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The characteristics of polysaccharide composition of red wines in China: Effects of grape varieties, origins and winemaking techniques

Hongyue Zhai [a,b], Mengqi Ling [a,b], Siyu Li [c], Bainian Chen [a], Xu Zhao [d], Wenzhe Tong [a,b], Chifang Cheng [e], Jin Li [f], Ying Shi [a,b], Changqing Duan [a,b], Yibin Lan [a,b,*]

[a] Center for Viticulture and Enology, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing 100083, China
[b] Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China
[c] Faculty of Food Science and Engineering, Kunming University of Science and Technology, Kunming 650500, China
[d] College of Life Sciences, Yantai University, Yantai, Shandong 264005, China
[e] Xinjiang CITIC Guoan Wine Co. Ltd., Manasi, Changji 832200, China
[f] Shandong Technology Innovation Center of Wine Grape and Wine, Yantai 264000, China

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ABSTRACT

In this work, the polysaccharide profile of different grapes and red wines in China was studied and the influences of two common winemaking techniques on the components of wine were analyzed. The soluble polysaccharide content in the skins of native grape species in China (non-*Vitis vinifera* grapes) was significantly higher than that of *Vitis vinifera* species, while the *terroir* effect on *V. vinifera* varieties was limited. The combination of the enzyme preparation and the addition of mannoproteins (MPs) at the beginning of alcoholic fermentation (MP1 + E) could increase the contents of MPs and acid polysaccharides (APS) compared to the control wines. Meanwhile, better color characteristics and higher level of anthocyanin derivatives were observed. However, MP1 + E treatment reduced the content of polysaccharides rich in arabinose and galactose (PRAGs) due to enzymatic hydrolysis. The study will provide useful information for winemakers to regulate the wine polysaccharide profile.

Introduction

Polysaccharide, one of the major macromolecules in wine, have been reported to play an important role in modulating wine quality (Jones-Moore, Jelley, Marangon, & Fedrizzi, 2022; Li, Duan, & Han, 2021; Zhai et al., 2023). On the one hand, grape cell wall composition may affect the efficiency of phenolic metabolites extraction from grape skins during the crushing and subsequent maceration-fermentation stage (Hensen, Hoening, Weilack, Damm, & Weber, 2022). On the other hand, the presence of polysaccharides contributes to the specific wine matrix and the perceived wine quality by influencing the colloidal state of the wine and interacting with phenolic and volatile compounds mainly through hydrogen bonding, hydrophobic effect and electrostatic interactions (Brandão et al., 2017; Fernandes et al., 2021). Their role in protein stability and protection against tartrate salt crystallization has been demonstrated (Dupin et al., 2000; Lankhorst et al., 2017), and practical applications of polysaccharides supplements have been widely carried out in the wine industry with the aim of regulating wine astringency (Alcalde-Eon, Ferreras-Charro et al., 2019) or improving wine color stability (Alcalde-Eon, García-Estévez, Puente, Rivas-Gonzalo, & Escribano-Bailón, 2014; Alcalde-Eon, Perez-Mestre et al., 2019). Most of the existing reports have focused on the effects of the application of commercial mannoprotein products, and the role of grape polysaccharides on the organoleptic qualities of wine has also gradually attracted the

Abbreviations: AGPs, arabinogalactan proteins; RG-II, type II rhamnogalacturonans; MPs, mannoproteins; HGs, homogalacturonans; RG-I, type I rhamnogalacturonans; PRAGs, polysaccharides rich in arabinose and galactose; APS, acid polysaccharides; TMS, total monosaccharides; SSP, skin soluble polysaccharides; PMP, 1-phenyl-3-methyl-5-pyrazolone; TFA, trifluoroacetic acid; CS, Cabernet Sauvignon; MA, Marselan; PV, Petit Verdot; ML, Merlot; CF, Cabernet Franc; SR, Syrah; SH, Shuanghong; SY, Shuangyou; YEER, Yeniang no.2; YELIU, Yeniang no.6; TIAN, Tianci; ZI, Ziqiu; BBH, Beibinghong; SDPL, Penglai, Shandong; HBCL, Changli, Hebei; HBHL, Huailai, Hebei; NXYC, Yinchuan, Ningxia; XJMS, Manasi, Xinjiang; Man, mannose; Rha, rhamnose; Glc A, glucuronic acid; Gal A, galacturonic acid; Glu, glucose; Gal, galactose; Xyl, xylose; Ara, arabinose; Fuc, fucose.

* Corresponding author at: Center for Viticulture & Enology, College of Food Science and Nutritional Engineering, China Agricultural University, No. 17 Tsinghua East Road, Beijing 100083, China.
E-mail address: lanyibin@cau.edu.cn (Y. Lan).

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attention of researchers (Manjón, Li, Dueñas, García-Estévez, & Escribano-Bailón, 2023). These studies indicate the crucial role of wine polysaccharides and that their effects on wine quality are strongly related to their composition and structure.

The most abundant polysaccharides identified in wine are grape-derived arabinogalactan proteins (AGPs) and type II rhamnogalacturonans (RG-II), and yeast-derived mannoproteins (MPs) (Apolinar-Valiente et al., 2014). The concentration of polysaccharide is critical, as either insufficient or excessive levels can destabilize the colloids and cause flocculation or precipitation, such as the loss of colloidal coloring matters and tannins (Ribereau-Gayon et al., 2000; Zhai et al., 2023). Wine polysaccharide composition is initially influenced by grape variety and maturity (Apolinar-Valiente, Romero-Cascales, Gómez-Plaza, López-Roca, & Ros-García, 2015a; Ortega-Regules, Ros-García, Bautista-Ortín, López-Roca, & Gómez-Plaza, 2008), and vineyard *terroir* (Apolinar-Valiente et al., 2013, Apolinar-Valiente, Romero-Cascales, Gómez-Plaza, López-Roca, & Ros-García, 2015b). Meanwhile, winemaking techniques are responsible for the greatest modification of wine polysaccharide profile throughout vinification (Apolinar-Valiente et al., 2013, 2014; Guadalupe, Palacios, & Ayestaran, 2007). Among these techniques, the application of commercial maceration enzymes is widely used in winemaking, with the aim of facilitating the skin degradation process and regulating the final wine quality (Apolinar-Valiente et al., 2013, 2014; Doco, Williams, & Cheynier, 2007; Ducasse et al., 2010). However, to the best of our knowledge, there are no comprehensive and systematic studies on the polysaccharide profile and its influencing factors of red wines in China.

China has a large area under wine grape cultivation (approximately 98,900 ha in 2018), accounting for 2.3 % of the world's wine grape cultivation area (Liu et al., 2021). The major wine regions in China have a wide longitude range (from 73°E to 135°E, a distance of approximately 4900 km), which contributes to a great diversity of *terroir* (Liang et al., 2014), resulting in the production of wines with different sensory characteristics. Although studies have investigated the phenolic characteristics of wine in China (Li, He, Zhu, Wang, & Duan, 2017a), data on the polysaccharide composition of grapes and wines are scarce so far. Considering that a better understanding of the polysaccharide characteristics is beneficial for the improvement of wine quality, in the present work, typical grape and red wine samples were collected (including *V. vinifera* species from different regions, and native grape species in China: *V. quingquangularis*, *V. amurensis*, *V. davidii*, and hybrids of *V. amurensis* and *V. vinifera*) to understand the polysaccharide profiles of grape/wine resources. Furthermore, winemaking experiments were conducted and two common techniques (enzyme preparation and commercial MPs addition during the maceration-fermentation stage) were applied to evaluate their effects on the polysaccharide profile, and the corresponding changes in the phenolic and volatile composition of wines. Therefore, this research aims to comprehensively investigate the polysaccharide characteristics of grapes and wines in China, accompanied by various winemaking experiments to explore an appropriate winemaking process from the viewpoint of wine polysaccharides to produce different characteristics of wines.

