F is the dilution ratio of the sample.

Determination of protein content Among acidic analytical reagent solutions, the dye Coomassie Brilliant Blue G250 combined with protein is mainly anionic, and the maximum absorption peak (λ_{max}) is at 590 nm. The color shade of the formed complex is directly proportional to protein concentration. Therefore, protein can be quantitatively determined by measuring the blue ion state of the dye.

A test tube without the addition of protein standard solution was set as the blank control, and its absorbance was measured at 595 nm. A standard curve was plotted with the concentration of standard solution as abscissa and absorbance as ordinate. Next, 5 ml of mulberry wine sample was added into a test tube, which was added with 5 ml of Coomassie Brilliant Blue G250 protein reagent. After mixing thoroughly, the absorbance was measured, and substituted into the standard curve equation to calculate the protein content in the sample. The content of protein in mulberry wine was calculated according to formula (5).

$$\text{Protein content (mg/L)} = [\text{Protein content obtained from standard curve/Sampling volume for determination}] \quad (5)$$

Determination of dry extract content The density was measured by the density bottle method[7]. The corresponding values were found in Appendix C (GB/T 15038-2006) to obtain the total extract content, and then the total sugar content was subtracted to obtain the dry extract content. (a) A density bottle was cleaned and dried, and weighing was performed after installing a thermometer and a side hole cover. Drying and weighing were repeated until the weight was constant, and the obtained weight was recorded as m. (b) The thermometer was taken out, and distilled water that had been boiled and cooled to about 15 ℃ was added into the density bottle with constant weight, and the thermometer was inserted to make it bubble-free. The density bottle was added in a water bath at 20.0℃ and kept for 10 min after the temperature of the sample was stable. The liquid leaking from the side tube was absorbed with filter paper, and the liquid level of the side tube was made flush with the side tube, and then, the side hole was immediately covered. The density bottle moved away, and the water on the surface of the bottle was absorbed with filter paper, and the weight was immediately measured and recorded as m_1. The water in the density bottle was poured out, and the density bottle was moistened with the sample for 3 – 5 times. Next, the density bottle was filled with the sample, and the same operation in (b) was repeated, and the final weight was measured and recorded as m_2. The sample density was calculated according to formula (6) and formula (8), and the correction value of air buoyancy was calculated according to formula (7) to avoid errors.

$$\rho_{Sample}(g/L) = \frac{m_2 - m + A}{m_1 - m + A} \times \rho_0 \quad (6)$$

$$A = \rho_\alpha \times \frac{m_1 - m}{997.0} \quad (7)$$

$$\rho_{Dealcoholized\ sample}(g/L) = 1.001\,80\ (\rho_{Sample} - \rho) + 1\,000 \quad (8)$$

In the formulas, ρ_0 is the density of distilled water at 20 ℃ (998.20 g/L); A is the correction value of air buoyancy; ρ_α is the density of dry air at 20 ℃ and 1 013.25 hPa ($\approx$1.2 g/L); 997.0 is the difference between the density of distilled water and dry air at 20 ℃ (g/L); ρ is the ethanol density measured in "Determination of ethanol content", unit: g/L. 1.001 80 is the correction coefficient of density bottle volume at 20 ℃.

Finally, according to the sample density, Appendix A was checked to get the ethanol content. Appendix C (GB/T 15038-2006) was checked according to the density of dealcoholized samples to get the total extract content, unit: g/L.

Determination of sulfur dioxide content First, 25.00 ml of NaOH solution was added into a 250 ml iodine volumetric flask, and 25.00 ml of mulberry wine was pipetted and added. The flask was covered tightly after shaking well. When it was stable, a small amount of crushed ice, 1 ml starch indicator solution and 10 ml of sulfuric acid solution were added in sequence, and after shaking well, titration was performed quickly with 0.1 mol/L I_2 standard titration solution until the solution was light blue, and the titration ended when the color remained unchanged for 30 s. The amount of I_2 standard titration solution was recorded as V. A blank control test was carried out by replacing the sample with the same volume of distilled water. The operation was the same as above, and the amount of I_2 standard titration solution was V_0. The content of sul-

fur dioxide in mulberry wine was calculated according to formula (9).

$$SO_2 \text{ content (mg/L)} = \frac{0.1 \times (V - V_0) \times 32}{25} \times 1\,000 \quad (9)$$

In the formula, 32 is the molar mass of SO_2, unit: g/mol; 25 is the sampling volume, unit: ml.

