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ORIGINAL ARTICLE

Exploring factors related to the browning of cherry (*Prunus pseudocerasus lindl*) wine during fermentation and aging

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Abstract
In order to explore factors related to the browning of cherry wine, the causes of browning in cherry wine fermentation and aging were investigated, and the main components of cherry wine browning in the aging process were analyzed using a model solution. The results showed that the browning degree of the heat-inactivated cherry wine and the control group increased from 0.500 to 0.891, 0.510 to 1.538 during fermentation, respectively ($P<0.05$). Both enzymatic browning and nonenzymatic browning were present during fermentation, and enzymatic browning dominated. During the aging process, the contents of amino acids, total phenols, and reducing sugars decreased by 31.4%, 75.6%, and 39.8%, respectively, while the browning degree increased by 22.6% ($P<0.05$). Model solution experiments revealed that chlorogenic acid and glucose are the primary factors influencing the browning of cherry wine under acidic conditions. These findings were useful for improving the quality of cherry wine and effectively alleviating the browning of cherry wine.

Keywords Browning · Cherry wine · Model solution

Introduction

The Chinese cherry (*Prunus pseudocerasus Lindl*) originates from China, its fruit is delicious and rich in nutrients (Yang et al. 2022). It is widely grown in many provinces of China, such as Shandong, Liaoning, and Hebei. However, Chinese cherries are highly perishable after harvesting, making them ideal candidates for processing into cherry wine. This approach not only enhances the derivative value of the cherries but also introduces a unique alcoholic product to the market. However, color changes (browning) can cause sensory quality defects and ultimately lead to deterioration of overall quality and marketability.

Color is often considered the basic attribute of wine, and the color is also helpful to reflect the quality of the wine. During the fermentation and aging processes, the browning of wine can affect its color alteration and lead to a decline in quality. The browning of wine is a complex oxidation process involving a variety of substances, such as oxygen, enzymes, sugars, amino acids, and phenolic substances (Yang et al. 2020). Color alteration (browning) in wine may be enzymatic or nonenzymatic (Celotti et al. 2022). Enzymatic reactions usually occur in the early period of fermentation. Polyphenol oxidase (PPO) plays a very important role in catalyzing the reaction to produce orthoquinone compounds with a high polymerization rate (Vlahou et al. 2022). After fermentation, nonenzymatic reactions may replace the enzymatic reaction due to PPO inactivation in wine (Paravisini and Peterson 2019). It is generally believed that the nonenzymatic browning of wine may be caused by the chemical oxidation of phenols and the polymerization of the subsequent oxidation products (Zhao et al. 2023). Some researchers have found that the sugars in wine could participate in a series of subsequent reactions with amino acids, which will also result in browning (Pereira et al. 2017a, b). Medina-Plaza et al. (2023) reported that after three months of aging at room temperature, the content of polymerized phenolic substances in rosé wine decreased significantly, and the color of rosé wine tends to change from rose-like to orange. Pérez-Magariño et al. (2023) verified that oxygen consumption will lead to a decrease in polyphenol content

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in wines and varying degrees of browning. Since browning is the most common color defect in wine and causes significant economic losses, identifying the factors that influence wine browning is of considerable industrial importance. However, various studies have emphasized the concerning browning of wine, while further research on the browning of cherry wine is still rare.

The purpose of this work was to elucidate the key factors of the browning reaction that occurs in cherry wine during fermentation and aging. The PPO activity and browning degree of wine from different pretreatment cherry mash during fermentation were measured. After cherry wine aging, the effects of browning on monosaccharide composition, free amino acids, and individual polyphenolics were studied. It provides data support for the study of the browning mechanism of cherry wine and offers a theoretical foundation for the industrial production of cherry wine and the enhancement of cherry's added value.

Materials and methods

Chemicals and standards solutions

Saccharomyces cerevisiae (DV10) was purchased from Shanghai Jatou Industrial & Commercial Co. Ltd. Free amino acid standards samples (glutamic acid, aspartic acid, L-arginine, leucine, valine, alanine, serine, lysine isoleucine, phenylalanine, tyrosine, glycine, proline, histidine, L-threonine, cysteine, DL-methionine), phenolic acid standards samples (gallic acid, chlorogenic acid, caffeic acid, p-hydroxybenzoic acid, vanillic acid, cinnamic acid, ferulic acid) and monosaccharide standards (L-arabinose, xylose, galactose, glucose) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. All other reagents used in this work were of analytical grade and purchased from Tianjin Kaitong Chemical Reagent Co., LTD., China.