Materials and methods

Reagents and standards

Analytical grade chemicals, including ethanol, sodium chloride, sodium hydroxide, acetone, and hydrochloric acid were purchased from Beijing Chemical Works (Beijing, China). Chromatographic grade solvents, including methanol (≥99.9 %) and acetonitrile (≥99.9 %) were purchased from Honeywell (Marris Township, NJ, USA). HPLC-grade formic acid (≥99 %) and trifluoroacetic acid (TFA) were purchased from ROE Scientific (Newark, NJ, USA) and TEDIA (Cincinnati, Ohio, USA), respectively. Potassium phosphate monobasic (KH_2PO_4), 1-phenyl-3-methyl-5-pyrazolone (PMP), D-glucose, L-rhamnose, D-xylose, L-arabinose, D-mannose, L-fucose, D-galactose, D-galacturonic acid, and D-glucuronic acid were purchased from Macklin (Shanghai, China).

Grape and red wine sample collection

A total of 36 grape samples and 41 red wine samples were kindly provided by wineries from several major grape origins in China (climate and soil characteristics were showed in Table S1), including four grape species. The number of *V. vinifera* varieties was three (Cabernet Sauvignon (CS), Marselan (MA) and Petit Verdot (PV)) in 2020 (n = 15) and six (CS, MA, PV, Merlot (ML), Cabernet Franc (CF), Syrah (SR)) in 2021 (n = 15), collected from different origins. The number of non-*V. vinifera* varieties was six (native grape species in China: Shuanghong (SH), Yeniang no.2 (YEER), Yeniang no.6 (YELIU), Tianci (TIAN), Ziqiu (ZI), Beibinghong (BBH)) in 2020 (n = 6), collected from their specific origin. In addition, the number of *V. vinifera* red wine samples was three (CS, MA and PV) in 2020 (n = 15) and six (CS, MA, PV, ML, CF, SR) in 2021 (n = 20), collected from different origins. The number of non-*V. vinifera* red wine samples was six (native grape species in China: SH, SY, YEER, TIAN, ZI, BBH) in 2020 (n = 6), collected from their specific origin (Table 1 and Fig. S1). The grapes were harvested at commercial maturity in the specific origins, and the physicochemical characteristics of the grapes were shown in Table S2. For each variety, three 150-berry samples were randomly collected from the 30 selected vines and placed in refrigerated bags, and carefully transported to the laboratory. The samples were immediately frozen and stored at −40 °C until polysaccharide extraction. All wines were produced with a single variety so that the varietal peculiarities could be reflected in the final wines and fermentation was carried out by each winery independently on an industrial scale and according to standard production practice. The winemaking process did not include any ageing process and the wines collected in triplicate were stored at 13–15 °C in glass bottles sealed with corks until analysis.

Vinification of red wine by using different techniques

To further investigate the influence of techniques on the wine

Table 1
Information of experimental grapes and red wines from different origins of China.

Grape species	Origins	Vintage	Grape berries	Red wines
V. vinifera	Penglai, Shandong (SDPL)	2020	CS, MA, PV	CS, MA, PV
		2021	Nc	MA, PV
	Changli, Hebei (HBCL)	2020	CS, MA, PV	CS, MA, PV
		2021	CS, MA	CS, MA, SR
	Huailai, Hebei (HBHL)	2020	CS, MA, PV	CS, MA, PV
		2021	CS, MA, PV, ML	CS, MA, PV, SR
	Yinchuan, Ningxia (NXYC)	2020	CS, MA, PV	CS, MA, PV
		2021	CS, MA, PV, CF, SR	CS, MA, PV, ML, CF, SR
	Manasi, Xinjiang (XJMS)	2020	CS, MA, PV	CS, MA, PV
		2021	CS, MA, PV, CF	CS, MA, PV, ML, CF
V. amurensis	Ji'an, Jilin	2020	SH	SH, SY
V. quingquangularis	Nanning, Guangxi	2020	YEER, YELIU	YEER
V. davidii	Huaihua, Hunan	2020	TIAN, ZI	TIAN, ZI
V. amurensis × *V. vinifera* hybrids	Ji'an, Jilin	2020	BBH	BBH

CS, Cabernet Sauvignon; MA, Marselan; PV, Petit Verdot; ML, Merlot; CF, Cabernet Franc; SR, Syrah; SH, Shuanghong; SY, Shuangyou; YEER, Yeniang no.2; YELIU, Yeniang no.6; TIAN, Tianci; ZI, Ziqiu; BBH, Beibinghong.
Nc: not collected.

polysaccharide profile and possible corresponding changes in other sensory components, Cabernet Sauvignon grapes, sourced from a commercial vineyard in Xinjiang in 2021 and harvested at 26.7 °Brix were selected. For each replicate (three replicates), destemmed and crushed grapes (50 kg) were homogenized and transferred to the 60 L stainless steel tanks. The fermentation-maceration process was carried out at a temperature of 24–26 °C for 10 days (commercial Lalvin strain D254 yeast, Laffort, Bordeaux, France, 200 mg/L). The post-fermentative maceration was carried out at 24–26 °C for 7 days. The wines were then separated from the pulp using a hydraulic bladder press (Zambelli, Bergamo, Italy) with a maximum pressure of 0.3 MPa. The free-run and pressed wines were combined and transferred to 30 L tanks with airlocks and inoculated with a commercial preparation of *Lactobacillus* (5 mg/L, Lalvin 31, Lallemand Inc, San Simeon, France) to initiate malolactic fermentation in a hermetic environment. After malolactic fermentation, the free SO_2 of all the wines was adjusted to 30 mg/L with potassium metabisulfite, and then the wines were bottled with synthetic corks (Nomacorc, Boston, USA) and stored in the cellar at 10–15 °C for two months until analysis. The physicochemical characteristics of the final wines were measured using a Foss Winescan (FT 120) rapid-scanning infrared Fourier-transform spectrometer (Foss Electric, Hillerød, Denmark) (Table S3).

The samples were divided into five groups (Fig. S2), namely: control, no addition of enzyme and MPs (C); enzyme preparation added only during maceration (E); enzyme preparation added during maceration and addition of commercial MPs at the beginning of alcoholic fermentation (MP1 + E) (when the relative density of the must was1.08); at the middle of alcoholic fermentation (MP2 + E) (when the relative density was 1.05); at the end of alcoholic fermentation (MP3 + E). The enzyme preparation used (1.2 g) was Vinozym® Vintage FCE from Novozymes, Copenhagen, Denmark (a mixture of pectinases and hemicellulases, cellulase and protease) and was added when the crushed grapes were transferred to the tanks (the addition time was the same for all groups of enzyme preparation). The commercial MPs were OptiRED® from Lallemand, San Simeon, France (300 mg/L, as recommended; polysaccharide purity was approximately 206 mg/g). Samples were taken in triplicate at the end of alcoholic fermentation, after post-fermentative maceration, and at the end of the malolactic fermentation and stored at 10–15 °C before analysis.