Determination of Fe content The Fe content in mulberry wine was determined referring to the phenanthroline colorimetric method of Wei *et al.* [11]. Fe standard solutions were used to draw a standard curve. Wet digestion sample preparation: First, 1.00 ml of sample (V) was added into a 1 ml flask, and slowly evaporated to dryness on an electric stove. After cooling slightly, 1 ml of concentrated sulfuric acid and 1 ml of hydrogen peroxide were added for digestion, which was performed with heating in a fume hood until the digestion solution became transparent. After cooling slightly, 10 ml of water was added, and the solution was boiled on soft fire for 3 min. Next, the absorbance values of the Fe standard solutions were measured at 480 nm. The standard curve was drawn using the concentration of the standard solution as abscissa and absorbance as ordinate. The absorbance A of the sample and the absorbance A_0 of the reagent blank were substituted into the standard curve to calculate the Fe content in the sample. The Fe content in mulberry wine was calculated according to equation (10).

$$\text{Fe content (mg/L)} = \frac{A - A_0}{V} \times 100\% \quad (10)$$

In the formula, V is the sampling amount, unit: ml.

Determination of methanol content Methanol is oxidized to formaldehyde under the action of fuchsin sulfurous acid solution, forming a blue purple compound, and the methanol content is determined by measuring the absorbance of the compound. The absorbance was measured at 590 nm, and a standard curve was drawn with the concentration of standard solution as abscissa and absorbance as ordinate. The content of methanol in mulberry wine was calculated according to formula (11).

$$\text{Methanol content (mg/L)} = \frac{m_1}{V_1} \times 1\,000 \quad (11)$$

In the formula, m_1 is the mass of methanol determined in the sample, unit: mg; V_1 is the sampling volume, unit: ml.

Determination of volatile acid content (calculated by acetic acid) First, 10 ml of sample was distilled to collect 100 ml distillate, which was heated to boiling, and 2 drops of phenolphthalein indicator was added. The sample was titrated to pink with 0.1 mol/L sodium hydroxide standard titration solution, and the titration ended when the color remained unchanged after 30 s. The volume of sodium hydroxide standard titration solution consumed was recorded as V_1. The measured volatile acid content in the sample was calculated according to formula (12), where 60.0 is the molar mass of acetic acid, unit: g/mol.

$$\text{Volatile acid content (calculated by acetic acid) (g/L)} = \frac{0.1 \times V_1 \times 60.0}{V} \quad (12)$$

Determination of total flavonoids The content of total flavonoids was determined referring to the method of Liang *et al.* [12]. A standard curve was drawn with rutin standard solutions. First, 1 ml of sample solution was diluted 10 times in a test tube, which was added with 5% NaNO solution. After standing for 6 min, 1% $Al(NO_3)_3$ solution was added. After standing for 6 min, 1 mol/L NaOH solution was added, and the obtained solution was diluted with 60% ethanol solution to constant volume. After standing for 15 min, with the test tube without sample solution as a blank control, the absorbance was measured at 510 nm. The measured absorbance was substituted into the regression equation of the standard curve to get content of total flavonoids in the sample solution. The content of flavonoids in the sample was calculated according to formula (13).

$$\text{Total flavonoids (g/L)} = c \times N \quad (13)$$

In the formula, C is the content of flavonoids in the sample solution obtained from the standard curve, unit: g/L; and N is the dilution ratio of the sample.

Determination of proanthocyanidin content The content of anthocyanins in mulberry wine was determined by the pH differential method[13]. Mulberry wine was diluted 25 times, and 10 ml of diluted sample solution was taken and diluted to 100 ml with potassium chloride-hydrochloric acid buffer (0.2 mol/L) with pH 1.0 and sodium acetate-hydrochloric acid buffer (0.2 mol/L) with pH 4.5, respectively. After equilibrium, the A values were determined at 520 and 700 nm, respectively, with distilled water without test sample as a blank control, and the content of anthocyanins was calculated according to formula (14).

$$\text{Proanthocyanidin content} = \frac{A \times 449.2 \times DF \times V}{\varepsilon \times L \times m \times 10^3} \times 1\,000 \quad (14)$$

In the formula, A is the total absorbance of proanthocyanidins in the sample solution, $A = (A520 - A700)\ \text{pH}\ 1.0 - (A520 - A700)\ \text{pH}\ 4.5$; 449.2 is the mass fraction of cyanidin-3-O-glucoside, unit: g/mol; DF is the dilution ratio; V is the sampling amount, unit: ml; L is the optical path, unit: cm; m is the weight of sample, unit: g; ε is the molar extinction coefficient of cyanidin-3-O-glucoside, 26 900 L/(mol · cm).