Chinese cherry wine samples

Chinese cherries (15.6 ºBrix, titratable acidity 3.64 g/L, pH 3.75) were purchased from a local farmers' wholesale market in Taian, China, and transported to the laboratory. Chinese cherry pulp was obtained after washing and crushing.

The process of pretreatment of the cherry pulp was as follows: Chinese cherry pulp was heated to 90°C for 20 min until the PPO activity reached 0 U/mL.

The fermentation parameters of Chinese cherry wine were described below: Chinese cherry pulp was treated by addition of SO_2 (80 mg/L), added saccharose to reach a total reducing sugar concentration of 230 g/L. Then, Chinese cherry musts were inoculated by adding *Saccharomyces cerevisiae* DV10 (0.25 g/L) at 22 ± 1°C. Until the total sugar content was less than 5 g/L, the Chinese cherry wine samples were obtained by pressing and stored at 18 ± 1°C.

The aging process of Chinese cherry wine was as follows: After the completion of fermentation, 250 mL cherry wine was filled into a clear glass wine bottle of 300 mL and sealed with corks, and the sample is divided into six sections with three groups of samples per section (pH 4.2). The cherry wines were stored at 18 ± 1°C, and cherry wines were measured every 10 d in the 60-d period.

Determination of browning

Chinese cherry wine samples were treated by centrifuge (4000 r/min, 15 min). The clear supernatant was placed into a cuvette and measured in a UV-2400PC spectrophotometer (Shimadzu Corporation, Suzhou, China) at 420 nm against distilled water as a blank (Tomic et al. 2017). The absorbance value is used to express the degree of browning.

Determination and extract of PPO activity

The methods reported by Micheloni et al. (2018) were used to determine PPO from Chinese cherry wine with some modifications. The reaction mixture consisted of 1 mL filtered sample and 0.6 mL of phosphate buffer (0.2 mol/L, pH 7.8, containing 2% PVPP). After adding 1.4 mL of catechol solution (0.05 mol/L) to the reaction mixture, the absorbance was recorded every 0.5 min over a period of 10 min at 410 nm using a UV-2400PC spectrophotometer (Shimadzu Corporation, Suzhou, China), with distilled water as a blank control. The PPO active enzyme unit is defined as a 0.001 per minute increase in the absorbance value of catechol at 410 nm.

Determination of amino acids content

Total amino acids content: According to the method of Gan et al. (2017), 5 mL of each Cherry wine sample (V) and 50 mL of water were added to two Erlenmeyer flasks. After adding three drops of neutral red indicator to the first one, titrated with 0.1 mol/L NaOH to produce amber color, and recorded the consumption volume of NaOH as V1. Add three drops of thymolphthalein indicator and 10 mL of neutral formaldehyde to the second. After being placed for 1 min, it was titrated with 0.1 mol/L NaOH to light blue, and the volume of NaOH consumed was denoted as V2.

$$\text{Total amino acids content } (\%) = 0.14 \times (\text{V2} - \text{V1})/\text{V} \quad (1)$$

Free amino acids: The method of Spáčil et al. (2008) was used to analyze the free amino acids. Cherry wine samples

(2 mL) were filtered through a 0.22 μm membrane filter. Free amino acids were detected using a fully automated amino acid analyzer (L-8900, Hitachi, Tokyo, Japan).

Determination of phenols compounds content

Total phenol content: According to the Folin-Ciocalteu method (Bernal et al. 1996). Gallic acid standard solution (40 mg/L) was diluted to 1, 2, 3, 4, 5, and 6 mg/L. Then, 1 mL of Folin-Ciocalteu's reagent was added, the volume of the solution was adjusted to 10 mL with 5% (w/v) sodium carbonate solution, and leave it for 10 min. The absorbance was measured at 765 nm using spectrophotometer. The linear regression equation as follows: $y=10.924x+0.0254$, $R^2=0.9974$. The absorbance of a 1 mL sample was determined according to the standard curve method. Gallic acid equivalents (GAE) represent the total phenolic content in cherry wine in mg/L.