Isolation of polysaccharides

Isolation of soluble polysaccharides from grape skin

Following the previously published method with slight modifications (Apolinar-Valiente, Romero-Cascales, López-Roca, Gómez-Plaza, & Ros-García, 2010), frozen grapes were peeled with a tweezer, and the skins were completely separated from the pulp and stored at −80 °C for subsequent extraction analysis. Grape skins (10 g) were homogenized and suspended in 60 mL of boiling water for 5 min, then centrifuged (10,610*g* for 5 min), and the procedure was repeated with 30 mL of boiling water. The supernatants were combined and one part was mixed with two parts of 96 % ethanol and extracted for 30 min at 40 °C. The crude alcohol-insoluble solids were separated by centrifugation and re-extracted with fresh 70 % ethanol for 30 min at 40 °C. The washing treatment with fresh 70 % ethanol was repeated several times until the Dubois test indicated no sugars in the 70 % ethanol phase. The alcohol-insoluble solids were then washed once with 96 % ethanol and acetone, respectively. Finally, the skin soluble polysaccharides (SSP) of the grapes were obtained and used for subsequent analysis after drying overnight in a fume cupboard. Polysaccharide extraction was performed in triplicate for each sample.

Isolation of total soluble polysaccharides (TSP) from wines

Wine polysaccharides were obtained by precipitation after ethanolic dehydration according to Guadalupe et al. (2012) with some modifications. Wine samples were first centrifuged to remove the insoluble materials and then concentrated to 1/5 in a vacuum concentrator (RayKol, Xiamen, China), and five times of cold acidified ethanol (96 % ethanol containing 0.3 mol/L HCl) was added and kept at 4 °C overnight. All the samples were then centrifuged (6790*g* for 10 min), the supernatants were discarded, and the pellets were washed twice with 96 % ethanol and once with acetone to remove the interfering materials. The total soluble polysaccharides (TSP) of the wine were obtained and used for subsequent analysis after drying overnight in a fume cupboard. This polysaccharide extraction was performed in triplicate for each sample.

Polysaccharide analysis

Determination of monosaccharide composition

The monosaccharides of the extracted polysaccharides were analyzed by high performance liquid chromatography (HPLC) according to the previously reported protocol (Zhao et al., 2017). Briefly, TSP extraction was hydrolyzed with 1 mL of 2 mol/L TFA at 110 °C for 4 h in a sealed tube. Then methanol was used to remove excess TFA and co-evaporated for three times (RayKol, Xiamen, China) after the hydrolysis was completed. Dry hydrolysates were dissolved in 1 mL of deionized water to proceed to the next derivatization step. The mixture of 100 μL hydrolysate, 120 μL 0.5 mol/L PMP and 100 μL 0.3 mol/L NaOH was derivatized at 70 °C for 1 h and neutralized with 100 μL 0.3 mol/L HCl. The derivative was extracted three times with 700 μL of methylene chloride to remove excess PMP. The derivatives were analyzed by HPLC (Agilent 1200, Santa Clara, USA) coupled with a variable wavelength detector (VWD) to determine the monosaccharide composition of the polysaccharides. The type of chromatographic column was Agilent Zorbax SB-C18 (4.6 × 250 mm, 5 μm). The mobile phase was a mixture of 0.1 mol/L KH_2PO_4 and acetonitrile (84:16, *v/v*). The flow rate was 1.0 mL/min and the column temperature was 35 °C. The absorbance of the samples was detected at 245 nm. D-glucose, L-rhamnose, D-xylose, L-arabinose, D-mannose, L-fucose, D-galactose, and D-galacturonic acid were derivatized and used as standards (Macklin, China) (Fig. S3).

Quantification of polysaccharide concentration

The concentrations of MPs, PRAGs, and APS in wine were estimated from the concentration of individual glycosyl residues, as determined by HPLC after hydrolysis and derivatization. All mannose was attributed to yeast MPs. PRAGs in wine, which mainly include AGPs, arabinogalactans, and arabinans, were estimated from the sum of galactose and arabinose residues (Apolinar-Valiente et al., 2013, 2014). The concentration of galacturonic acid was used to estimate the major acid polysaccharide from grape pectin in wine polysaccharide (APS) (Guadalupe et al., 2007). For grape polysaccharides, pectin concentration was calculated from the concentrations of uronic acids (measured as galacturonic acid), galactose, arabinose and rhamnose (Li et al., 2021; Ortega-Regules et al., 2008). Hemicellulose concentration was calculated from the concentrations of xylose, fucose, mannose and non-cellulosic glucose (Li et al., 2021; Ortega-Regules et al., 2008). In addition, TMS was calculated from the sum of all monosaccharide residues.

Analysis of phenolic compounds

Phenolic compounds were detected using high performance liquid chromatography triple-quadrupole tandem mass spectrometry (HPLC-QqQ-MS/MS) on an Agilent 1200-6410B (Agilent Technologies, Santa Clara, USA) according to the previous research with slight modifications (Tong et al., 2022) including non-anthocyanin phenolic compounds, monomeric anthocyanin compounds including their acylated (acetyl/coumaroyl) forms and anthocyanin derivatives. The separation of phenolic compounds was performed on an Agilent Poroshell 120 EC-C18 column (150 mm × 2.1 mm, 2.7 μm). Mobile phase A: 1 mL/L formic acid in water; mobile phase B: 1 mL/L formic acid in 1:1 (v/v) methanol:

acetonitrile. The wine samples were filtered through a 0.22 μm polyethersulfone filter. The elution gradient of non-anthocyanin phenolic compounds was from 10 % to 46 % B for 28 min, from 46 % to 10 % B for 1 min, and re-equilibration at 90 % A and 10 % B for 5 min. The elution gradient of monomeric anthocyanin was from 10 % to 100 % B for 10 min and re-equilibrated to the initial conditions for 5 min. The injection volume of above two method was 5 μL and the flow rate was 0.4 mL/min. For anthocyanin derivatives, the elution gradient was as follows: 0 % to 25 % B for 3 min, then to 30 % B up to 15 min, and 100 % B up to 20 min, and maintained eluting with 100 % B for 5 min and re-equilibrated to the initial conditions for 5 min. The injection volume was 10 μL and the flow rate was 0.3 mL/min. The column temperature was all set at 55 °C.

The MS was operated in positive mode for anthocyanins and their derivatives, and negative mode for non-anthocyanin phenolic compounds with electrospray ionization (ESI) source at 150 °C. The capillary voltage was 4 kV. The drying gas temperature was 350 °C and the drying gas flow rate was 12 L/h. The nebulizer pressure was 0.24 MPa. The multiple reaction monitoring (MRM) mode was used to complete the acquisition of mass spectra of targeted phenolic compounds. All anthocyanins were quantified on the basis of the calibration curve of malvidin-3-*O*-glucoside and non-anthocyanins phenolic compounds were quantified according to the calibration curves of their own reference compounds (Table S4). Samples were analyzed in triplicate and quantified by peak areas based on calibration curves.

Analysis of volatile compounds

Volatile compounds were extracted using headspace solid-phase microextraction (HS-SPME) with a 2 cm DVB/CAR/PDMS 50/30 μm SPME fiber (Supelco, Bellefonte, PA., USA), and analyzed using an Agilent 7890 gas chromatography equipped with Agilent 5975 mass spectrometer (GC–MS system) according to the previous research (Tong et al., 2022). Briefly, 5 mL of wine mixed with 1.5 g NaCl and 10 μL of internal standard 4-methyl-2-pentanol (1 g/L) was added to a 20 mL vial capped with a PTFE-silicon septum. The vial was equilibrated at 40 °C for 30 min, and then the fiber was injected into the headspace of vial to extract volatile compounds for 30 min with stirring at 500 rpm. Then, the SPME fiber was desorbed in GC injector for 8 min at 250 °C with 5:1 split mode.