Determination of aromatic substances Extraction of aromatic substances: Aromatic substances in mulberry wine were extracted with n-hexane using a rotary evaporator and determined by gas chromatography-mass spectrometry (GC-MS). In specific, 500 ml of mulberry wine was extracted with n-hexane, and the extraction operation was repeated for three times. The organic phases were combined, and distilled under reduced pressure at 35 ℃ to obtain crude aromatic substances. Steam distillation was performed to obtain the crude product of aromatic substances, and the distillate was condensed to obtain an oil-water mixture, which was centrifuged at 10 000 rpm for 20 min, and the oil layer was collected and dried with anhydrous sodium sulfate to obtain the aromatic substances.

Component analysis: Thermo Trace DSQ GC-MS was adopted. The column temperature was initially set at 60 ℃ for 3 min, and then it was increased at the rate of 5 ℃/min to 200 ℃, which was increased at a rate of 10 ℃/min to 230 ℃, which was kept for 1 min. Helium was used as the carrier gas, and the flow rate was 1 ml/min. MS conditions: ionization voltage 70 eV (EI); scanning mode: full scanning; mass range: 20 – 550 amu; solvent delay 3 min. According to the peak areas in GC, the relative percentage contents of aromatic substances were calculated, and the

components were identified by comparing mass spectra and retention time in MS library.

Results and Analysis

Components of active substances in mulberry wine

The composition and contents of active substances in mulberry wine are shown in Table 1.

Table 1 Composition and content of aromatic substances in mulberry wine (the units are g/L except for alcohol by volume)

Active substance//g/L	Content
Total sugar	53.4 ± 0.08
Total acids	2.91 ± 0.10
Ethanol content//% vol	14.00 ± 0.10
Total phenols	20.70 ± 4.25
Resveratrol	0.010 5 ± 0.000 6
Protein content	0.381 ± 0.011
Dry extract	17.1 ± 1.22
Sulfur dioxide	0.032 ± 0.001 7
Fe	0.003 2 ± 0.000 1
Volatile acids (calculated by acetic acid)	0.3 ± 0.04
Total flavonoids	0.406 ± 0.018
Proanthocyanidins	0.855 ± 0.022
Methanol	0.07 ± 0.011

Total sugars According to the determination of total sugars, the total sugars were (53.4 ± 0.08) g/L, and the polysaccharide content was ≥45.1 g/L. The total sugar content met the wine standard[14], which indicated that the mulberry wine made by fermentation was sweet.

Total acids The content of total acids was (2.91 ± 0.10) g/L according to the "Determination of total acids".

Ethanol content The ethanol content measured according to "Determination of ethanol content" was (14 ± 0.1)% vol.

Total phenols The content of total phenols in mulberry wine was (20.7 ± 4.25) g/L, which was 18.3 times higher than the total phenol content (0.201 ± 1.133) g/L in grape wine measured by Li[16] and 7.75 times higher than that (1.72 – 2.67 g/L) in the research by Shi *et al.*[17]. Because polyphenols can regulate the redox state of cells and reduce the occurrence of body damage and diseases[15], the content of total phenols in wine reflects its good nutritional and health care value indirectly.

Resveratrol content The content of resveratrol measured according to "Determination of resveratrol content" was (10.5 ± 0.6) mg/L, which was 8.95 times higher than the content of resveratrol (0.121 – 1.172 8 mg/L) in grape wine measured by Ying *et al.*[18] and 4.77 times higher than that (1.0 – 2.2 mg/L) in mulberry wine measured by Lu *et al.*[19]. Modern pharmaceutical experiments have proved that resveratrol has cardiovascular protection, anti-cancer and anti-aging effects. This study shows that mulberry wine made by fermentation method has good medicinal value.

