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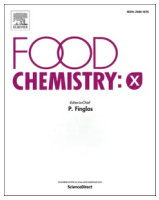

Enhancing the color and astringency of red wines through white grape seeds addition: Repurposing wine production byproducts

Nong-Yu Xia [a,b], Ao-Yi Liu [a,b], Meng-Yao Qi [a,b], Hua-Lin Zhang [a,b], Yong-Ce Huang [a,b], Fei He [a,b], Chang-Qing Duan [a,b], Qiu-Hong Pan [a,b,*]

[a] Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China
[b] Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China

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ABSTRACT

The clear juice fermentation technique for white wines suggests that white grape seeds, rich in flavan-3-ols and proanthocyanidins, are not effectively utilized in the winemaking process. This study incorporated 'Gewürztraminer' grape seeds into 'Cabernet Sauvignon' must before cold soak to investigate how the resultant red wines' phenolic compound profiles, color, and astringency were affected. The results showed that adding seeds primarily inhibited the leaching of flavan-3-ols from both skins and seeds. A significant increase in the levels of flavan-3-ols, tannins, and phenolic acids, as well as direct and aldehyde-bridged flavan-3-ol-anthocyanin polymers, were observed in the wines with additional seeds. This led to the improvement in the wine' red hue and its resistance to SO_2 bleaching. Furthermore, the wine added with seeds exhibited stronger astringency compared to those without. The findings provide a promising winemaking strategy to improve color stability and intensify the astringency of red wines through the utilization of grape seeds.

1. Introduction

The color and astringency of red wine are crucial quality characteristics that are primarily influenced by phenolic compounds present in wine (Casassa & Harbertson, 2014; de Freitas & Mateus, 2011; Qi et al., 2023; Zhang et al., 2022). Among these phenolic compounds, anthocyanins represent the principal coloring substances. However, monomeric non-acylated and acylated anthocyanins derived from grapes are highly unstable and gradually decrease until they disappear during wine fermentation and aging stages (Alcalde-Eon et al., 2006). The color of aged wine is primarily influenced by anthocyanin derivatives that are produced during both the fermentation and aging processes. These derivatives consist mainly of pyranoanthocyanins and polymeric pigments (Zhang et al., 2022). The polymeric pigments present in wine include anthocyanin-flavan-3-ols (A-F type), flavan-3-ols-anthocyanin (F-A type), anthocyanin ethyl-linked flavan-3-ols (A-e-F type) and anthocyanin ethyl-linked anthocyanins (A-e-A type), as identified by Zhang et al. (2021). The A-F and F-A types are considered direct-linked pigments, while the A-e-F and A-e-A types are considered bridge-linked. Anthocyanins, flavan-3-ols, tannins, and hydroxycinnamic acid found in

Abbreviations: EGC, (−) -epigallocatechin; C, (+) -catechin; EC, (−) -epicatechin; ECG, (−)-epicatechin gallate; GC, (+)-gallocatechin; Cy-glc, Cyanidin-3-*O*-glucoside; Cy-ac-glc, Cyanidin-3-*O*-acetylglucoside; Pe-glc, Peonidin-3-*O*-glucoside; Pe-ac-glc, Peonidin-3-*O*-acetyl glucoside; Pe-co-glc, Peonidin-3-*O*-coumaroyl glucoside; Dp-glc, Delphinidin-3-*O*-glucoside; Dp-ac-glc, Delphinidin-3-*O*-acetyl glucoside; Pt-glc, Petunidin-3-*O*-glucoside; Pt-ac-glc, Petunidin-3-*O*-acetyl glucoside; Pt-co-glc, Petunidin-3-*O*-coumaroyl glucoside; Mv-glc, Malvidin-3-*O*-glucoside; Mv-ac-glc, Malvidin-3-*O*-acetyl glucoside; Mv-co-glc, Malvidin-3-*O*-coumaroyl glucoside; (E)cat-Mv, (Epi)catechin-malvidin-3-*O*-glucoside; (E)cat-MvA, (Epi)catechin-malvidin-3-*O*-acetyl glucoside; Mv-e-di(e)cat, Malvidin-3-*O*-glucoside-ethyl-di(epi)catechin; Mv-e-(e)cat, Malvidin-3-*O*-glucoside-ethyl-(epi)catechin; MvC-e-(e)cat, Malvidin-3-*O*-coumaroyl glucoside-ethyl-(epi)catechin; MvA-e-(e)cat, Malvidin-3-*O*-acetyl glucoside-ethyl-(epi)catechin; Mv-py acid, Malvidin-3-*O*-glucoside-pyruvic acid; MvC-py acid, Malvidin-3-*O*-coumaroyl glucoside-pyruvic acid; MvA-py acid, Malvidin-3-*O*-acetyl glucoside-pyruvic acid; Mv-aldehyde, Malvidin-3-*O*-glucoside-acetaldehyde; MvA-aldehyde, Malvidin-3-*O*-acetyl glucoside-acetaldehyde; MvC-vpol, Malvidin-3-*O*-coumaroyl glucoside-4-vinylphenol; Mv-vcol, Malvidin-3-*O*-glucoside-4-vinylcatechol; Mv-vpol, Malvidin-3-*O*-glucoside-4-vinylphenol; Mv-vgol, Malvidin-3-*O*-glucoside-4-vinylguaiacol; MvA-vpol, Malvidin-3-*O*-acetyl glucoside-4-vinylphenol; MvA-vgol, Malvidin-3-*O*-acetyl glucoside-4-vinylguaiacol.

