Effects of Oak Chip Treatments on Quality of Dry White Wines During Aging

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Introduction

Longyan dry-white wine is fermented with pure Longyan grape juice. With its yellowish and green color, clarity and translucence and fresh fruity aroma, it is recognized as “Oriental Wine” by European and American wine experts. After fermentation, this wine adopts a sour and poor taste and is unsuitable for drinking, therefore, its aging takes a long time. The aging process is one of the fundamental steps to obtained high-quality wine and it promotes wine maturity and improves sensory properties (Bautista-Ortín et al., 2008; Cerdán and Ancín-Azpilicueta, 2006; Alamo-Sanza et al., 2019). Through aging, the wine obtains a stable color, complex aroma and improved taste because it loses its astringency and bitterness (Crump et al., 2015). The aging of wine is usually conducted in oak barrels. However, aging wine in barrels requires long periods of contact time. Traditional maturation systems are costly and laborious due to the high price of barrels, limited lifetime, large space and maintenance requirement. Therefore, oak products, such as oak planks, blocks and slices, have been used in recent years instead of oak barrels to save money and short the aging time (Coelho et al., 2019; Guchu et al., 2005).

Abstract: The objective of the research was to evaluate the effect of different oak chip treatments on quality of Longyan dry white wine during aging. Phenolic substances, antioxidant activity and Cu²⁺ reduction force were measured. The results showed that wines treated with French oak chips (6 g/L) had significantly higher contents of total phenols (0.083 mg/mL) and flavonoids (0.063 mg/mL), also the highest total antioxidant capacity (47.853 U/mL) was observed. However the scavenging rate of DPPH free radicals and hydroxyl radical scavenging rate were the highest in samples aged with American oak chips (6 g/L) and Yanshan oak chips (2 g/L), respectively. As for 12 monomer phenols determined by high-performance liquid chromatography, French oak chips can contribute to the formation of monomer phenols, especially the generation of syringaldehyde (15.134 mg/L) and guaiacol (17.345 mg/L). Cu²⁺ reduction ability of wine sample increased with the increase of oak chips content. Correlation analysis revealed that the monomer phenols had a strong correlation with total antioxidant capacity, DPPH radical scavenging rate and hydroxyl radical scavenging rate. Understanding the influence of oak chips on wine properties could aid to lay a theoretical foundation for the effective use of oak chips in wine.

Keywords: Longyan Dry White Wine, Aging, Phenols, Antioxidation

Currently, the two most important varieties of oak chose for wine aging are the French Quercus Robur and the American Quercus fabri (Bozalongo et al., 2007; Cerdán et al., 2002; Gordillo et al., 2016). French and American oaks have important differences in terms of chemical composition. French oak has more phenolic compounds, ellagiotannins and extractable soluble substances and the lignin modified phenolic resin extracted from oak have the metal adsorption capacity of Cd²⁺ with sustainability, low production cost and environmental control (Arasaretanam and Kirudchayini, 2019). Differences have also been found between them in terms of C13 compounds and norisoprenoids composition (Cerdán et al., 2002). China’s domestic oak is known as eucalyptus, which is mainly distributed in the secondary forest species in the northeastern forest area of China. The Chinese eucalyptus, French oak and the American oak belong to the same family. The Chinese eucalyptus is cold-tolerant, drought-tolerant and growth-resistant. In terms of shape, fruit, texture and scent, Chinese eucalyptus is similar with imported oak and has considerable potential for wine aging.
Phenolic compounds are important substances in wine because they not only directly or indirectly affect the color, taste and aroma of wines but are also closely related to their antioxidant activity. DPPH method is widely used for quantitative determination of antioxidant capacity and DPPH can be used as a substance to monitor reactions in chemical reactions containing free radicals (Mahboubi and Mahboubi 2015). Meanwhile, hydrogen peroxide scavenging activity can be used to evaluate antioxidant capacity and some authors reported that phenols have the ability to scavenge hydrogen peroxide (Prahadeesh et al., 2018). The relationship between phenols and antioxidant capacity can be demonstrated by a multivariate statistical analysis, the multivariate statistical analysis is generally used to evaluate the correlation between variables. For example, a multi-objective optimization model is developed to allocate the agricultural and environmental water (Sedghamiz et al., 2018). The phenolic substances in wine differ with the variety of grapes, brewing process and method of aging. With oak-aged wine, the lignin in oak degrades to form volatile phenols and phenolic compounds (Canas et al., 2019; Gómez García-Carpintero et al., 2012). However, there is little known about wine produced from longyan white grape varieties and specifically about the aging of the wine.