Analysis of individual phenolic compounds: Determination was performed as described by Yoo with some modifications (Yoo et al. 2010). Cherry wine samples were treated with 0.22 μm membrane filter and analyzed using an Acquity Ultra Performance Liquid Chromatography (UPLC) system with a binary solvent manager and a sample manager that was coupled to a tunable ultraviolet (UV) detector (Waters, Prague, Czech Republic). The C18 column (2.1 × 100 mm, 1.7 μm; Waters Corporation, Milford, USA) was maintained at 50°C. An injection volume of 4 μL in a 10 μL loop was used. The mobile phase generated from water with 0.1% formic acid and methanol was mixed directly in the instrument at a flow rate of 0.45 mL/min. The UV detection wavelength was set at 280 nm, and the data acquisition rate was 40 Hz. The chromatographic data were treated using Empower 2 software.

Determination of reducing sugar content

The amount of reducing sugar: The reducing sugar content was determined by direct titration (GB/T 15038 − 2006).

Monosaccharide composition: The method used was reported by Shen et al. (2011), with a slight modification. Before use, Sep-Pak (C18, NH2, CN, Florisil and silica) was activated using 10 mL methanol and nanopure water. Sixty microliters of melezitose (10 g/L) were added to 3 mL of cherry wine sample and filtered through 0.45 μm filters, followed by Sep-Pak cartridges. Twenty microliters of the eluate were injected into high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). The separation was performed on a CarboPac PA100 (2 × 250 mm) equipped with a guard column (2 mm × 50 mm) and a borate trap precolumn (50 × 4 mm). The mobile phases were prepared from nanopure water (solvent A) and 0.2 mol/L NaOH (solvent B). The elution was initiated with 100% solution A at time 0 with a linear gradient of 0–100% B over 45 min and then held at 100% B for 10 min. The elution flow rate was maintained at 1 mL/

Table 1 Cherry wine simulation system

Simulation system	
[1]Glu+GLU+AA+CA	[9] Glu+AA+CA
[2] Glu+GLU+AA	[10] Glu+AA
[3] Glu+GLU+CA	[11] Glu+CA
[4] Glu+GLU	[12] Glu
[5] GLU+AA+CA	[13] AA+CA
[6] GLU+AA	[14] AA
[7] GLU+CA	[15] CA
[8] GLU	

The composition of 12% ethanol/0.1 M citrate (pH 4.2) was glucose (Glu), 0.30 g/100 mL; glutamic acid (GLU), 195 mg/100 mL; ascorbic acid (AA), 0.39 mg/100 mL; and chlorogenic acid (CA), 0.6 mg/100 mL

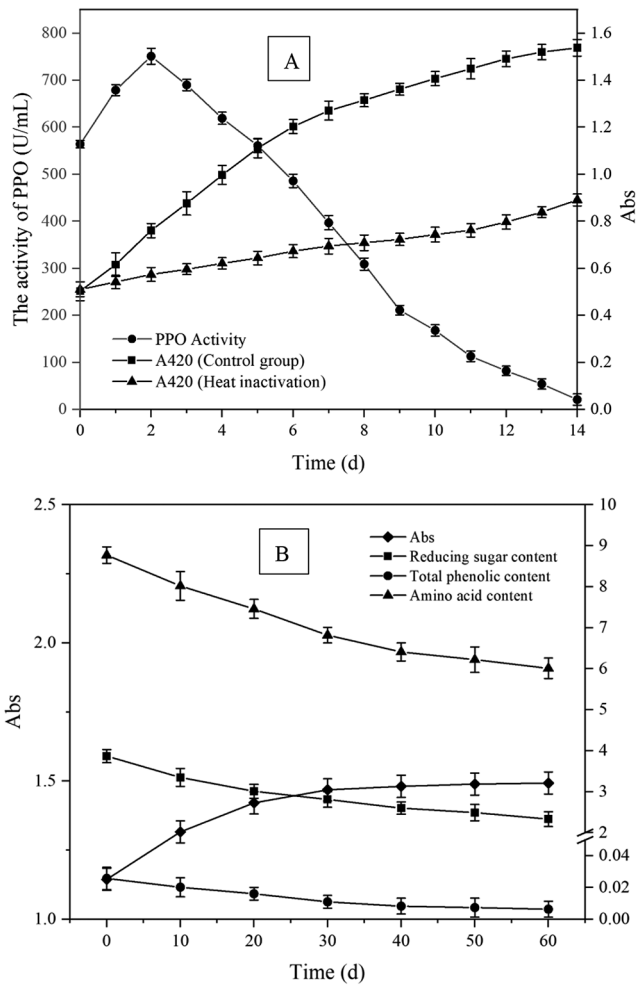

Fig. 1 **A**: PPO activity and absorbance at 420 nm during fermentation; **B**: Variations in browning degree, total phenol content, reducing sugar content, and total amino acid content during the aging process of cherry wine. Significance level at $P<0.05$