* Corresponding author at: Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China

E-mail address: panqh@cau.edu.cn (Q.-H. Pan).

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grapes all contribute to the formation of anthocyanin derivatives, which possess red or purple-red hues (Escribano-Bailon & Santos-Buelga, 2012). Their high levels are significant for stabilizing the color of red wine (Zhang et al., 2022). One current area of research in the field of wine color is how to enhance the production of consistent wine pigments.

Flavan-3-ols and condensed tannins present in grape seeds are critical in the co-pigmentation process for wine color (Rousserie et al., 2019). The mean degree of polymerization (DP) of proanthocyanidins (PA) in grape seeds varies from 2.3 to 30.3, while that of PA in grape skins ranges from 2.1 to 85.7, with a higher degree of polymerization (Rousserie et al., 2019). Bindon et al. (2014) revealed that tannins with low molecular weight decreased during the late stage of wine fermentation while stable wine pigments increased. This suggests that a lower DP of PA facilitates interaction with anthocyanins, leading to a more stable wine hue. Seed tannins, being a rich source of low-degree polymerized proanthocyanidins, can act as high-quality copigments in wines and encourage the production of polymeric pigments. Thus, the appropriate incorporation of grape seeds during the fermentation process becomes a desirable technique to enhance the stability of wine color. Despite exhibiting a lower extraction rate in comparison to skin tannin, the tannins from grape seeds still represent a higher proportion in wine due to their abundant content (Gombau et al., 2020).

Regarding astringency, it is commonly held that the sensation of dryness and puckering in the mouth is caused by the interaction between oral proteins and phenolic compounds found in wines. To determine the level of astringency, tannins and artificial salivary proteins can be employed for testing (Qi et al., 2023). Most hypotheses suggest that this is achieved by altering salivary lubrication, activating the mechanoreceptors within the oral mucosa, or through tannin interacting with oral epithelial cells (González-Muñoz et al., 2022). Although flavonol components such as quercetin-3-*O*-glucoside (Ferrer-Gallego et al., 2016), syringetin-3-*O*-glucoside, and quercetin-3-*O*-rutinoside (Casassa & Harbertson, 2014), as well as monomeric anthocyanins (González-Muñoz et al., 2022) have been reported a positive association with astringency, proanthocyanidins are widely acknowledged as determinants of astringency in wine (Casassa & Harbertson, 2014; González-Muñoz et al., 2022; Qi et al., 2023). Furthermore, flavan-3-ols and tannins, which were predominantly derived from grape seeds, are also widely known to contribute to the bitterness of wines (Li & Duan, 2019). In summary, flavan-3-ols and tannins play a crucial role in the astringency and bitterness of wines.

Based on the plasticity of phenolic compounds in grapes and wines, different cultivation and vinification techniques have been applied to improve wine's color and astringency characteristics by altering the phenolic profile of wines (Alcalde-Eon et al., 2019; Ferrer-Gallego et al., 2016; Gordillo et al., 2021; Kovac et al., 1995; Lu et al., 2022; Lurton et al., 2002; Smith et al., 2015). Researchers have been investigating the effects of adding grape seeds and grape tannin on phenolic compounds and color representation of wine for the past three decades based on the fact that seeds are the primary source of flavan-3-ols and tannins in wines (Kovac et al., 1995; Lurton et al., 2002). Mature seeds are generally preferred over immature seeds due to the lower level of bitterness compounds such as low polymeric tannins and flavan-3-ol monomers like epigallocatechin gallate (Liu et al., 2023). Kovac et al. (1995) investigated the effects of adding varying amounts of mature grape seeds on the concentrations of phenolic compounds, such as catechins, proanthocyanidins, and anthocyanins, in the wines of different red grape varieties. Subsequently, some scientists investigated the effects of pre-fermentative (Rivero et al., 2017) and post-fermentative (Alcalde-Eon et al., 2019; Gordillo et al., 2021; Rivero et al., 2019) addition of seeds from *Vitis vinifera* L. cv. Pedro Ximénez grapes in the 'Syrah' wines on the phenol compounds (flavan-3-ols, flavonols, procyanidins, anthocyanins, and phenolic acids) and the color parameters (CIELab) at different sampling points during and after the maceration. Among them, the differences between single and double seed addition (Rivero et al., 2019), overripe grape seeds (43°Brix) and mannoproteins (Alcalde-Eon et al., 2019), ripe grape seeds (16°Bé) and overripe grapes seeds (23°Bé) (Gordillo et al., 2021) were investigated. All of these research reported that adding seeds whether pre- or post-fermentative could lead to the increase of flavan-3-ols and procyanidins concentrations, as well as a positive impact on the wine color (such as darker color). However, the knowledge about the effect of adding seeds on the color and astringency of sensory evaluation is still limited. Furthermore, at the harvest stage in most wine regions in China, the total soluble solids content of white grapes is much lower than the overripe grapes mentioned above. In the wine production in the winery, the wines are separated from the pomace at the end of alcoholic fermentation. Therefore, the post-fermentative addition of seeds adds extra steps and undoubtedly makes the enology procedure more complicated and thus increases production costs. Additionally, it remains unclear whether the changes in wines solely resulted from the leaching of phenolic compounds from external seeds or if it also concurrently changed the leaching of these compounds in the red grape materials.