The aim of this study was to determine the effects of (i) three types of oak slices (Yanshan, American and French) and (ii) different addition amount (2, 4 and 6 g/L) on the final quality of Longyan dry white wine over the course of a 60 day aging, total phenolics, total flavonoids, total flavanols, monomeric phenol, antioxidant activity and Cu²⁺ reduction force were measured and correlation analysis was used to evaluate the contribution of phenolic substances measured to the antioxidant capacity. The research method of this paper is showed in Fig. 1. Further it lay the foundation for the extensive use of oak chips in aged wine in wine industry.

**Materials and Methods**

**Chemicals and Reagents**

Gallic acid, protocatechuic acid, catechin, vanillic acid, syringic acid, coumaric acid, syringaldehyde, ferulic acid, guaiacol, benzoic acid, salicylic acid, quercetin, ρ-DMACA, neocuproin, a water-soluble analog of Vitamin E and DPPH were all purchased from Shanghai Yuanye Biotechnology Co., Ltd. Total antioxidant capacity kit was purchased from Nanjing Institute of Bioengineering. Other reagents were of analytical grade.

**Samples**

Samples of Longyan dry white wine from the 2017 vintage were industrially manufactured in a winery named Aristocratic Manor, Huailai County, Hebei Province and China. The base parameters of the
Longyan dry white wine were as follows: Alcohol content 12% vol, pH 3.63, total acidity 7.11 g/L and reduced sugar 0.18 g/L. American/French oak slices were purchased from Saiprisin (Beijing) Technology Co. Ltd., with dimensions of approximately 10×5×2 mm, moderately baked 200±10°C and baked for 30 min. Yanshan oak slices were purchased from the 20-year-old eucalyptus base of Guojiatan Town, Longhua County, Hebei Province, China, with specifications of approximately 10×5×2 mm, moderately baked 200±10°C for 30 min.

**Aging of Wine**

A total of three kinds of oak slices were used for aging. These oak slices were added to the base wine according to addition amounts of 2, 4 and 6 g/L and aged for 60 days at room temperature. Afterward, the samples were analyzed. Each oak treatment was set in three parallels and compared with wines aged at the same temperature and storage time without oak slices.

**Determination of Total Phenolics, Total Flavonoids and Total Flavanols**

Total phenols were determined by folin-phenol colorimetry with 752 UV-visible spectrophotometer (Shanghai Jinghua Technology Instrument Co., Ltd.) (Baiano et al., 2015). A total of 0.1 mL sample was added into a 10 mL volumetric bottle for taste and then 6 mL distilled water, 0.5 mL folin-phenol reagent and 1.5 mL Na₂CO₃ were quickly added after fully mixing the solution. The capacity was finally fixed with distilled water. The solution was placed at 20°C for 2 h and then UV-visible spectrophotometer was used to determine the absorbance value of each solution at the wavelength of 765 nm. The results were expressed in terms of gallic acid equivalent.

The determination of total flavonoids was performed according to the methodology (Sánchez-Palomo et al., 2017). A total of 0.5 mL sample was added for taste and 0.5 mL ethanol and 0.15 mL 0.5 mol/L NaNO₂ were added to the 10 mL volumetric bottle with fully mixed. A total of 0.15 mL 0.3 mol/L AlCl₃ was then added 5 min later, followed by the addition of 1 mL 1 mol/L NaOH and 2 mL distilled water after 10 min. The absorbance was determined at 510 nm and read against a blank solution. Concentrations were calculated in terms of catechin.

**Determination of Monomeric Phenol**

A total of 10 mL of the wine was took and extracted thrice with 10 mL ethyl acetate. Afterward, the organic phase was combined and concentrated by rotary evaporation to dryness. The mixture was reconstituted with 3 mL chromatographic methanol and stored at-20°C in the dark for liquid chromatography.