The separation of volatile compounds was performed on a HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 μm, J&W Scientific, Folsom, CA, USA). The flow rate of the carrier gas (helium) was 1 mL/min. The temperature program was as follows: hold 50 °C for 1 min, increase at a rate of 3.0 °C/min to 220 °C, and hold 220 °C for 5 min. The mass detector was operated in the full scan mode with a mass range of *m/z* 30–350. Volatile compounds were identified by comparing the obtained mass spectra and retention indices (RI) with those of reference standards and compounds in the NIST 11 MS database. Calibration curves of each aroma standard were obtained according to twelve succession dilutions with the synthetic matrix (120 mL/L ethanol with 2 g/L glucose and 7 g/L tartaric acid, pH 3.5) (Table S5). All samples were analyzed in triplicate.

Color analysis

The quantitative description of wine chromatic properties was evaluated with the CIELab space. Wine samples were filtered through polyethersulfone filters (0.45 μm, Jinteng Experimental Equipment Co., Ltd, Tianjin, China) and measured in a 2 mm path length glass cuvette using a UV–visible spectrophotometer (UV-2450; Shimadzu Corporation, Kyoto, Japan). The visible absorption spectra (400–700 nm) of the wine was recorded with 1 nm intervals and the values of L^*, a^* and b^* were obtained through calculation formula according to the previous method (Ayala, Echavarri, & Negueruela, 1997).

Statistical analysis

Significant differences between samples were evaluated by one-way analysis of variance (ANOVA) using the SPSS 24.0 software (SPSS, Chicago, IL, USA). Differences between means were compared using Duncan's test. $P < 0.05$ was considered statistically significant. The pooled standard deviation (also known as the combined variance) was used to estimate variance of several different samples more precisely and better visualization. The calculation formula is as follows.

$$s_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \cdots + (n_k - 1)s_k^2}{(n_1 - 1) + (n_2 - 1) + \cdots + (n_k - 1)}}$$

where n_1, n_2, …, n_k are the sizes of the data subsets at each level of the variable s_1^2, s_2^2 …, s_k^2 are their respective variances. The graphs were drawn using GraphPad Prism 9 (GraphPad Software Inc).

Results and discussion

Effect of grape species/varieties on wine polysaccharidic profile

Grape skin cell walls are generally considered to be the major source of polysaccharides in grapes because of their tight carbohydrate structure in the skin tissue (Vidal, Williams, O'Neill, & Pellerin, 2001). In addition, from a cultivation point of view, ''mg/berry'' was chosen to represent the polysaccharide concentration in the grape skin of each berry, which was calculated as ''mg/g skin'' multiplied by the skin rate (skin weight/berry weight, Table S2). Overall, a clear separation was obtained by principal component analysis (PCA) model between the native grape species in China and the *V. vinifera* species, while the points of *V. vinifera* species were clustered together (Fig. S4A). Galacturonic acid, galactose and arabinose were the major carbohydrates detected in all the grape SSP and the main polysaccharide family was pectin, accompanied by a small amount of hemicellulose (Table 2). The concentration of pectin was significantly higher in the skin of the Chinese native grape (average 0.23 mg/berry) than that of the *V. vinifera* species (average less than 0.06 mg/berry). There was no significant difference in the concentration of pectin in grape skins among *V. vinifera* varieties, except MA and ML. Considering the widespread use of pectin in the food industry due to their techno-functional and/or bioactive properties (Megías-Pérez, Ferreira-Lazarte, & Villamiel, 2023), and purified grape polysaccharides as an adjuvant in winemaking where permitted (Hensen et al., 2022; Manjón et al., 2023), native grape varieties in China, especially BBH, can be considered as a rich source of pectin raw materials to increase their commercial value. In addition, two characteristic ratios were calculated to elucidate the sugar structure of different grape SSP (Table 2). The (Ara + Gal)/Rha ratio was used to estimate the amount of the neutral side chains relative to the rhamnogalacturonan backbone, since most of the arabinose and galactose content was associated with pectin hairy regions (Apolinar-Valiente et al., 2015a; Canalejo et al., 2022). The Rha/GalA ratio was used to indicate the relative abundance of polysaccharides as homogalacturonans (HGs) versus rhamnogalacturonan-like structures (Canalejo et al., 2022). The (Ara + Gal)/Rha ratio was significantly higher in *V. vinifera* grapes, suggesting that the SSP of *V. vinifera* grapes carried more neutral lateral chains compared to the skins of native grapes in China. In addition, SH, YELIU and YEER grapes showed a significantly lower Rha/GalA ratio than most *V. vinifera* grapes (except SR). In combination with these two ratios, there may have been more "smooth region" (HGs) polysaccharides in the skins of native grapes in China, which was the main structural domain of pectin. In addition, pectin polysaccharides with high linearity and low neutral side chains are reported to have a stabilizing effect on anthocyanin color with higher binding affinity (Fernandes et al., 2021). However, this structure is easily hydrolyzed into fragments during the grape-to-wine transformation process by endogenous or exogenous added enzymes (Vidal et al., 2001). This could

Table 2
Monosaccharide composition (mg/berry) × 100 of the extracts determined by HPLC, polysaccharides families (mg/berry) × 100 and two molar ratios in grape skin of different varieties of China.

Grape varieties	Man	Rha	GlcA	GalA	Glu	Gal	Xyl	Ara	Fuc	TMS	Pectin	Hemicellulose	(Ara + Gal)/Rha	Rha/Gal A
CS	0.262 g	0.305 ef	0.288 ef	1.70 ef	0.364 e	1.18 ef	0.166 cde	0.892 fg	0.056 ab	5.21 ef	4.08 fg	0.848 gh	7.54b	0.212 ab
PV	0.388 fg	0.348 ef	0.329 ef	1.44 ef	0.482 e	1.59 de	0.207 cde	1.14 fg	0.150 a	6.07 ef	4.51 fg	1.23 gh	8.97b	0.274 a
MA	0.479f	0.429 e	0.440 e	2.43 e	0.595 e	1.78 de	0.271 cd	1.34 ef	0.154 a	7.92 e	5.98f	1.50 g	8.02 ab	0.209 ab
SR	0.422f	0.255 ef	0.358 ef	2.14 ef	0.303 e	1.27 ef	0.139 def	0.878 fg	0.123 ab	5.89 ef	4.54 fg	0.987 gh	9.20 a	0.140 bc
CF	0.389 fg	0.282 ef	0.363 ef	1.45 ef	0.374 e	1.19 ef	0.227 cde	0.958 fg	0.132 ab	5.37 ef	3.88 fg	1.12 gh	8.41 ab	0.233 a
ML	0.148	0.208f	0.209f	1.14f	0.305 e	0.923f	0.092 fg	0.633 g	0.087 ab	3.75f	2.90 g	0.632 h	8.17 ab	0.213 ab
SH	0.504f	0.997 d	0.665 d	11.0c	1.45 d	2.11 d	0.301c	1.88 d	tr	18.9 d	16.0 d	2.25f	4.42 cde	0.106c
BBH	1.34 e	2.97 a	1.32c	25.7 a	3.03c	2.72c	Tr	5.65 a	tr	42.7 a	37.0 a	4.37 d	3.23 e	0.135 bc
YELIU	3.69 a	1.75c	2.42 ab	16.4b	6.01 a	4.32 a	2.00 a	3.86b	tr	40.4 a	26.3b	11.7 a	5.18c	0.124c
YEER	3.37b	1.64c	2.27b	16.8b	3.97b	3.69b	1.74b	3.28c	tr	36.8b	25.4b	9.09b	4.70 cd	0.114c
TIAN	3.03c	2.23b	2.52 a	11.9c	3.70b	3.77 ab	tr	3.20c	tr	30.4c	21.1c	6.73c	3.45 de	0.218 ab
ZI	1.72 d	1.03 d	1.28c	8.26 d	1.53 d	1.82 de	tr	1.78 de	tr	17.4 d	12.9 e	3.26 e	3.87 cde	0.147 bc
Pooled standard deviation	0.154	0.112	0.101	0.738	0.297	0.406	0.086	0.335	0.077	1.78	1.32	0.461	0.923	0.056