In China, red wines commonly face challenges such as a relatively thin and less persistent taste structure, as well as rapid fading of red-purple color. For white wines, they were fermented from the clear juice of white grapes, which is separated from skins and seeds. In the scope of the white winemaking process, high amounts of pomace are generated, which are the major waste from vinification (Besrukow et al., 2022). Meanwhile, estimates from the China Alcoholic Drinks Association indicate that roughly 3,000,000 tons of white wine are produced annually in China, potentially resulting in 720,000 tons of pomace. These highly polyphenolic pomaces serve as valuable sources of polyphenols that can be utilized. It is hypothesized that adding seeds of 'Gewürztraminer' grapes could be an effective way to improve the color stability and astringency intensity of red wine. Therefore, in this study, white grape 'Gewürztraminer' seeds were added during the pre-fermentation stage of 'Cabernet Sauvignon' wine, and different maceration duration was designed. Our research findings will elucidate the leaching patterns of exogenous seed tannins and their impact on the leaching of phenolic compounds from wine grapes themselves, as well as the impact on phenolic profiles, color parameters and stability, and sensory characteristics (taste and color) of the red wines during the fermentation and bottle storage. This study also allows for a clear understanding of which compound changes affect the final color and astringency of wine. Consequently, it provides an effective solution for the utilization of white grape seeds and the improvement of color and astringency in red wine without incurring additional costs, thus increasing product profitability and economic benefits.

2. Materials and methods

2.1. Chemicals and standards

Analytical-grade chemicals including acetone, sodium bisulfite, methylcellulose, sulfurous acid, ethylenediaminetetraacetic acid (EDTA), ethyl ether, L-ascorbic acid, L (+)-tartaric acid and lactoferrin were purchased from Beijing Lanyi Chemical Factory (Beijing, China). Lysozyme and bovine serum albumin were purchased from Sangon Biotech (Shanghai, China). Folin-Ciocalteu reagent was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). 4-Dimethylaminocinnamaldehyde (DMACA), poly-L-proline, and α-amylase, as well as HPLC grade phenolic standards, were purchased from Sigma Aldrich Chemical Co. (St Louis, MO, USA). HPLC-grade trifluoroacetate and acetic acid were purchased from Tedia (Ohio, USA), and formic acid was purchased from Roe Scientific Inc. (Newark, NJ, USA). Methanol and acetonitrile were purchased from Honeywell Burdick & Jackson (Morris Plains, NJ, USA). Precast-Gel (12%) was purchased from Solarbio Science & Technology Co., ltd. (Beijing, China).

2.2. Grape and seed materials

The clusters of *Vitis vinifera* L. cv. Cabernet Sauvignon (CS) were harvested from the Zhuozhou experimental station (39°28′N, 115°51′E) of China Agricultural University in 2022. The experimental site belongs to a temperate continental monsoon climate and the soil type is sandy loam. The own-rooted 'Cabernet Sauvignon' vines for the experiment were planted in 2018 and were trained to a uniformly modified vertical shoot positioning (M-VSP) system. The vineyard was oriented north-south with row and vine spacing 3 × 1 m.

The white grape seeds of 'Gewürztraminer' used for exogenous addition were gained from the COFCO Chateau SunGod GreatWall in Huailai, Hebei province, during the 2022 wine production season. Following the extraction of the grape juice, the residue was quickly washed with water to remove the grape skin and approximately 5 kg of seeds were collected. The moisture content of the seeds was around 20%. After washing and drying with paper, some of the white grape seeds were immediately stored at −20 °C for subsequent analysis of condensed tannins and flavan-3-ols. The remaining seeds were vacuum-sealed in bags and stored at 0–4 °C for wine-making experiments.

2.3. Experimental design and small-scale vinification

After manually destemming and crushing the grape clusters, the resulting must was distributed evenly into nine 20 L stainless steel tanks, with each tank containing 15 kg of grapes. The fermentation tanks were then divided into three groups, each consisting of three tanks. The control group (labeled as C) did not receive any added seeds, while the treatment groups had 'Gewürztraminer' seeds placed in a white nylon mesh bag, with 300 g in each tank (2%). Afterward, the bag was submerged in the 'Cabernet Sauvignon' must until it was removed at the end of alcohol fermentation (CA label) or the end of malolactic fermentation (CM label). At the end of alcoholic fermentation, the wines were separated from the 'Cabernet Sauvignon' pomace, and 10 L of wines were transferred into the new 10-L tank to conduct the malolactic fermentation. Meanwhile, 100 g of 'Gewürztraminer' seeds were removed from the bag in the CM treatment group for each biological replicate for subsequent flavan-3-ol determination. The bag containing the remaining 200 g of seed was continued back into the CM-treated wines to continue maceration at the same seed proportion (2%) until the end of malolactic fermentation. In the winemaking process, both the white grape seeds in the nylon bag and grape marc were punched down three times a day to ensure the diffusion of their contents.