Protein content According to "Determination of protein content", the content of protein was (381 ± 11) mg/L, which was 19.49 times higher than the protein content of 0.49 – 19.55 mg/L in grape wine measured by Liu *et al.*[20].

Content of dry extract According to "Determination of dry extract content", the dry extract content was (17.1 ± 1.22) mg/L, which met the requirement of dry extract content ≥16.0 mg/L in wine standard[14].

Sulfur dioxide content According to "Determination of sulfur dioxide content", the sulfur dioxide content was (32 ± 1.7) mg/L, which met the requirement of sulfur dioxide content ≤250 mg/L in the hygienic standard of fermented wine[21].

Fe content The Fe content measured according to "Determination of Fe content" was (3.2 ± 0.1) mg/L, which was 1.4 times higher than the Fe content of 1.06 – 2.29 mg/L in grape wine measured by Li *et al.*[16] and 1.07 times higher than that of 0.5 – 3.0 mg/L in mulberry wine measured by Lu *et al.*[19], and met the requirement of Fe content ≤8.0 mg/L in wine standard[14].

Methanol content The methanol content measured according to "Determination of methanol content" was (70 ± 11) mg/L, which met the requirement of methanol content ≤ 250 mg/L in wine standard[14].

Volatile acid content (calculated by acetic acid) The volatile acid content measured according to "Determination of volatile acid content" was (0.3 ± 0.04) g/L, which was 1.06 times lower than the volatile acid content of 0.318 – 0.766 g/L measured in grape wine by Li *et al.*[16], 4 times lower than the volatile acid content of 1.2 – 1.6 g/L in grape wine measured by Pei *et al.*[22], and 1.03 times lower than that of 0.31 – 1.1 g/L in mulberry wine measured by Shi *et al.*[17].

Total flavonoid content According to "Determination of total flavonoids", the content of total flavonoids was (406 ± 18) mg/L, which was 3.76 times higher than that of 97.90 – 108.12 mg/L in black wolfberry and mulberry compound wine measured by Ji[23].

Proanthocyanidin content The content of proanthocyanidins measured according to "Determination of proanthocyanidin content" was (855 ± 22) mg/L, which was 34.60 times higher than the proanthocyanidin content of 23.04 – 24.71 mg/L in mulberry dry red wine measured by Guo *et al.*[26], 11.83 times higher than the proanthocyanidin content of 16.2 – 72.3 mg/L in mulberry wine measured by Lu *et al.*[19], and 1.63 times higher than that of 240.85 – 524.87 mg/L in mulberry wine measured by Shi *et al.*[17]. Because proanthocyanidins have obvious free radical scavenging effect[24-25], the mulberry wine in this study also has strong free radical-scavenging potential.

Components of aromatic substances in mulberry wine prepared by fermentation

The components and relative contents of aromatic substances in mulberry wine are shown in Table 2.

The aromatic substances in mulberry wine were a light yellow transparent liquid, which emitted a unique aroma. The specific gravity of the extract was 0.033 mg/ml. According to GC-MS analysis, 28 kinds of aroma components were detected, accounting for 94.6% of the total aroma components, including 15 kinds of esters, 7 kinds of alcohols, 3 kinds of acids and 3 others, and esters, alcohols and acids accounted for 52.8%, 24.1% and 7.6% of the total aroma components, respectively. Ethyl formate (9.9%) and ethyl acetate (7.6%) were the main components,

followed by acetal (6.2%), propanol (5.7%), isobutanol (5.6%) and isoamyl acetate (5.3%). These six substances were the main aroma components of mulberry wine, and 5.4% of the aroma components were not identified as specific substances, and the relative contents of phenylethanol, hexaethylene glycol and butyl benzoate were all less than 1%. Wu *et al.* [27] applied GC-MS to determine the aroma components in mulberry beverage after ultra-high pressure treatment, and found that the esters were mainly ethyl acetate (1 403.905 μg/L) and isoamyl acetate (253.063 μg/L), the main alcohol was isoamyl alcohol (853.964 μg/L), and the main acid was acetic acid (550.342 μg/L). However, the high content of aroma components detected in the wine samples in this study was in good agreement with Wu Meng's research results, and the contents of ethyl acetate, isoamyl acetate, isoamyl alcohol and acetic acid were, respectively, 2 533.33, 1 766.66, 1 600 and 866.66 μg/L, which were higher than previous data. Wei *et al.* [28] also analyzed the aroma components in mulberry wine, and the results showed that the 12 components with the highest contents were different from the results of this study. Among them, the relative content of phenylethanol was only 0.9%, which might be caused by the different extraction conditions, mulberry varieties used and fermentation process conditions.