CS, Cabernet Sauvignon; MA, Marselan; PV, Petit Verdot; ML, Merlot; CF, Cabernet Franc; SR, Syrah; SH, Shuanghong; BBH, Beibinghong; YELIU, Yeniang no.6; YEER, Yeniang no.2; TIAN, Tianci; ZI, Ziqiu. Man, mannose; Rha, rhamnose; Glc A, glucuronic acid; Gal A, galacturonic acid; Glu, glucose; Gal, galactose; Xyl, xylose; Ara, arabinose; Fuc, fucose.
TMS was calculated from the sum of all the monosaccharide residues. Pectin was calculated from the sum of galacturonic acid, galactose, arabinose, and rhamnose residues; Hemicellulose was calculated from the sum of xylose, fucose, glucose, and mannose residues. The (Ara + Gal)/Rha ratio was used to estimate the amount of the neutral side chains relative to the rhamnogalacturonan backbone and the Rha/GalA ratio was used to indicate the relative abundance of polysaccharides as homogalacturonans (HGs) versus rhamnogalacturonan-like structures.
Data are expressed as mean and Pooled standard deviation. Different letters in the same row represent significant differences between different grape varieties (Duncan, $P < 0.05$).
tr: trace.

explain the high level of pectin in the skins of native grapes in China, but the relatively low level that entered into the wine (Table 3).

The transformation from grape to wine induces several changes in the structure and concentration of carbohydrates, while grape-derived pectin could be hydrolyzed, leaving mainly PRAGs (estimated from the sum of galactose and arabinose residues) and APS (estimated from galacturonic acid) (Table 3). Mannose, galactose, and arabinose were the major glycosyl residues in wine TSP, with minor contributions of rhamnose and galacturonic acid, which were consistent with other studies (Apolinar-Valiente et al., 2013, 2014; Li, Bindon, Bastian,

Table 3
Monosaccharide composition (mg/L) of the extracts determined by HPLC and polysaccharides families (mg/L) in red wines of different grape varieties of China.

Grape varieties	Man	Rha	GlcA	GalA	Glu	Gal	Xyl	Ara	Fuc	TMS	PRAGs
CS	142 bc	32.8 e	16.6 abcd	39.6 cd	47.0b	84.7b	3.06 ab	65.9 a	5.50 bc	437 cd	151b
PV	190b	35.3 de	21.2 abc	43.7 cd	57.1b	100b	3.02 ab	59.8 a	5.62 bc	516 bcd	160 ab
MA	169 bc	35.3 de	20.1 abcd	45.2 cd	52.4b	98.6b	3.39 a	58.0 a	5.17c	487 bcd	157b
SR	158 bc	27.4 e	11.1 d	29.7 d	54.1b	82.4b	3.51 a	59.8 a	5.44 bc	431 cd	142b
CF	146 bc	39.1 cde	17.0 abcd	50.0 bcd	56.7b	90.3b	5.07 a	52.0 a	8.19 abc	465 bcd	142b
ML	170 bc	41.7 cde	19.8 abcd	56.7 abc	47.3b	90.4b	4.20 a	54.0 a	7.95 abc	492 bcd	144b
SH	128c	35.9 de	13.2 bcd	38.0 cd	48.9b	92.2b	3.83 a	57.9 a	10.9 ab	381 d	134b
SY	152 bc	34.2 de	12.7 cd	30.4 d	57.3b	96.4b	3.56 a	58.7 a	8.98 abc	427 cd	142b
BBH	298 a	56.6 ab	22.9 ab	68.6 ab	47.4b	139 a	tr	88.8 a	13.2 a	734 a	228 a
YEER	148 bc	69.7 a	24.4 a	77.2 a	75.7 a	103b	tr	88.7 a	5.05c	592b	192 ab
TIAN	123c	48.8 bcd	18.1 abcd	51.7 bcd	45.0b	108 ab	2.35 ab	70.1 a	7.23 bc	475 bcd	178 ab
ZI	153 bc	51.9 bc	18.8 abcd	59.4 abc	52.0b	108 ab	4.04 a	82.8 a	13.0 a	543 bc	191 ab
Pooled standard deviation	34.6	10.3	6.36	15.0	12.3	22.2	2.24	29.2	3.60	94.2	46.9

CS, Cabernet Sauvignon; MA, Marselan; PV, Petit Verdot; ML, Merlot; CF, Cabernet Franc; SR, Syrah; SH, Shuanghong; BBH, Beibinghong; YELIU, Yeniang no.6; YEER, Yeniang no.2; TIAN, Tianci; ZI, Ziqiu. Man, mannose; Rha, rhamnose; Glc A, glucuronic acid; Gal A, galacturonic acid; Glu, glucose; Gal, galactose; Xyl, xylose; Ara, arabinose; Fuc, fucose.
TMS was calculated from the sum of all the monosaccharide residues. MPs was estimated from mannose residue; PRAGs were estimated from the sum of arabinose and galactose residues; APS was estimated from the main acid monosaccharide, galacturonic acid.
Data are expressed as mean and Pooled standard deviation. Different letters in the same row represent significant differences between different grape varieties (Duncan, $P < 0.05$).
tr: trace.

Jiranek, & Wilkinson, 2017). The reduced galacturonic acid proportion was attributed to the hydrolysis effects of endogenous or exogenous enzymes, while an increased mannose proportion indicated the release of yeast MPs during winemaking (Guadalupe & Ayestaran, 2008; Vidal et al., 2001). In contrast to the results obtained for the grape SSP, the concentration difference of carbohydrates between native grapes in China and *V. vinifera* grapes was smaller in wine TSP (Different wine samples could not be better separated by the PCA model, Fig. S4B). Among the native grape varieties in China, the concentration of PRAGs in BBH wine and APS in YEER wine were significantly higher than those in most of *V. vinifera* varieties. There was no significant difference of concentration among *V. vinifera* varieties in all the carbohydrates (except APS between ML and SR). In general, the concentration of wine polysaccharides depends not only on the grape variety, but may also be affected by other factors (such as the resistance of different composition and structure of grape polysaccharides to winemaking techniques) that influence the transformation of grape pectin during wine production.

Effect of grape regions on wine polysaccharidic profile

The soluble polysaccharide profile of grape skins and wines varied according to grape origin. The five main wine origins in this study have a wide range of longitude from XJMS to SDPL (from 86°E to 120°E), which contributes to the great distinction in *terroir*. The eastern regions with lower altitudes, have higher temperatures and humidity, while the western regions with higher altitudes far from the sea have lower average temperatures and humidity (Liang et al., 2014). These differences may affect the biosynthesis of grape polysaccharides possibly related to the different berry size and skin thickness. However, the PCA model showed that the five grape origins could not be well separated, indicating that the differences were not significant (Fig. S5). Specifically, the concentration of pectin and hemicellulose of CS were significantly higher in NXYC than in other origins, while the PV concentration was significantly lower in SDPL. However, there was no significant difference in the pectin concentration of MA among different origins (Fig. 1A, B, C). Apolinar-Valiente et al., (2013,2015b) found differences in the skin cell wall material of Monastrell grapes from different origins in the southeastern Spain, suggesting a possible *terroir* effect on grape polysaccharide composition. Although little literature has analyzed the underlying relationship between environmental factors and grape skin polysaccharide characteristics, the diversity of climatic conditions could affect the accumulation of soluble polysaccharides in grape skins by influencing berry size or skin texture characteristics. For example, water deficit generally decreases grape berry size and increases skin thickness, resulting in a higher skin/pulp ratio and influencing the polysaccharide profile in grape berries (Zsofi, Palfi, & Villango, 2021).