The fermentation process was consistent across all nine tanks and was conducted as follows: the must was added to the tanks and then potassium metabisulfite (Enartis Winy, Italy) was introduced to reach a final concentration of SO_2 at 40 mg/L. After this, 0.02 g/L of pectinase (Optivin, Australia) was added. The tanks were placed in a cold storage facility where the temperature was controlled, and the cold soak process began, with the temperature being maintained between 5 and 10 °C. The 0.3 g/L yeast (EnartisFerm Red Fruit, Italy) was introduced after four days of cold soak to commence alcohol fermentation at 22–26 °C. When the specific gravity of the must had dropped below 0.993, the pomace of 'Cabernet Sauvignon' was eliminated, and the resulting wine was moved to another 10-L tank. Subsequently, 6 mg/L *Oenococcus oeni* (Enartis-Ml UNO, Italy) was introduced to begin malolactic fermentation. At the end of malolactic fermentation, the wines were bottled and stored in a cellar with a regulated temperature of 10–15 °C and humidity of around 70%.

Sampling was conducted at seven-time points in this study, namely (1) before cold soak (referred to as Must), (2) at the end of cold soak, i.e. post cold-soak (referred to as PCS), (3) when the must specific gravity reached about 1.050 (referred to as SG1050), (4) when the must specific gravity reached about 1.020 (referred to as SG1020), (5) at the end of alcohol fermentation (referred to as EA), (6) at the end of malolactic fermentation (referred to as EM), and (7) after four months of bottle aging (referred to as BS). At each sampling point, 50 mL of grape juice or wine samples were collected for each biological replicate to conduct the subsequent determination of color parameters and phenol compounds. For the determination of oenological parameters of EA and EM wines, 300 mL of samples were collected for each biological replicate. Following the assessment of color parameters, the samples were stored in a freezer set at −40 °C. The Must, PCS, SG1050, SG1020, EA, EM, and BS stages occurred at 0, 4, 7, 9, 16, 41, and 161 days after grape harvest, respectively. All vinification processes were consistent and synchronized between the control and different treatment groups, except for the maceration times of the white grape seeds.

2.4. Determination of oenological parameters of wines

The pH of wines was measured by PB-10 m (Sartorius, Germany). The alcohol degree, total acidity, volatile acidity, and residual sugar in wine samples at EA and EM stages were measured using the method outlined in the national standard of China (GB/T 15038–2006) for analytical methods of wine and fruit wine. In brief, the alcohol degree was determined using the pycnometry method, residual sugar through titration using Fehling's reagent, and total acidity and volatile acidity through acid-base titration using an indicator solution, respectively. The total phenol content was determined using the Folin-Ciocalteu reagent with some modifications (Ainsworth & Gillespie, 2007; Ma et al., 2019). The determination details of oenological parameters (alcohol degree, total acidity, volatile acidity, and residual sugar) and total phenol are described in **Supplementary Method S1 and Method S2, respectively.**

2.5. Determination of soluble tannin in 'Gewürztraminer' seeds

The soluble tannin in seeds was extracted following our previous description (Liu et al., 2023). To extract soluble tannin, 0.1 g of seed powder was placed in a 2 mL centrifuge tube for each sample, followed by adding 1 mL of 70% acetone (containing 0.1% acetic acid). After that, the supernatant was collected. The quantification of soluble tannin was performed by using a microplate reader (SpectraMax 190, Molecular Devices, San Jose, CA, USA) to measure the absorbance at 640 nm, and using the standard procyanidin B1 as a reference.

2.6. Extraction of phenolic compounds in grape skins and seeds

The extraction of anthocyanins and flavonols in grape skins, as well as flavan-3-ols in grape skins and seeds, were performed according to the methods outlined by Lu et al. (2022). The phenolic acids were extracted following the method described by (Song et al., 2013). The details of the extraction of these phenol compounds in grapes were described in **Method S3**.

2.7. Determination of phenolic compounds in grapes and wines

These extracts obtained above were used for the following analysis, while the wine underwent direct filtration through a 0.22 μm polyethersulfone filter before analysis. The instrumental parameters for phenolic compound determination were set following our previous reports (Lu et al., 2022; Yao et al., 2024). An Agilent 1200 series HPLC system equipped with Agilent 6410 series triple-quadrupole tandem mass spectrometry (Agilent, Santa Clara, CA, USA) was utilized to determine the phenolic compounds in grapes and wines. The column was a Poroshell 120 EC-C18 column (150 × 2.1 mm, 2.7 μm, Agilent Technologies), and the temperature was set at 55 °C. The HPLC separation of monomeric anthocyanins and non-anthocyanin phenolic compounds was conducted with the binary mobile phase: (A) 0.1% formic acid water solution, (B) acetonitrile/methanol (50:50, *v/v*) containing 0.1% formic acid, at the flow rate of 0.4 mL/min. The gradient elution program was as follows: 10% solvent B at 0 min, 46% B at 28 min, and 10% B at 29 min, with 5 min of post-running time. Electrospray