Table 2 Changes in the free amino acid, phenolic acid, and monosaccharide content of cherry wine during aging for 0 and 60 d

Free amino acid species	**Content (g/L)**		
	0 d	**60 d**	**Reduction (%)**
Glutamic acid	1.95 ± 0.04	0.94 ± 0.03	-51.8
Aspartic acid	0.41 ± 0.01	0.24 ± 0.01	-41.5
L-Arginine	0.35 ± 0.01	0.30 ± 0.01	-14.3
Leucine	0.60 ± 0.02	0.48 ± 0.01	-20
Valine	0.57 ± 0.01	0.51 ± 0.02	-10.5
Alanine	0.53 ± 0.02	0.40 ± 0.01	-24.5
Serine	0.52 ± 0.02	0.51 ± 0.02	-1.9
Lysine	0.42 ± 0.01	0.18 ± 0.03	-57.1
Isoleucine	0.41 ± 0.02	0.22 ± 0.03	-46.3
Phenylalanine	0.40 ± 0.03	0.34 ± 0.02	-15
Tyrosine	0.38 ± 0.02	0.20 ± 0.02	-47.4
Glycine	0.36 ± 0.01	0.19 ± 0.01	-47.2
Proline	0.35 ± 0.01	0.30 ± 0.02	-14.3
Histidine	0.30 ± 0.01	0.18 ± 0.01	-40.0
L-Threonine	0.27 ± 0.02	0.24 ± 0.03	-11.1
Cysteine	0.16 ± 0.01	0.13 ± 0.01	-18.8
DL-Methionine	0.13 ± 0.01	0.07 ± 0.01	-46.2
Total Amino Acids	8.11 ± 0.03	5.43 ± 0.04	-33.0
Monosaccharide composition	**Content (g/L)**		
	0 d	**60 d**	**Reduction %**
L-Arabinose	0.19 ± 0.01	0.17 ± 0.01	-10.52
Xylose	0.22 ± 0.01	0.15 ± 0.02	-31.8
Galactose	0.07 ± 0.01	0.06 ± 0.01	-14.3
Glucose	2.96 ± 0.02	1.68 ± 0.01	-43.2
Phenolic acid species	**Content (mg/L)**		
	0 d	**60 d**	**Reduction (%)**
Gallic acid	1.498 ± 0.011	0.306 ± 0.013	-79.57
Chlorogenic acid	6.019 ± 0.021	0.117 ± 0.018	-98.06
p-Hydroxybenzoic acid	4.076 ± 0.032	1.754 ± 0.025	-56.97
Caffeic acid	2.442 ± 0.018	0.662 ± 0.014	-72.89
Vanillic acid	1.494 ± 0.026	0.013 ± 0.029	-99.12
Cinnamic acid	0.083 ± 0.011	0.0336 ± 0.010	-59.52
Ferulic acid	2.313 ± 0.012	0.515 ± 0.021	-77.73

Results were presented in the form of means ± standard deviation (SD)

min. The carbohydrates were dissolved in nanopure water, and the concentration of stock solutions was 10 g/L. Standard solutions were obtained through sequential dilution with deionized water. Melezitose was used as an internal standard for the quantitative analyses of cherry wine, and the final concentration was 200 mg/L in both the standard and the samples. Then, the mixture was extracted by the solid-phase extraction described above.

Preparation and analysis of model solution

The model solution (12% ethanol and 0.1 mol/L citrate solution, pH 4.2) was prepared according to the main components and contents that affect the browning of cherry wine after fermentation (Pereira et al. 2017a, b). Chlorogenic acid, glucose, glutamic acid, and ascorbic acid were added to the buffer solution at 6.02 mg/L, 2.96 g/L, 1.95 g/L, and 3.90 mg/L, respectively. The browning model system was established as shown in Table 1. The model solution was stored at a constant temperature of 50°Cfor 50 d(to speed up the test), and the absorbance value at 420 nm was determined.