The determination of monomeric phenol was carried out by Waters 1525 HPLC system equipped with UV detector and an Agilent column C-18 (250×4.6 mm, 5 μm) at 30°C. The detection was performed at 280 nm. The mobile phase consisted of a 2% glacial acetic acid (eluent A) and an acetonitrile (eluent B). The flow rate was 1 mL/min. The solvent gradient was as follows: 97-90% A (0-5 min), 90-85% A (5-15 min), 85-70% A (15-35 min), 70-97% A (35-40 min).

**Determination of Antioxidant Activity**

The total antioxidant capacity was determined according to the kit instructions. The absorbance value of the reaction system was increased by 0.01 per mg of solution per minute at 37°C as a unit of total antioxidant capacity.

The determination of DPPH free radical-scavenging rate was determined. A total of 1 mL 0.2 mmol/L DPPH ethanol solution, 1 mL distilled water and 1 mL sample wine were added to the plug-in tube, fully mixed and then incubated at room temperature for 20 min in the dark. The Absorbance (As) was then measured at 517 nm. The blank group used 1 mL absolute ethanol instead of DPPH ethanol solution and the Absorbance (Ax) was measured; by contrast, the control group used 1 mL distilled water instead of the sample to measure the Absorbance (Ao). The blank was finally zeroed with an equal volume of distilled water and absolute ethanol mixture.

The hydroxyl radical-scavenging rate was determined according to the methodology (Apak et al., 2004). The reaction mixture comprised the addition of 100 μL 0.02 mol/L FeSO₄, 45 μL 0.15% H₂O₂ and 1 mL 8 mmol/L salicylic acid into 4 mL distilled water. The solution was mixed thoroughly and then 120 μL sample wine was added. The absorbance was read at 593 nm after 4 min incubation at an ambient temperature of 3°C in the dark against a blank of distilled water and control of absolute ethanol.

The determination of the Cu²⁺ reduction ability was performed according to the methodology of Apak et al. (2004). The reaction mixture contained 200 μL sample wine, 2 mL distilled water, 0.3 mL 5 mmol/L CuSO₄ and read against a blank solution. Concentrations were calculated as catechin.
and 0.3 mL 3.75 mmol/L neocuproin. The absorbance was read at 450 nm after 30 min. The results were expressed in terms of the water-soluble analog of vitamin E.

**Statistical Analysis**

The results were presented as mean ± standard deviation (SD). The data were performed using one-way analysis of variance (ANOVA) followed by Tukey’s test at $P<0.05$. All analyses were conducted using SPSS 20.0 for Windows.

**Results and Discussion**

**Effects of Different Oak Chips on Total Phenolics, Total Flavonoids and Total Flavanols**

Fig. 2-4 showed the effects of different species and amounts of oak slices to total phenolics, flavonoids and flavanols. Fig. 2 showed that samples aged in oak slices have higher total phenolic content compared with control sample (0.038±0.002 mg/mL). It has been reported that wines aged with oak chips during 45 days presented higher total phenolic content compared with the control wine, wines treated 3 g/L of oak chips increased phenolic content and the wines containing with 5 g/L had a lower phenolic content (Dumitriu et al., 2016). However, in this study, the total phenolic contents increased along with oak chips content and the highest total phenol content (0.083±0.001 mg/mL) was observed when 6 g/L of the French oak slices was used. The inconsistency between the results may be due to the difference of oak chip wood and baking degree. Fig. 3 showed that the addition of the oak slices can increase the total flavonoid content in the Longyan dry white wine. In the aging process of American and French oak slices, the 6 g/L French oak slices had the highest total flavonoid contents (0.063±0.001) mg/mL. The results coincides with previous research, which pointed out that no difference in total flavonoid content was due to their participation in the oxidation reactions and American oak chips presented very low increment as the extraction proceeded. Fig. 4 showed that the aging effect of the oak slices on the total flavanol contents varied with the type and content of oak. Regardless of 2, 4, or 6 g/L of American oak slices were added, no effect was observed on the total flavanol content. The total flavanol content in the control sample was only 0.004 mg/mL and the effect of oak slices treatment on total flavanol was not significant ($P < 0.05$).