ionization (ESI) was employed to produce ions in positive mode for monomeric anthocyanins, and in negative mode for non-anthocyanin phenolic compounds. For anthocyanin derivatives, which was determined as reported by Zhang et al. (2021). The elution program of the detection of anthocyanin derivatives with 0.3 mL/min of flow rate was set as: 0% solvent B for 0–1 min, 25% B at 3 min, 30% B at 15 min, and 100% B at 20 min. The ion source parameters were as follows: capillary voltage of 4 kV, a spray gas of 12 L/h, a nebulizer of 35 psi, source temperature of 350 °C, and gas temperature of 150 °C. The injection was 5 μL. The mass spectrometric acquisition of target phenolic compounds mentioned above was performed in Multiple Reaction Monitoring (MRM) mode.

Phenolic compound analysis was conducted by utilizing Qualitative Analysis 10.0 and QqQ Quantitative Analysis software 10.0 (Quant-My-Way) (Agilent Technologies Inc., Palo Alto, CA, USA) and aligning with their external standards and gradient concentration curves. Both qualitative and quantitative data were obtained. Anthocyanins were calculated in malvidin-3-*O*-glucoside equivalents, flavonols in quercetin-3-*O*-glucoside equivalents, flavan-3-ols and proanthocyanidins in terms of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-*O*-gallate, and (+)-gallocatechin equivalents, and phenolic acids in terms of gallic acid and caffeic acid respectively. The concentrations of these compounds in grapes and wines were measured in mg/kg fresh weight and mg/L, correspondingly. The detailed data regarding MRM, retention time, standards, and calibration curves for the phenolic compounds were presented in **Fig. S1**, and **Tables S1-S4**.

The determination of condensed tannins in wines was conducted using the methylcellulose precipitable assay (MCP), as described by Sarneckis et al. (2006). The content was quantified as (−)-epicatechin equivalent via a calibration curve. The details were listed in **Method S4**.

2.8. Determination of colorimetric parameters of wines

Before determination, the must and wine samples were filtered through 0.45 μm polyethersulfone filters (Jinteng Experimental Equipment Co., ltd, Tianjin, China) in 2 mm path-length quartz cells. The colorimetric parameters of wines were assessed using a CM-3700 A spectrophotometer (Konica Minolta, Japan). The L^* value indicates lightness, ranging from 0 (black) to 100 (white). The positive/negative a^* value indicates red/green intensity, while the positive/negative b^* value indicates yellow/blue intensity. Additionally, we calculated the saturation (C^*_{ab}) and hue angle (h_{ab}*) using the same device. The h_{ab}* value was applied to signify a particular chromaticity or tone, with red at 0°, yellow at 60°, green at 120°, cyan at 180°, blue at 240°, and magenta at 300°.

The effect of SO_2 bleaching on the wines was determined according to Alcalde-Eon et al. (2023). To conduct the study, wines were diluted with acidified water (pH 1.4, HCl) in the ratio of 1:1 (*v/v*). Subsequently, 1.2 g of $NaHSO_3$ was added to 3 mL of wine, and the solution was kept in the dark for 45 min. Absorption spectra of the samples in 10 mm quartz cells were measured using a UV–visible spectrophotometer at 440 nm, 530 nm, and 600 nm. The CIELab parameters were calculated according to the methods described by Yao et al. (2024) as listed in Eqs. (1)–(2), and the differences in these parameters between diluted wines and bleached wines were analyzed.

$$C^*_{ab} = \sqrt{\left[(a^*)^2 + (b^*)^2\right]} \tag{1}$$

$$h^*_{ab} = arctan\left(\frac{b^*}{a^*}\right) \tag{2}$$

2.9. Determination of artificial saliva precipitation index (ASPI) of wines

The wine astringency was evaluated by using the artificial saliva precipitation index (ASPI), as described by Qi et al. (2023). A buffer solution of artificial saliva (AS) was prepared by dissolving $NaHCO_3$ (5.208 g), $K_2HPO_4{\cdot}3H_2O$ (1.369 g), NaCl (0.877 g), KCl (0.477 g), and $CaCl_2{\cdot}2H_2O$ (0.441 g) in 1000 mL of deionized water, and adjusting the pH to 6.6. AS was prepared by adding 145.7 mg/L of lactoferrin, 19.3 mg/L of lysozyme, 425.8 mg/L of α-amylase, 103.2 mg/L of bovine serum albumin (BSA), and 233.6 mg/L of poly-L-proline to the buffer solution. A mixture of 200 μL of artificial saliva (AS) and 25 μL of wines was vortex-mixed, then incubated for 5 min at 37 °C, before being centrifuged for 2.5 min at 10,000 rpm at 4 °C. The binding reaction of AS and deionized water was conducted under the same conditions as the wines and was labeled as the blank. The obtained supernatant was used to conduct sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and HPLC analysis.