The samples containing chlorogenic acid with or without glucose were incubated at a constant temperature of 50°C. The relationship between chlorogenic acid and glucose was analyzed by measuring the content of chlorogenic acid in samples. After 12 d, the model solutions were filtered using a microfiltration membrane with a 0.45 μm pore size (Xuntong, Shanghai, China). The content of chlorogenic acid in the sample was detected by a high-performance liquid chromatography (HPLC) with Kromasil C18 (250 mm×4.6 mm column; Posai Inc, Beijing, China) and Agilent 1100 diode array detector (Agilent, Shanhai, China). The mobile phase

Table 3 Browning rate of the model solution after 50 d of incubation at 50°C

Model solution	Browning rate[a] (A420 nm/d)	Determination coefficient (r^2)
Glu+GLU+AA+CA	0.00432[ef]	0.95
Glu+GLU+AA	0.00381[a]	0.97
Glu+GLU+CA	0.00323[bc]	0.96
Glu+GLU	0.00296[c]	0.98
GLU+AA+CA	0.00182[d]	0.98
GLU+AA	0.00184d[e]	0.98
GLU+CA	–	–
GLU	–	–
Glu+AA+CA	0.00257[f]	0.93
Glu+AA	0.00184[ab]	0.97
Glu+CA	0.00305[d]	0.96
Glu	–	–
AA+CA	0.00179[c]	0.98
AA	0.00180[a]	0.97
CA	–	–

Three replicates on average. In Duncan's multirange test, the values of different letters in this column were significantly different ($P<0.05$). -, could not be detected

was acetonitrile/ethanoic acid (35/65, v/v), the flow rate was 0.9 mL/min, and the temperature was 28°C.

Analysis of the effects of individual phenolic compounds and monosaccharide

The sample model solutions containing glucose and chlorogenic acid, glucose and phlorizin, chlorogenic acid, glucose, and xylose were incubated at 50°C for different periods. The absorbance value at 420 nm was used to evaluate the browning degree.

Statistical analysis

All these experiments were statistically analyzed three times and the results were expressed as the average standard deviation. The statistical analysis of the data was carried out by variance analysis (ANOVA). At the same time, the Duncan's multirange test was employed to analyze the statistically significant differences between the samples at the significance level of $P<0.05$. The statistical analyses were carried out using SPSS 23 and the figures were created using Origin8.0.

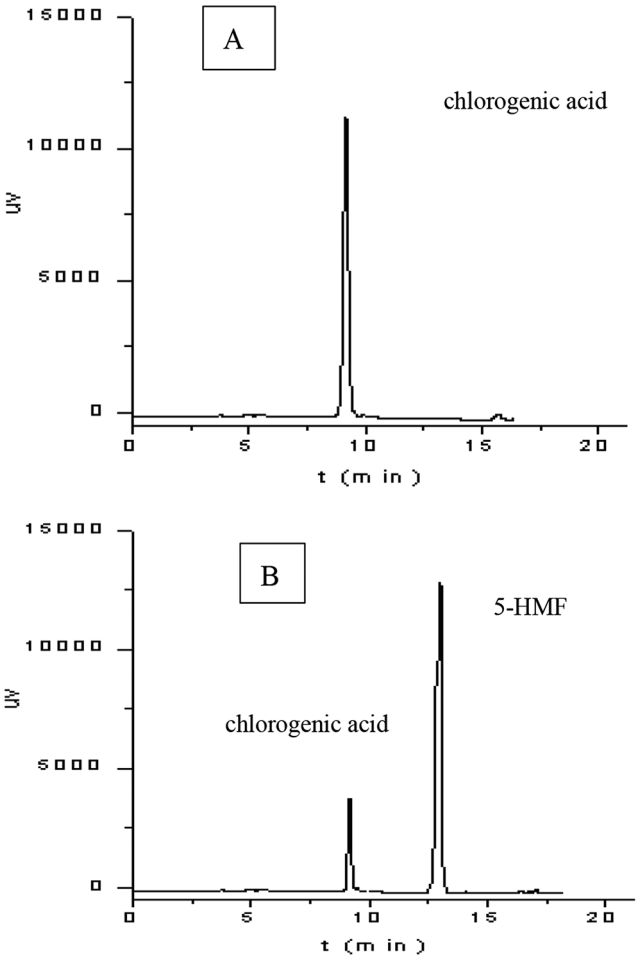

Fig. 2 HPLC patterns of model solutions containing chlorogenic acid (**A**) and chlorogenic acid and glucose (**B**) after incubation at 50°C for 12 days. Significance level at $P<0.05$