**Fig. 2**: Effect of different oak slices on total phenol content during aging of Longyan dry white wine. Different superscripts indicate statistical differences between different additions of the same oak chips ($P < 0.05$)
**Effects of Different Oak Chips on Monomeric Phenol**

The effects of different oak slices on 12 monomeric phenols were showed in Table 1. In the Table 1, the content of most monomeric phenols was higher than that of the control wine sample. This finding showed that phenolic substances in aged oak were effectively immersed in wine samples. Among these phenols, the largest increase was guaiacol because oak is rich in guaiacol and guaiacol content increased with the added amount of oak slices. This result is inconsistent with that observed by previous research, the most abundant monomeric phenol in control wine samples were ellagic acid and gallic acid and the most abundant compounds were ellagic acid and sinapic aldehyde in wine samples aged with oak chips (Sanzal et al., 2004). This may be due to different types of wood containing various low molecular phenolics, the release of...
the phenolic substances from wood to wine depends on the type of chips and the degree of toast during red wine aging. Derivatives of benzoic and cinnamic acid (such as benzoic acid, gallic acid, syringic acid, salicylic acid, coumaric acid and ferulic acid) are products of sugar degradation during wood roasting (Araptisas et al., 2004; Natali et al., 2006). Therefore, after aging, the content of these derivatives increased and the wine sample aged with 6 g/L French oak slices increased most, these results agree with those observed by other researches (Jordão et al., 2006; Jordão et al., 2012). This phenomenon was due to the high levels of lignin, cellulose, hemicellulose and other sugars degraded by medium-baked French oak (Baiano et al., 2015; Ortega-Heras et al., 2004). The syringaldehyde is considered as markers in distilled beverages during aging and syringaldehyde and vanillin comes from the degradation of lignin, it is used to evaluate the quality of aged samples (Doussot et al., 2002). Some authors reported that a ratio (syringicaldehyde/vanillin) from 1.4 to 2.5 indicates a balance in the lignin degradation (Michel et al., 2011). In this study, the syringaldehyde in the wine sample considerably changed after aging. French oak chips treated wine samples contain higher content of syringaldehyde, being higher than that reported (Michel et al., 2011). The syringaldehyde will turn into syringic acid after oxidation and the syringic acid in the wine sample did not substantially change, thus indicated that the wine sample had less contact with oxygen during aging, leading to low oxidation. Moreover, the overall change effect of various monomer phenols was similar to those of total phenol, flavonoids and flavanols.

**Effect of Different Oak Chips on Antioxidant Capacity**

Fig. 5 showed the effect of different oak slices on the total antioxidant capacity of Longyan dry white wine. The wines aged with 6 g/L French oak slices had the strongest total antioxidant capacity of (47.853±0.360) U/mL, reaching thrice that of the control sample. The effects of Yanshan and American oak chips on the total antioxidant ability of wine samples were not extremely different. These results can be due to the influence of some parameters, as toasting intensity and origin of the oak wood piece studied. Fig. 6 showed the effect of different oak slices on DPPH free radical-scavenging rate. As shown in this figure, the aged wine with 6 g/L American oak slices increased the DPPH free radical-scavenging rate from (94.841%±0.324) to (97.545%±0.331). However, some authors reported that light or medium toasting intensity of the oak chips presented higher antioxidant activity, the content of oak chips had little effect on the antioxidant capacity (António et al., 2012). DPPH free radicals are reduced by providing hydrogen atoms through antioxidant compounds, suggested that different species and contents of oak slices are considerably affected by the hydrogen supply capacity of antioxidant molecules in wine samples. Fig. 7 showed the hydroxyl radical-scavenging rate of different oak slices on Longyan dry white wine. As shown in this figure, the clearance rate (74.731%±0.430) of hydroxyl radicals was the highest in the aged sample with 2 g/L Yanshan oak slices and the scavenging rate of hydroxyl radicals in the wine samples decreased as the amount of Yanshan oak slices increased. In the wine samples aged with American and French oaks, the scavenging rate of hydroxyl radicals increased as the amount of oak slices increased. And values for the clearance rate of hydroxyl radicals in French oak chips coincides with results reported by other authors in cognacs (Da Porto et al., 2000).