SDS-PAGE was applied to determine the intensity reduction rates of lactoferrin, albumin, α-amylase, and lysozyme in AS, labeled as X1, X2, X3, and X4, respectively. Additionally, the loss peak area rate of poly-L-proline in AS, denoted as X5, was assessed using an Agilent 1100 series HPLC system featuring a Zorbax 300SB-C18 column (4.6 × 250 mm, 5 μm) (Agilent Technologies Inc., Palo Alto, CA, USA) at 40 °C, with the mobile phase running at a flow rate of 0.4 mL/min. The details were listed in **Method S5.**

Finally, ASPI was calculated using the following eq. (3):

$$Y = 0.623X_1 + 3.3409X_2 + 3.1954X_3 + 1.2168X_4 + 1.0526X_5 - 0.6648 \tag{3}$$

2.10. Electronic tongue analysis of wines

An electronic tongue (TS-5000Z, Intelligent Sensor Technology, Inc., Atsugi, Japan) was utilized in this study, comprising an astringency sensor (AE1), sourness sensor (CA0), bitterness sensor (AC0), and bitterness aftertaste sensor (C00)(Chay et al., 2018). This apparatus was employed to evaluate the taste profile of wines after four months of bottling. A mixture solution containing 30 mmol/L KCl and 0.3 mmol/L tartaric acid was employed as both a cleaning agent and a reference standard solution. Approximately 35 mL of each wine sample was transferred into the designated beaker, with two replicates per biological replicate sample, and positioned in the injection positioning plate for automated injection. After the activation, testing, and calibration of the instrument, the determination procedure was applied, including measurement of the reference solution (*Vr*), initial taste voltage values (Vs) for the samples, and aftertaste voltage values (*Vr'*). The relative voltage values (*R*) of the initial and aftertaste values of the wine samples with respect to the reference solution were obtained using eqs. 4 and 5. The taste values were calculated by inputting the voltage values into the TS-5000Z Library search software (Intelligent Sensor Technology, Inc., Atsugi, Japan). The measurements for all samples were technically repeated four times. In accordance with the supplier's recommendation, the initial run was excluded from the statistical analysis, and the final three cycles were used to determine the values.

$$R_{initial\ taste} = V_s - V_r \tag{4}$$

$$R_{aftertaste} = V_{r'} - V_r \tag{5}$$

2.11. Sensory evaluation of wines

The sensory evaluation of wines that underwent four-month bottle aging was performed by using the wine-tasting glasses of the International Standards Organization (ISO 3591: 1977) that held 30 mL of wines. The testing was conducted in a controlled environment with white lighting and at room temperature (20 °C). The evaluation was performed at the individual block. A panel of seventeen judges, with a background in wine tasting, was selected from the Center for Viticulture and Enology (CFVE) at China Agricultural University. The panel consisted of nine females and eight males, whose ages ranged from 23 to 31

years old. Each sensory evaluator voluntarily participated in this study and provided full consent, following the 1975 Declaration of Helsinki. Ethical approval for the participation of human subjects in this study was granted by the China Agricultural University Research Ethics Committee (reference number CAUHR-20231103). All judges had prior experience in wine tasting and regularly underwent training at CFVE.

The color and taste profiles of wines were evaluated on a scale of 0 to 5 using the China Rating System for Global Wine (**Table S5**). Before the formal sensory evaluation, panelists underwent a training session to familiarize themselves with the evaluation standards outlined in **Table S5**. The training session included the examination of three different 'Cabernet Sauvignon' wines to standardize the scoring criteria. Subsequently, the panelist evaluated and discussed the color profiles, including clarity, color saturation, and wine hue, as well as the taste profiles, including balance, taste structure, wine body satiation, roundness, tannin intensity, tannin texture, and layers of these three wines, ultimately arriving at a consensus. The wines from three biological replicates from the same treatment group were mixed uniformly, labeled with three random digits, and presented to tasters for sensory analysis in a random order under blind conditions. A brief pause of approximately 1–2 min was observed between each wine sample. Subjects utilized water and soda crackers to cleanse their mouths and restore a neutral oral environment.

2.12. Data analysis

The 'agricolae' package in R statistical environment (3.6.2) (R Foundation for Statistical Computing, Vienna, Austria) was performed to conduct the one-way ANOVA (*Duncan*, $p < 0.05$). Graphpad prism 8 (GraphPad Software, San Diego, California, USA) and Origin 2023 software (OriginLab Corporation, Northampton, USA) were used for plotting and graphing. *K*-means analysis was performed by using 'fviz_nbclust' function in the 'factoextra' package in R statistical environment (3.6.2). Adobe Illustrator 2022 (Adobe Systems Incorporated, San Jose, California, USA) was utilized for color card retrieval and creation. Partial Least Squares Regression (PLSR) analysis was conducted by using SIMCA 14.1 (MKS Umetrics AB, Umea, Sweden).