Results and discussion

PPO activity and browning degree of cherry wines in the fermentation process

Figure 1(A) shows the changes in PPO enzymatic activity and browning degree during cherry wine fermentation. The enzymatic activity of PPO fermented mash without heat inactivation reached its maximum value (751 U/mL) on the second day of fermentation and then gradually decreased until the end of fermentation when the activity was reduced to almost 0 U/mL. The browning degree of cherry wine with and without heat inactivation all increased during fermentation. After fermentation, the absorbance value at 420 nm was significantly different ($P<0.05$) between the control group and the heat inactivation group. The absorbance of the heat inactivation group at 420 nm increased from 0.510 to 0.896 after 15 d. However, the increase in the absorbance at 420 nm in the control group was more significant, and the

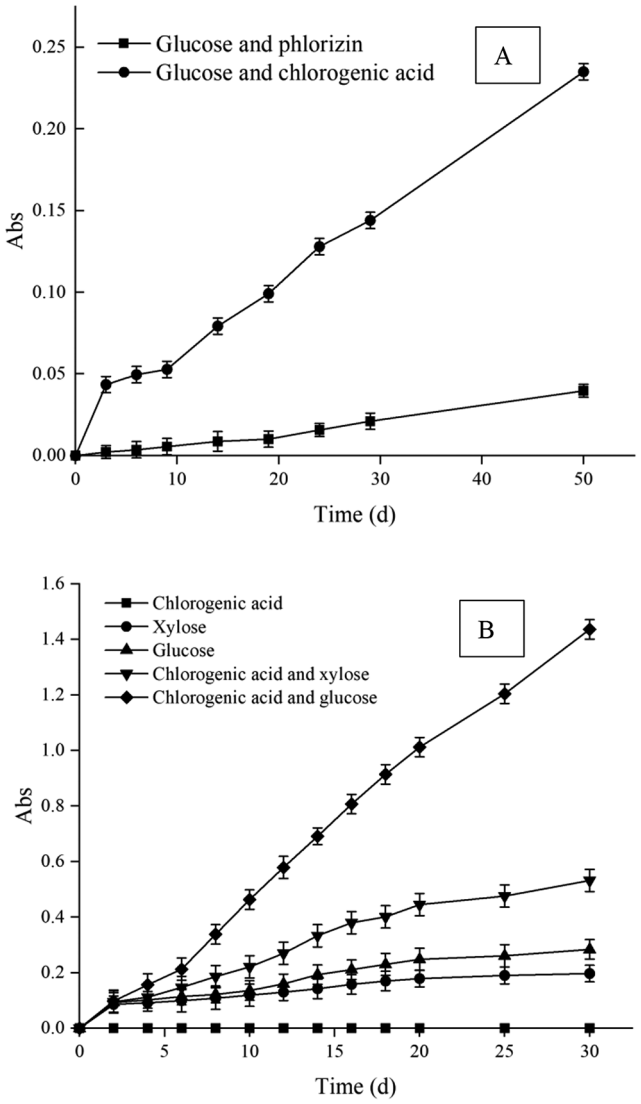

Fig. 3 **A**: Browning in model solutions containing glucose, chlorogenic acid, and phlorizin during incubation at 50°C for up to 50 days; **B**: Browning in model solutions containing chlorogenic acid, glucose, and xylose during incubation at 50°C for up to 30 days. Significance level at $P<0.05$

absorbance at 420 nm varied from 0.510 to 1.543 after 15 d, indicating that enzymatic browning existed in the fermentation process of cherry wine. The above research results showed that enzymatic browning and nonenzymatic browning existed simultaneously during the whole fermentation process.

Browning of cherry wine during aging

Browning degree and the composition of cherry wines during aging

The changes in total amino acid, total phenol, and reducing sugar contents during storage of cherry wine samples are shown in Fig. 1(B). During storage, the contents of amino acids, total phenols, and reducing sugars decreased with the increase in absorbance. The rate of increase in browning degree at the beginning of aging was significantly higher than the rate at the end of aging. After aging, the absorbance at 420 nm in cherry wine samples increased 22.6% ($P<0.05$). The original content of total phenols in cherry wine was 25.73 mg/L, and after 60 d, the content of total phenols decreased to 6.27 mg/L ($P<0.05$). The decrease in total phenolic contents may be due to the oxidative polymerization of phenolic substances during aging, which in turn caused the larger particles to settle, manifesting in the darkening of the cherry wine color (Moreira et al. 2018). During aging, the total amino acid content in cherry wine decreased by 31.4% ($P<0.05$). The decrease in amino acid content might be due to decarboxylation or deamination reactions during aging (Perestrelo et al. 2020). After storage, the reducing sugar content of the cherry wine samples decreased from 3.87 g/L to 2.33 g/L ($P<0.05$). Therefore, reducing sugars, amino acids and total phenols may be the key factors affecting the browning of cherry wine. However, the specific components need further experimental analysis.