The polysaccharide profile in CS, PV and MA wines was not completely consistent with the distribution trend of the grape skins (Fig. 1D, E, F). With regard to grape-derived polysaccharides, the PRAGs concentration of the three varieties in NXYC was similar to that in SDPL and HBCL, but significantly lower than that in HBHL and XJMS (except CS). In addition, they all showed significantly higher APS concentration in XJMS, whereas there was no significant difference among the other four origins. The changes in polysaccharide distribution indicated that the transformation from berry to wine is complicated and influenced by many factors, in particular PRAGs concentration is susceptible.

Effect of enological techniques on wine polysaccharide profile and sensory compounds

Polysaccharide profile

Fig. 2 showed the concentration of TMS, MPs, PRAGs and APS of wine samples with different treatments. The polysaccharide content released by the addition of enzyme supplement (E) was similar to that of control (C) at the end of alcoholic fermentation (Fig. 2A), however, as winemaking progressed, the two groups tended to differ significantly. Analyses of the polysaccharides of enzyme-treated group (E) at the end of malolactic fermentation showed a significant decrease in PRAGs (about 60 %) and APS (about 51 %) compared to control wine (C), indicating an extensive degradation of pectic polysaccharides of HGs and PRAGs (or possibly other arabinose-rich polymers) in the grape cell wall (Fig. 2C) (Ducasse et al., 2010; Guadalupe et al., 2007; Li, Bindon, Bastian, Jiranek, & Wilkinson, 2017b). The results were similar with some studies (Kassara et al., 2019; Li et al., 2017b), which observed that

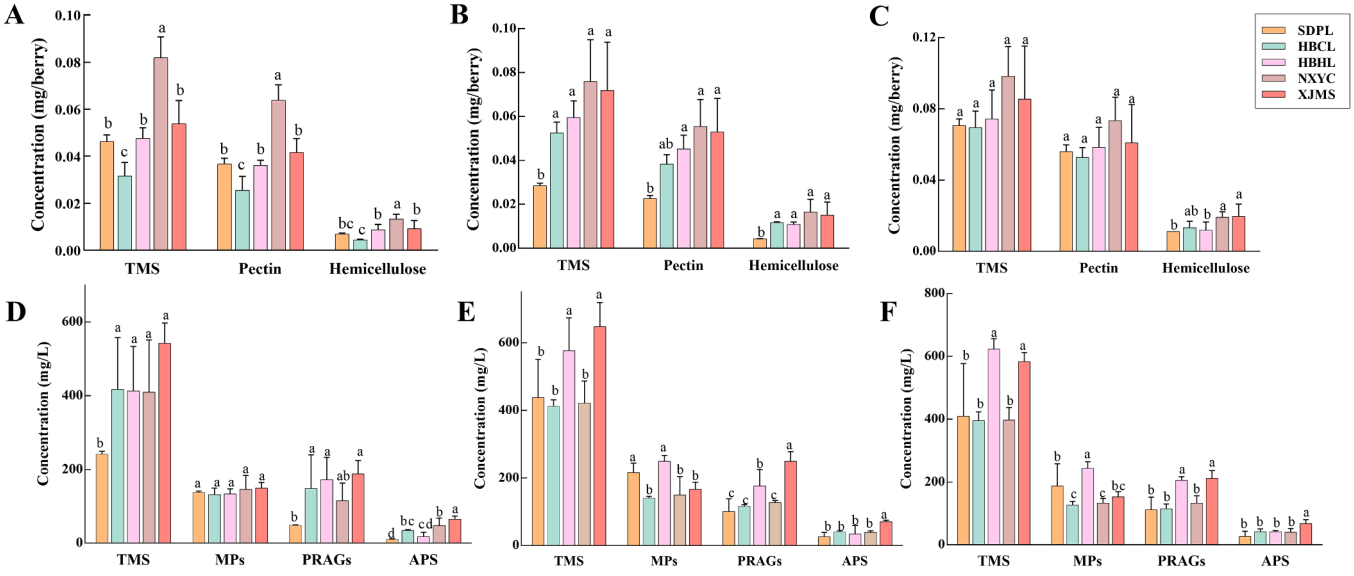

Fig. 1. The polysaccharide content in grape skins and red wines of Cabernet Sauvignon (A and D), Petit Verdot (B and E), and Marselan (C and F) from five major wine regions in China. Pectin was calculated from the sum of galacturonic acid, galactose, arabinose, and rhamnose residues; Hemicellulose was calculated from the sum of xylose, fucose, glucose, and mannose residues; MPs was estimated from mannose residue; PRAGs were estimated from the sum of arabinose and galactose residues; APS was estimated from the main acid monosaccharide, galacturonic acid. TMS was calculated from the sum of all the monosaccharide residues. SDPL, Penglai, Shandong; HBCL, Changli, Hebei; HBHL, Huailai, Hebei; NXYC, Yinchuan, Ningxia; XJMS, Manasi, Xinjiang. Data are expressed as mean ± SD (n = 3) *Different letters represent significant differences in the data between different grape regions (Duncan, $P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

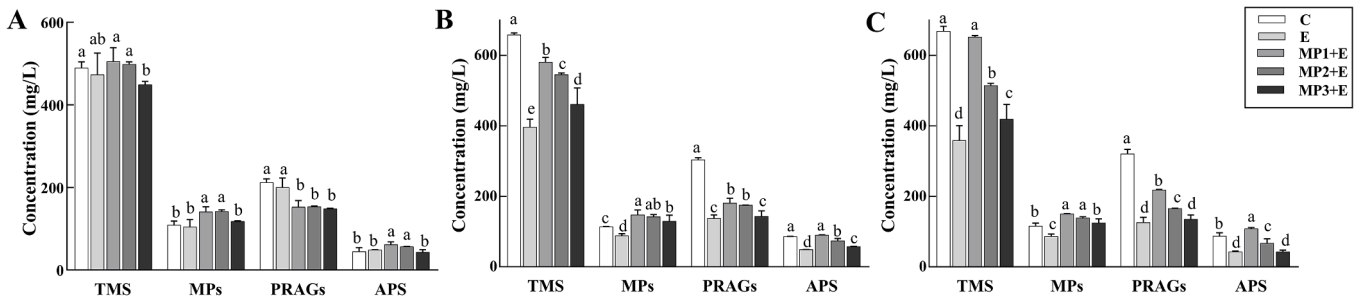

Fig. 2. The polysaccharide content in wines with different treatments at the end of alcoholic fermentation (A); after post-fermentative maceration (B); at the end of malolactic fermentation (C). TMS was calculated from the sum of all the monosaccharide residues. MPs was estimated from mannose residue; PRAGs were estimated from the sum of arabinose and galactose residues; APS was estimated from the main acid monosaccharide, galacturonic acid. C, control, no addition of enzyme and MPs; E, enzyme preparation added only during maceration; MP1 + E, MP2 + E, and MP3 + E mean the treatments of enzyme preparation added during maceration and addition of commercial MPs at the beginning, middle, and end of acholic fermentation, respectively. Data are expressed as mean ± SD (n = 3) *Different letters represent significant differences in the data between different treatments (Duncan, $P < 0.05$).