3. Results

3.1. Leaching ratio of tannins from white grape seeds

To examine the leaching amount of tannins from added seeds to wines after maceration, we detected the concentrations of five flavan-3-ol components, including (−) -epigallocatechin, (+) -catechin, (−) -epicatechin, (−) -epicatechin gallate, and (+) -gallocatechin, as well as soluble tannins in 'Gewürztraminer' seeds that were not macerated (marked as G-Seeds), and at the end of alcoholic fermentation (EA) and end of malolactic fermentation (EM) stages (Fig. 1 & **Table S1**). Fig. 1 showed that during maceration until the end of alcoholic fermentation, notable reductions in all identified compounds were observed compared to unmacerated seeds (G-Seeds). Specifically, the leaching ratio ranged from 78.22%–80.79% for total free flavan-3-ols and 73.89%–76.05% for soluble tannins. When the seeds were macerated until the end of malolactic fermentation and then removed, there was a further increase in the leaching ratio by 7.03%–9.61% for total free flavan-3-ols, 10.01%–12.26% for total terminal subunits, 9.35%–9.57% for total extension subunits, and 13.79%–15.95% for soluble tannins, respectively.

3.2. Leaching of phenolics from 'cabernet sauvignon' grapes

To assess the extent of phenolic leaching from 'Cabernet Sauvignon' (CS) grapes, we compared the concentrations of phenolics in seeds (**Table S2**) and skins (**Table S3**) from pre-fermentation grapes and post-fermentation marc. A total of 14 flavonols, nine phenolic acids, and 15 anthocyanins were identified in CS skins. Additionally, three forms of five flavan-3-ols components, namely free-form, terminal, and extension

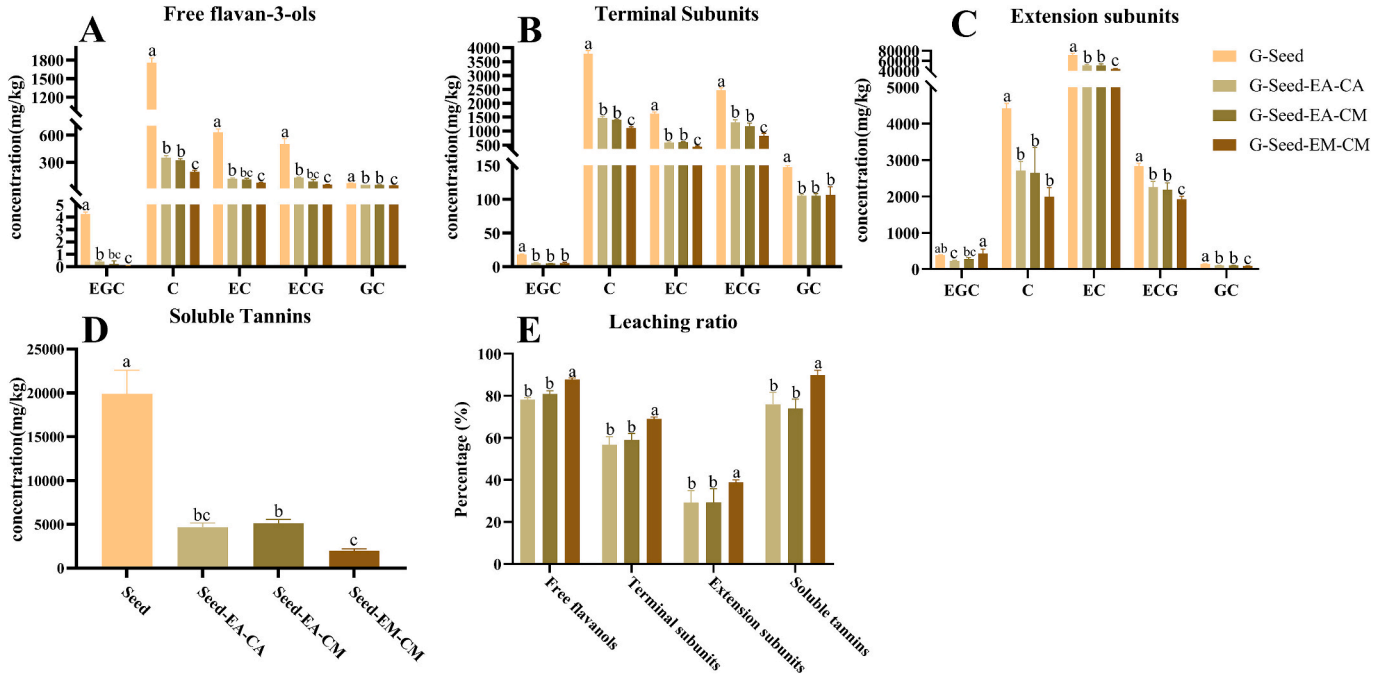

Fig. 1. The concentrations of **(A)** free flavan-3-ols and proanthocyanidins (**(B)** terminal subunits and **(C)** extension subunits), **(D)** soluble tannins, as well as **(E)** leaching ratio of each flavan-3-ol compound in the 'Gewürztraminer' seeds before and after maceration. Different lower letters indicate a statistical difference between different treatments (*Duncan*, $p < 0.05$). The legend G-Seed represents the 'Gewürztraminer' seeds before maceration. The legends G-Seed-EA-CA and G-Seed-EA-CM refer to the 'Gewürztraminer' seeds that are macerated until the end of alcoholic fermentation (EA) in the CA and CM treatments, respectively. The legends G-Seed-EM-CM indicate the 'Gewürztraminer' seeds that are macerated until the end of the malolactic fermentation (EM) in the CM treatment. The details of CA and CM treatments are explicated in the "Materials and Methods" section.

subunits, were examined in CS skins and seeds.