![Fig. 5: Effect of different oak slices on total antioxidant capacity during aging of Longyan dry white wine.](image_url)

Different superscripts indicate statistical differences between different additions of the same oak chips ($P < 0.05$)
Fig. 6: Effect of different oak slices on DPPH radical scavenging rate during aging of Longyan dry white wine. Different superscripts indicate statistical differences between different additions of the same oak chips (P < 0.05).

Fig. 7: Effect of different oak slices on hydroxyl radical scavenging rate during aging of Longyan dry white wine. Different superscripts indicate statistical differences between different additions of the same oak chips (P < 0.05).

Fig. 8: Effect of different oak slices on Cu^{2+} reduction force during aging of Longyan dry white wine. Different superscripts indicate statistical differences between different additions of the same oak chips (P < 0.05).
Table 1: Evolution of monomer phenols in Longyan dry white wines treated with different oak chips for 60 days

| Monomeric phenol (mg/L) | 2 g/L | 4 g/L | 6 g/L | 2 g/L | 4 g/L | 6 g/L | 2 g/L | 4 g/L | 6 g/L |
|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Gallic acid 1.20±0.005 | 1.08±0.017 | 1.27±0.026 | 2.01±0.021 | 1.23±0.017 | 1.54±0.007 | 1.99±0.025 | 1.53±0.018 | 1.56±0.017 | 2.04±0.010 |
| Protocatechuic acid 3.27±0.023 | 3.42±0.026 | 4.03±0.015 | 6.12±0.037 | 3.32±0.006 | 4.04±0.041 | 4.53±0.011 | 3.45±0.024 | 4.37±0.001 | 5.43±0.023 |
| Catechin 1.32±0.035 | 1.03±0.009 | 1.37±0.002 | 2.19±0.011 | 1.37±0.014 | 1.43±0.037 | 1.78±0.019 | 1.96±0.005 | 1.69±0.005 | 1.84±0.016 |
| Vanillic acid 2.13±0.005 | 2.76±0.014 | 3.74±0.007 | 5.37±0.029 | 3.02±0.021 | 5.15±0.015 | 5.38±0.033 | 3.01±0.032 | 4.75±0.029 | 6.02±0.019 |
| Syringic acid 1.75±0.012 | 1.92±0.043 | 2.13±0.005 | 2.53±0.032 | 2.01±0.099 | 2.51±0.012 | 3.17±0.012 | 2.03±0.009 | 3.02±0.003 | 3.91±0.028 |
| Coumaric acid 2.12±0.027 | 2.42±0.007 | 2.40±0.003 | 3.01±0.004 | 3.25±0.018 | 3.20±0.023 | 4.09±0.007 | 2.3±0.017 | 2.5±0.001 | 2.67±0.020 |
| Syringalddehyde 0.02±0.039 | 3.12±0.065 | 9.43±0.522 | 11.58±1.301 | 3.03±0.065 | 10.80±0.655 | 12.95±0.655 | 3.41±0.023 | 12.21±0.038 | 15.13±0.023 |
| Ferulic acid 1.35±0.006 | 1.47±0.011 | 1.39±0.021 | 1.53±0.011 | 1.35±0.015 | 1.41±0.008 | 1.78±0.013 | 1.43±0.004 | 1.45±0.011 | 1.52±0.008 |
| Ginseng 2.90±0.026 | 2.52±0.013 | 3.13±0.060 | 1.20±0.058 | 7.64±0.023 | 10.56±0.028 | 13.05±0.050 | 9.34±0.014 | 9.91±0.015 | 9.7±0.019 |
| Benzoic acid 3.71±0.022 | 3.94±0.015 | 4.37±0.001 | 6.09±0.047 | 4.12±0.047 | 5.67±0.006 | 8.31±0.050 | 5.34±0.022 | 8.76±0.014 | 11.35±0.034 |
| Salicylic acid 5.20±0.001 | 5.83±0.029 | 6.719±0.043 | 9.34±0.099 | 6.15±0.026 | 7.18±0.022 | 11.70±0.021 | 7.80±0.013 | 8.14±0.037 | 13.21±0.101 |
| Quercetin 0.57±0.030 | 0.91±0.003 | 0.58±0.010 | 0.87±0.001 | 0.53±0.011 | 0.57±0.015 | 0.59±0.025 | 0.62±0.014 | 0.59±0.008 | 0.73±0.004 |