Composition of cherry wine after 60 d

Amino acids are not only nutrients in wine but also precursors of aroma compounds that are directly beneficial to the taste, aroma, and appearance of wine. Studies have shown that amino acids participate in nonenzymatic browning and are closely related to the browning of wine (Yang et al. 2019). Amino acids underwent a series of complex reactions with reducing sugars (carbonyl compounds) in wine to form brown or black macromolecular substances (Kwak and Lim 2004). Table 2 summarizes the content of free amino acids in samples from the beginning stage of aging and at the age of 60 d. At the beginning of aging, glutamic acid (1.95 g/L) showed the highest amino acid content in the wine samples, followed by leucine (0.60 g/L). The content of glutamic acid (0.94 g/L) was still the highest at the end of the experiment. The contents of lysine, glutamic acid, tyrosine, glycine, isoleucine, methionine, aspartic acid and histidine significantly decreased by 57.1%, 51.8%, 47.4%, 47.2%, 46.3%, 46.2%, 41.5% and 40.0%, respectively ($P<0.05$). In the free amino acids detected, the most significant declines were glutamic acid and lysine. The rate of glutamic acid decline was lower

than that of lysine (Table 2), while the decline in glutamic acid content was significantly higher than that of lysine. At the end of aging, the contents of glutamic acid and lysine decreased to 1.01 g/L and 0.24 g/L ($P<0.05$), respectively. Therefore, glutamic acid was selected for preparing the model solutions.

Phenolic compounds contributed directly or indirectly to the color, astringency, and bitterness of wines (Sun et al. 2011). They are indispensable factors of fruit wines, not only in enzymatic reactions but also in nonenzymatic browning (Xiao et al. 2015). The phenolic acid content in cherry wine was presented in Table 2. At the beginning of aging, the highest content of phenolic acids was chlorogenic acid, followed by p-hydroxybenzoic acid. At the end of aging, the content of p-hydroxybenzoic acid was the largest, followed by caffeic acid. The decrease in the content of chlorogenic acid in the cherry wine was very significant, the content decreased from 6.019 mg/L to 0.117 mg/L after 60 d of aging and decreased by 98.06% ($P<0.05$). The content of p-hydroxybenzoic acid decreased from 4.076 mg/L to 1.754 mg/L and decreased by 56.97% ($P<0.05$), the rate of decline was the smallest. In the Table 2, the contents of gallic acid, ferulic acid, caffeic acid and cinnamic acid in the cherry wine samples decreased by 79.57%, 77.73%, 72.89%, and 59.52% ($P<0.05$), respectively. Although the rate of decline in vanillic acid was the highest (Table 2), its content in cherry wine was much lower than that of chlorogenic acid. During the process of nonenzymatic oxidation of wine, o-diphenols were oxidized to o-quinones and produced semiquinone free radicals. The unstable quinones may further react to form colored compounds (Arvisenet et al. 2019). The major reason for browning might be due to the regenerative capacities of phenols coupled in oxidation reactions with other compounds and their nonoxidative browning reactions (Sirén et al. 2015).

Sugar concentrations influence the release of aroma compounds and sensory output (Liu et al. 2018). The contents of main monosaccharide composition in cherry wines were listed in Table 2. At the beginning of storage, the contents of L-arabinose, xylose, galactose, and glucose were 0.19 g/L, 0.22 g/L, 0.07 g/L, and 2.96 g/L, respectively. The contents of these four monosaccharides decreased to different degrees after aging, which decreased by 10.52%, 31.8%, 14.3%, 43.2% ($P<0.05$), respectively. In acidic environments, monosaccharides may dehydrate and degrade to form smaller molecules, such as furanic compounds (Apolinar-Valiente et al. 2014). In addition, monosaccharides can react with free amino acids to form flavorant, such as furfuraldehyde (Yu et al. 2017). In terms of both content and reduction, glucose and xylose were the most abundant. Hence, in the simulation experiment, glucose and xylose were selected to represent all the reducing sugars in cherry wine.