arabinose-rich polysaccharides and galacturonic acid were reduced in enzyme-treated wines. The above results indicate that the presence of enzymes led to an increased extraction of pectin from the red grape skin. However, this was accompanied by further degradation, resulting in reduced levels of arabinose, galactose, and galacturonic acid. In addition, the arabinose/galactose (Ara/Gal) ratio has been used as a characteristic of wine PRAGs, and higher values of this ratio indicate higher levels of arabinose or arabinose-rich structures (such as AGPs and arabinans) (Apolinar-Valiente et al., 2013; Canalejo et al., 2022). At the end of alcoholic fermentation, the Ara/Gal ratio of the enzyme-treated wine was extremely low (0.66) compared to the control wine (1.20), indicating a change in the total PRAGs composition of the wine (calculated by the molar ratio of Ara and Gal, data not shown). The results were consistent with Doco et al. (2007) who also found a dramatic decrease in PRAGs and the Ara/Gal ratio in enzyme-treated wines. Unexpectedly, the MPs content was also slightly lower in the enzyme-treated wine compared to the control wine (Fig. 2C), whereas other studies found no significant effect of enzyme treatment (Doco et al., 2007; Ducasse et al., 2010) or opposite results (Kassara et al., 2019; Li et al., 2017b). Kassara et al. (2019) claimed that pectinase may induce changes in the wine matrix that affect yeast metabolism and facilitate the release of MPs, but the influencing mechanism needs further investigation. The above conflicting results may be due to interactions between different polysaccharides, and the varying composition of the enzyme products may lead to a different colloidal state of the wine.

Compared to the E group (the concentration of MPs was 86.5 mg/L), the addition of MP supplement at different stages during malolactic fermentation (MP1 + E, MP2 + E and MP3 + E groups) increased the content of MPs in wines, and the earlier the addition stage (MP1 + E) (150 mg/L), the more conducive to the accumulation of MPs (Fig. 2C). The release of yeast polysaccharides continued throughout the alcoholic fermentation and reached its maximum at the end of alcoholic fermentation, resulting in a large accumulation of MPs (Guadalupe & Ayestaran, 2008). Therefore, when exogenous MPs was added at this time (MP3 + E), it would immediately cause the precipitation of co-aggregates of MPs or mannoprotein-tannin complexes (Guadalupe & Ayestaran, 2008), resulting in the lower content of MPs compared to the other two groups (MP1 + E and MP2 + E) (Fig. 2A). Finally, the content of MPs was relatively stable in the later stage, and this trend was maintained at the end of malolactic fermentation (Fig. 2C).

When considering the combination of the addition of both enzyme and MP supplements, there was a noticeable regulation of the wine polysaccharide composition (especially MP1 + E groups) compared to the C groups, i.e. to increase the MPs and APS content, but reduce the content of PRAGs. However, when compared with the single enzyme treatment (E), the MP1 + E group had higher PRAGs content, indicating that MP1 + E treatment could reduce the loss of PRAGs caused by enzyme degradation (Fig. 2C). Many studies have reported the improved effects of commercial enzyme products on wine quality due to their accelerated extraction of phenolic compounds in a relatively short maceration time (Ducasse et al., 2010; Guadalupe et al., 2007; Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2012). Nevertheless, wine polysaccharides have been extremely focused on due to their influence on the colloidal state of wine and their interaction with other compounds in the wine system (Brandão et al., 2017; Fernandes et al., 2021). The modification of their structure by the winemaking process is likely to have a direct impact on their properties and, consequently on wine quality (Doco et al., 2007). Considering the widespread use of enzymes and the underlying role of wine polysaccharides, the combination of both industrial mannoproteins and enzymes could be considered as a potential application in the red wine maceration process to regulate the wine polysaccharide composition. In addition, the techniques applied will also lead to changes in the phenolic and volatile composition of wines, which may be related to the change in polysaccharide profile and wine matrix composition caused by the techniques.

Color parameters and phenolic composition of wines

The contents of the main phenolic compounds and color parameters of the wines with different treatments are shown in Table 4 and they can be well separated by PCA model (Fig. S6). Group E and MP1 + E are clustered on the left-hand side of the plot, while the other three groups are clustered on the right-hand side. Group E exhibited higher a^* values and lower $L^*$and b^* values than all the other groups (except for the similar b^* value with group C), indicating a better color quality (higher color intensity with more reddish and purplish characteristics). In addition, the combination group of MP1 + E showed higher a^* value and lower L^* value than the group C. Enzyme maceration has been widely reported as a valuable tool in winemaking to increase the color intensity of wine (Ducasse et al., 2010; Romero-Cascales et al., 2012), whereas the effects of MPs addition on wine color characteristics were not consistent, possibly related to the specific composition (different mannan to protein ratio) of commercial MP products and different wine matrix conditions (Alcalde-Eon et al., 2014; Alcalde-Eon, Perez-Mestre et al., 2019; Guadalupe et al., 2007; Zhai et al., 2023).

Unexpectedly, the content of phenolic compounds was not highest in the enzyme-treated wines (Table 4), which was inconsistent with the results that enzyme-maceration increased the extraction of phenolic compounds in most of the literatures (Romero-Cascales et al., 2012; Li et al., 2017b). However, some studies also reported that there was no significance in the total polyphenols index and color intensity between enzyme-treated samples and control wines (Apolinar-Valiente et al., 2013) or the lower content of monomeric anthocyanins in enzyme-treated samples (Parley et al., 2001). Therefore, it seems that in some cases the commercial enzyme was not sufficient to release phenolic compounds, possibly due to the grape skin structure or the composition

Table 4
CIELab parameters and the concentration of major categories of phenolic compounds (mg/L) and volatile compounds (μg/L) of wines with different treatments at the end of malolactic fermentation.

	C	E	MP1 + E	MP2 + E	MP3 + E	Pooled standard deviation
CIELab parameters						
*L**	57.8c	47.5 e	53.2 d	66.1 a	65.1b	0.316
*a**	34.8c	43.7 a	40.1b	29.7 e	31.2 d	0.249
*b**	13.5 d	13.8 d	14.0c	14.6b	16.1 a	0.145
Monomeric anthocyanins						
Total non-acylated	139 a	100 d	106c	137 a	120b	1.576
Total acylated	86.6c	61.9 e	81.3 d	103 a	94.1b	0.364
Total monomeric anthocyanins	226b	162 e	188 d	240 a	214c	1.378
Anthocyanin derivatives						
A-F/F-A/A-e-F	4.50 d	7.02 a	6.69b	5.22c	4.43 d	0.088
A-v-F	0.794 e	1.27 a	1.16b	1.00c	0.839 d	0.009
Vitisins	10.5 d	32.5 a	20.7b	13.1c	10.5 d	0.404
Pinotins	4.05c	4.97 a	4.14b	4.05c	2.91 d	0.036
Total anthocyanin derivatives	20.3 d	46.2 a	33.2b	23.9c	19.2 e	0.502
Non-anthocyanin phenolics						
Flavan-3-ols	132 a	124b	127b	117c	123b	1.984
Flavonols	48.2b	49.6 a	43.8c	33.1 e	41.2 d	0.529
Hydroxycinnamic acids	3.46 a	1.79b	1.45 d	1.60c	0.954 e	0.032
Hydroxybenzoic acids	22.6 d	23.9b	23.0c	25.1 a	21.5 e	0.187
Total non-anthocyanin phenolics	206 a	199b	195c	177 e	187 d	2.481
Volatile compounds						
C6/C9 alcohols	3029b	2607c	3294 a	2991b	3222 a	109
Higher alcohols	597,261 a	601,985 a	624,426 a	645,146 a	619,265 a	38,277
Aromatic compounds	135,304 a	62,953b	157,550 a	86,660b	175,154 a	31,842
Acids	11,167b	6609b	9611 ab	6860b	11,811 a	2799
Terpenes & norisoprenoids	8.04 a	4.58c	6.36b	4.84c	8.34 a	0.943
Acetate esters	573 ab	546b	602 a	552b	559 ab	34.9
Ethyl esters	2043 a	1574 bc	1723 bc	1551c	1798b	178