Different superscripts indicate statistical differences between different additions of the same oak chips (P < 0.05)

Table 2: Correlation between phenols substances and antioxidation

| Phenols         | Total antioxidant capacity | DPPH free radical scavenging rate | Hydroxyl radical scavenging rate | Cu²⁺ reduction ability |
|-----------------|---------------------------|----------------------------------|---------------------------------|------------------------|
| Gallic acid     | 0.961                     | 0.923                            | 0.909                           | 0.352                  |
| Protocatechuic acid | 0.697                  | 0.664                            | 0.567                           | 0.246                  |
| Catechin        | 0.770                     | 0.761                            | 0.641                           | 0.079                  |
| Vanillic acid   | -0.434                    | -0.549                           | -0.595                          | -0.335                 |
| Syringic acid   | 0.730                     | 0.871                            | 0.847                           | 0.164                  |
| Coumaric acid   | 0.677                     | 0.752                            | 0.654                           | -0.110                 |
| Syringaldehyde  | -0.806                    | -0.892                           | -0.839                          | -0.181                 |
| Ferulic acid    | 0.922                     | 0.970                            | 0.937                           | 0.247                  |
| Guaiacol        | -0.812                    | -0.816                           | -0.761                          | 0.021                  |
| Benzoic acid    | -0.283                    | -0.061                           | -0.051                          | -0.294                 |
| Salicylic acid  | 0.242                     | 0.334                            | 0.223                           | 0.032                  |
| Quercetin       | 0.937                     | 0.931                            | 0.936                           | 0.548                  |

Excessive metal ions in wine, such as Cu²⁺, are indirectly involved in oxidation through the catalysis of oxygen free radicals, resulted in the oxidation and deterioration of wine (Pettit et al., 2013). Cu²⁺ reduction ability is the provision of electrons through antioxidants to restore Cu²⁺. Fig. 8 showed the effect of different oak chips on the Cu²⁺ reduction ability of wine samples. The Cu²⁺ reduction ability of increased with the increase of oak chips. A total of 4 and 6 g/L American oak slices and 6 g/L French oak slices had considerable influence on the Cu²⁺ reduction ability of wine samples and the maximum value reached 0.59 mg/mL with American and French oak, Yanshan oak had less influence on the Cu²⁺ reduction ability of the wine sample.

Correlation Between Phenolic Substances and Antioxidation in Longyan Dry White Wine

Numerous studies have shown that the oxidation resistance of phenolic substances is mainly reflected in two aspects. On the one hand, the oxygen content in the environment is reduced by redox reaction (Oberholster et al., 2015; Aruwa et al., 2019). In this process, phenolic substances simultaneously act as hydrogen donors to release hydrogen into the environment. The combination of free radicals terminates the chain reaction initiated by free radicals, thereby preventing the commencement of the oxidation process. On the other hand, polyphenols can effectively remove excessive free radicals in the body, inhibit oxidation and protect against free radical-induced damage to biological macromolecules (Navarro et al., 2016; Pérez-Coello et al., 2000).

In order to evaluate the contribution of phenolic substances measured to the antioxidant capacity, correlation analysis was used. Table 2 showed that a certain correlation existed between phenolic content and antioxidation. This result showed that a strong positive correlation was observed between gallic acid, ferulic acid, quercetin and total antioxidant capacity, as well as in DPPH free radical-scavenging rate and hydroxyl radical-scavenging rate, is inconsistent with other results. According to other authors, positive correlations were observed between the concentration of total ellagitannins quantified and the total antioxidant activity (António et al., 2012). Additionally, syringaldehyde was negatively correlated with total antioxidant capacity, DPPH radical scavenging rate and hydroxyl radical scavenging rate. The correlation between various phenolic substances and Cu²⁺ reduction ability was weak. These findings may be related to the participation of Cu²⁺ in the oxidation reaction through the catalytic action of oxygen acts.

Conclusion

The phenolic substances and antioxidant activities of Longyan dry-white wine aged by three different oak slices were determined. The results showed that samples aged in oak slices have higher total phenolic content.
The authors declare that they have no competing interests.

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