The correlation between the degree of browning and the contents of total phenols, amino acids and reducing sugars in Fig. 1(B) can be calculated. The correlation coefficients of browning degree with total phenolics, reducing sugars and amino acids were 0.953, 0.943 and 0.969, respectively ($P<0.01$). The results indicated that the degree of browning had a significant correlation with reducing sugars, total phenols, and amino acids in the cherry wine samples.

Browning in model solutions

The type of nonenzymatic browning in cherry wine was investigated by model solutions. The specific brown rates of the sample solutions are shown in Table 3. Among them, the browning rate of Glu+GLU+AA+CA is the highest, which is 0.00432, and the browning rate of the simulated solution (Glu+GLU) was 0.00296. The simulated solution containing glutamic acid and glucose (Glu+GLU+AA+CA, Glu+GLU+AA, Glu+GLU+CA, Glu+GLU) all showed browning, so the Maillard reaction was one of the factors affecting browning. On the other hand, browning occurred in solutions containing only ascorbic acid, and the browning rate was 0.00180 after 50 d, indicating the significance of the degradation and oxidation of ascorbic acid. The data also showed that the interaction between glucose and chlorogenic acid and the browning rate was 0.00305 after 50 d. When the pH of the solution is less than 7, the carbohydrate substance is easily converted to 5-hydroxymethyl furfural (5-HMF) and reacts with phenolic substances to form colored substances. (Lee and Nagy 1988). And the results showed that no browning occurred in the simulated solutions (GLU+CA, GLU, Glu, CA).

The HPLC patterns of the model solutions with chlorogenic acid and the model solutions with chlorogenic acid and glucose after incubation at 50°C for 12 d are shown in Fig. 2. A new peak appeared in the model solution containing chlorogenic acid and glucose (Fig. 2(B)). The newly formed peak was determined to be 5-hydroxymethyl furfural (5-HMF) by comparison with the standard product. These results demonstrate that the reaction between reducing sugars and phenols may occur through the formation of 5-hydroxymethyl furfural (5-HMF).

Effects of individual phenolic compounds and monosaccharide on browning degree

The browning degree in a simulated solution (chlorogenic acid and phlorizin, glucose and chlorogenic acid) is shown in Fig. 3(A). The browning degree in both model solutions showed an increasing trend within 50 d of storage, but

chlorogenic acid was more conducive to reacting with glucose than phlorizin. The absorbance at 420 nm for the solution containing glucose and phlorizin increased to 0.0397 after 50 d ($P<0.05$). However, the absorbance at 420 nm significantly increased to 0.2350 after 50 d ($P<0.05$) in the solution containing glucose and chlorogenic acid.

Except for the model solution with only chlorogenic acid, the absorbance at 420 nm of all model solutions showed a gradually increasing trend during the 30-d storage period, as shown in Fig. 3(B). The solution containing only glucose or xylose was slightly browned, and the sample with glucose was slightly more browned than the xylose. The presence of chlorogenic acid increased the browning rate in a solution that contained reducing sugars. At the end of storage, the absorbance at 420 nm of the sample containing chlorogenic acid and glucose was 1.436 ($P<0.05$), and the absorbance of the sample containing chlorogenic acid and xylose was 0.532. The results showed that glucose could promote the browning of chlorogenic acid more than xylose.

Conclusions

In the present study, the relationship between the PPO enzymatic activities and browning degree during fermentation showed that enzymatic browning and nonenzymatic browning existed simultaneously during fermentation, and enzymatic browning was the main mechanism. Through dynamic monitoring of the contents of reducing sugars, total phenols, and total amino acids and the browning degree of cherry wine during the aging, it was found that non-enzymatic reaction played a key role in the browning of cherry wine during storage. Further, it can be inferred from the model solution that the browning in cherry wine is mainly caused by the oxidative polymerization of ascorbic acid, the reaction of phenolic substances with reducing sugars, and the Maillard reaction. Chlorogenic acid and glucose were more potent than the other compounds in enhancing the browning of cherry wine. Research has shown that regulating polyphenol oxidase activity during cherry wine fermentation can effectively control color changes in the wine. Additionally, further studies should focus on strategies to effectively mitigate the interactions between reducing sugars, total phenols, and total amino acids during the aging process. This work provides a theoretical basis for addressing the browning issue in the fruit wine industry and contributes to further exploration of substances that inhibit browning and natural antioxidants in fruit wines.

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Code availability Not applicable.

Declarations

Ethical approval Not applicable.

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Conflict of interest The authors declare that they have no competing interests.

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