C, control, no addition of enzyme and MPs; E, enzyme preparation added only during maceration; MP1 + E, MP2 + E, and MP3 + E mean the treatments of enzyme preparation added during maceration and addition of commercial MPs at the beginning, middle, and end of acholic fermentation, respectively.
A-F/F-A/A-e-F represent different forms of anthocyanin-flavan-3-ol condensation products. A-v-F, Flavanyl-pyranoanthocyanins.
The specific data of the contents of relevant phenolic and volatile compounds in each category is shown in Table S5 and S6.
Data are expressed as mean and pooled standard deviation (n = 3). Different letters in row represent significant differences in data between different treatments (Duncan, $P < 0.05$).

of the enzymatic products. In Table 4, the levels of total monomeric anthocyanins, flavan-3-ols, and hydroxycinnamic acids were significantly lower in the group E than in the group C. However, the presence of higher contents of anthocyanin derivatives, flavonols, and hydroxybenzoic acids in the group E indicated more stable pigments and the occurrence of copigmentation effect in wine (Zhao, He, Zhang, Shi, & Duan, 2022). The contradiction between the better color quality exhibition and the lower concentration of monomeric anthocyanins in the enzyme-treated wines, possibly indicates that the enzyme treatment resulted in a higher anthocyanin extraction followed by their conversion into other pigments. The decrease in monomeric anthocyanins, flavan-3-ols and hydroxycinnamic acids may be related to the formation of anthocyanins derivatives (Table 4), which have been reported to be crucial for the long-term color expression of red wine (Zhang et al., 2022). Ducasse et al. (2010) also observed similar results and suggested that wine color properties are not related to anthocyanin concentration but depend on the proportions of anthocyanins derivatives and the copigmentation phenomenon. In addition, the color stability of anthocyanin solutions is reduced in the presence of flavan-3-ol monomers in the long-term progress but increased in the presence of flavonol copigments (Zhao et al., 2022).

The addition of MPs at different stages of alcoholic fermentation was beneficial to increase the content of monomeric anthocyanins ($P < 0.05$), but showed negative effects on anthocyanin derivatives and total non-anthocyanin phenolics ($P < 0.05$) compared to the E group (Table 4). The results were contrary to those of Alcalde-Eon et al. (2014), who observed higher anthocyanin derivatives in wine possibly from the colloidal point of view of MPs. Furthermore, among the different addition stages of MPs, the earlier addition (MP1 + E) seemed to benefit the synthesis of anthocyanin derivatives and the accumulation of non-anthocyanin phenolics compared to MP2 + E and MP3 + E. With the gradual accumulation of polysaccharide content in the late period of alcoholic fermentation, the phenomenon of co-aggregates could occur, resulting in a decrease in the synthesis of anthocyanin derivatives or the precipitation of phenolic compounds. The protective effect of MPs on wine colloidal matters is ambiguous and some studies find a decreased phenomenon of anthocyanins and tannins content (Li et al., 2017b; Guadalupe & Ayestaran, 2008). The concentration and composition of polysaccharides in wine are crucial for their effect on wine quality, as they can destabilize the colloids and cause co-aggregation with phenolic colloidal matters (Zhai et al., 2023). When comparing the MP1 + E group with the C group, the former had higher levels of anthocyanin derivatives, but lower levels of monomeric anthocyanins and flavan-3-ols.

Volatile compounds

The major volatile compounds detected in the different wines were classified into seven categories according to their structural characteristics (Table 4). The addition of MPs at different stages of alcoholic fermentation was able to maintain or even increase the concentrations of all the volatile categories compared to the E group to varying degrees, especially on C6/C9 alcohols, aromatic compounds, terpenes and

norisoprenoids. Contrarily, the content of ethyl lactate was significantly lower in all the groups with the addition of MPs than that in the E group (Table S6). Guadalupe et al. (2007) found that there was retention effect of MPs on the volatility of fruity, floral, and green aromas in Tempranillo wines, but there was no detection data of volatile compounds. It has been suggested that MPs may modify the volatility of aroma compounds and improve aroma revelation through a retention or salting-out effect, depending on the hydrophobicity and content of both volatile and polysaccharide compounds (Li et al., 2021; Jones-Moore et al., 2022; Zhai et al., 2023). However, the enzyme-treated wines showed lower levels in all volatile categories except higher alcohols, possibly due to the enzymatic degradation of wine polysaccharides which have reduced their retention effect on volatile compounds (Jones-Moore et al., 2022). It was possibly due to the composition difference of the enzymatic products, especially the type and proportion of glycosidases. Interestingly, the combination of enzyme preparation and MPs addition (MP1 + E) significantly increased the contents of C6/C9 alcohols to the C group, while reducing the loss of terpenes, norisoprenoids and ethyl esters caused by enzyme treatment (Table 4). Further studies will focus on the influence of different types of wine polysaccharides on volatile compounds and their possible interactions.

Conclusion

The polysaccharide profile is critical for its influence on wine quality and there are no systematic studies on the polysaccharide profile and its influencing factors of red wines in China. In general, this work has shown that enological techniques possibly have a much greater impact on the wine polysaccharide profile than grape raw material. The contents of pectin and hemicellulose in the grape skin of native grape species in China were significantly higher than those of *V. vinifera* grapes, which could be considered as a rich source of pectin raw materials to increase their commercial value in the food industry. However, the difference of polysaccharide profile of grapes decreased in wine and there was no significant difference in the polysaccharide content of grape skins and wines for most *V. vinifera* varieties, and the *terroir* trend from western to eastern origins of China was not fully consistent. The combination of both enzyme preparation and MPs addition (added at the beginning of alcoholic fermentation) could increase the content of MPs and APS compared to the control wines. Meanwhile the loss of PRAGs content in wines caused by enzyme treatment was reduced, and better color properties and higher levels of anthocyanin derivatives were observed ($P < 0.05$). The results indicate that the combination of winemaking techniques is important for improving wine quality, and can compensate for the shortcomings of any single process. In addition, the enological techniques used can greatly influence the transformation of carbohydrates from grape to wine, resulting in changes in the wine polysaccharide profile. This study will provide useful information for the winemakers to obtain different characteristics of wines modulated by the polysaccharide profile by using different grape raw materials and winemaking techniques. Further studies will focus on the interaction mechanism between wine polysaccharides and other wine components to reveal their actual effects on wine quality and to directly regulate the polysaccharide profile of wines.

CRediT authorship contribution statement

Hongyue Zhai: Writing – original draft, Investigation, Data curation, Conceptualization. **Mengqi Ling:** Writing – review & editing, Investigation. **Siyu Li:** Writing – review & editing, Supervision, Investigation. **Bainian Chen:** Investigation, Formal analysis. **Xu Zhao:** Writing – review & editing, Validation. **Wenzhe Tong:** Writing – review & editing, Validation. **Chifang Cheng:** Validation, Resources. **Jin Li:** Validation, Resources. **Ying Shi:** Writing – review & editing, Validation, Supervision. **Changqing Duan:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Yibin Lan:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101283.

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