Table 2 Components and contents of aromatic substances in mulberry wine

No.	Name	Retention time	Relative content//%
1	Ethyl formate	5.79	3.5
2	Ethyl formate	6.55	9.9
3	Acetal	6.65	1.3
4	Propanol	9.45	2.5
5	Isobutanol	10.91	2.6
6	Isoamyl acetate	11.81	7.6
7	Isoamyl alcohol	12.98	6.2
8	Ethyl hexanoate	14.51	3.8
9	Hydroxypropanone	16.48	5.7
10	Butyl succinate	16.56	3.9
11	Ethyl lactate	17.43	2.5
12	Hydroxyethyl acrylate	19.13	5.3
13	Etheyl octanoat	19.65	0.6
14	Acetic acid	20.43	2.5
15	Formic acid	21.79	3.9
16	2,3-Butanediol	22.53	2.9
17	Ethyl decanoate	24.89	2.4
18	Isobutyl benzoate	25.29	0.9
19	Butanoic acid	25.67	1.4
20	Ethyl butyrate	25.99	0.8
21	2,5-Dimethyl-3-hexanol	27.54	1.7
22	Benzyl alcohol	27.48	4.8
23	Ethyl palmitate	27.89	5.6
24	Ethyl laurate	29.57	2.4
25	Butyrolactone	30.74	2.6
26	Phenylethyl alcohol	31.65	3.6
27	Hexaethylene glycol	32.97	2.1
28	Butyl benzoate	34.405	1.6
29	Not detected		5.4

Conclusions and Discussion

In this study, the physical and chemical indexes of mulberry wine prepared by fermentation reached the national standard of wine and the hygienic standard of fermented wine, and the active substances and aromatic composition of mulberry wine were preliminarily explored. The results showed that the contents of resveratrol, protein, total flavonoids, anthocyanins and Fe were, respectively, (10.5 ± 0.6), (381 ± 11), (406 ± 18), (855 ± 22) and (3.2 ± 0.1) mg/L, which were 8.95, 19.49, 3.76, and 1.63 times higher than grape wine, respectively. Its aromatic substances were mainly composed of ethyl formate (9.9%), ethyl acetate (7.6%), acetal (6.2%), propanol (5.7%), isobutanol (5.6%), and isoamyl acetate (5.3%). It indicates that mulberry wine contains potential nutritional value and enormous economic benefits. Since there is only grape wine standard in the fruit wine market at present, follow-up research will focus on the formulation of the industry standard of mulberry wine and the establishment of a unified standard of high-quality mulberry wine production, so as to promote the healthy and sustainable development of mulberry wine industry.

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their experimental operation ability, but also promote the cultivation of teamwork and communication skills.

Meanwhile, it is very important to create a positive academic discussion atmosphere and a good interactive atmosphere in the experimental classroom. Teachers can encourage students to ask questions, share their experimental experience or organize the demonstration of experimental results to promote the communication between students and the sharing of learning results. Meanwhile, establishing a regular feedback mechanism to listen to students' opinions and suggestions on teaching content and experimental process is helpful to adjusting teaching strategies in time and improving teaching effect and learning experience.

Conclusions

According to the actual needs of food quality and safety courses and the relevance of course knowledge and the development of modern food science and technology, experimental schemes can be adjusted in time, so that students can better integrate their knowledge and cultivate their innovative consciousness. We look forward to further improving the quality of physical chemistry experiment teaching of food quality and safety major through continuous teaching reform and practical exploration. It is necessary to strengthen the forefront and practicality of experimental contents continuously and closely combine with the development trend of the industry and scientific and technological innovation, so as to ensure the close connection between teaching content and practical application. Meanwhile, we should continue to optimize teaching methods and means, and introduce more interactive teaching and personalized learning techniques to meet the learning needs and academic interests of different students.

In a word, through the efforts of teaching reform, we believe that the physical chemistry experiment teaching of food quality and safety major will usher in a broader development space and make positive contributions to cultivating more high-quality and professional talents.

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