Compared to grape skins, CS seeds had 26.61, 2.33, 10.40, and 2.59 times more total free-form, extension subunits, terminal subunits, and total flavan-3-ols, respectively. To the end of alcohol fermentation, it was estimated that CS seeds leached 45.74%–57.65% for total free flavan-3-ol, 23.95%–24.73% for total extension subunits, 39.05%–42.70% for total terminal subunits, and 26.36%–26.89% for total flavan-3-ols into the wines. Compared to the seeds in CK pomace, CA and CM treatments reduced the leaching ratio of flavan-3-ol compounds to varying degrees by the end of the alcoholic fermentation process (**Table S2**).

Not all flavan-3-ol components were effectively extracted from the skins during the winemaking process **(Table S3)**. Post-fermentation marc skins contained higher concentrations of free (−)-epicatechin gallate and two terminal subunit components ((−)-epicatechin and (−)-epicatechin gallate) in comparison to pre-fermentative grape skins. This suggests that the pomace potentially absorbed tannins from the seeds that were added. Compared with the control group, the leaching of most flavan-3-ols from the CS skins in CA and CM treatment groups decreased. Reductions ranged from 14.03% to 33.42% for total free flavan-3-ols, 18.20% to 29.65% for total extension subunits, 26.41% to 42.76% for total terminal subunits, and 18.36% to 29.95% for total flavan-3-ols.

Regarding other phenolic compounds, only the phenolic acid compound 4-hydroxybenzoic acid was found in higher concentrations in marc skins compared to pre-fermentative grape skins. A considerable amount of the remaining phenolic acids, monomeric anthocyanins, and flavonols were transferred to the wines during maceration until the end of alcohol fermentation. Externally added seeds resulted in a decreased leaching ratio of total flavonols (4.64%–10.48%), total phenolic acids (0.84%–5.56%), and total anthocyanins (0.70%–2.60%) in CS skins, as compared to the control groups without additional seeds. Among them, the extraction levels of peonidin-3-*O*-(6-*O*-*p*-coumaryl) glucoside, malvidin-3-*O*-(6-*O*-*p*-coumaryl) glucoside, and total coumaroylated anthocyanins were significantly reduced compared to the control group (**Table S3**).

3.3. Effects on physicochemical indicators of wines

Compared to the control wines, the addition of seeds did not significantly impact most oenological parameters. For example, alcohol degree, total acidity, volatile acidity, and residual sugar were not impacted by adding seeds, as shown in Table 1. However, the CA-treated wines showed an increase in pH at the end of malolactic fermentation, compared to the control group. As expected, the total phenol concentration in the CA- and CM-treated wines increased by 27.31%–35.68% during the EM stage compared to the control wines. This indicates that phenolic compounds are extracted from 'Gewürztraminer' seeds and integrated into the 'Cabernet Sauvignon' wines.

3.4. Effects on phenolic compounds in wines

All phenolic compounds in wines, including individual components and their total quantities in various categories, were categorized into seven distinct classes using *K*-means. The categorization was based on the pattern of evolution during the winemaking process and affected by seed addition (Fig. 2 **A&B**).

3.4.1. Anthocyanins

A total of 15 monomeric anthocyanins were identified in the wines, including the nonacylated, their acetylated, and coumaroylated forms of delphinidin-type, cyanidin-type, petunidin-type, peonidin-type, and malvidin-type glucoside. Among them, malvidin-type anthocyanins were the most abundant components. Malvidin-3-*O*-glucoside, malvidin-3-*O*-(6-*O*-acetyl) glucoside, and malvidin-3-*O*-(6-*O*-*p*-coumaryl) glucoside accounted for 58.16%–65.09%, 26.54%–30.87%, and 1.16%–5.21% of the total monomeric anthocyanins in the wines at EM and BS stages, respectively. Total monomeric nonacylated and acylated anthocyanins derived from grapes were all grouped into Clusters 4 and 7, as shown in Fig. 2B. Total nonacylated anthocyanins, total acetylated anthocyanins, and total monomeric anthocyanins increased rapidly during the cold soak phase, then slightly increased during the early and middle stages of alcohol fermentation, before decreasing subsequently (**Fig. S2D**). Total levels of coumaroylated anthocyanins elevated quickly from the must to the SG1050 stage, stabilized during the middle of alcohol fermentation, and decreased after the SG1020 stage (**Fig. S2G**). Of these compounds, only total coumaroylated anthocyanins, peonidin-3-*O*-(6-*O*-*p*-coumaryl) glucoside, and malvidin-3-*O*-(6-*O*-*p*-coumaryl) glucoside exhibited notable increase at the EM and BS stages in CA treated-wines compared to the CK wines (**Fig. S2G** and **Table S4**).

Anthocyanin derivatives were produced during the winemaking process. A total of 17 anthocyanin derivatives, including two F-A components, four A-e-F components, five vitisin components, and six pinotin components were identified and grouped into Clusters 1, 2, 3, and 7 (Fig. 2B **& Table S4**). Cluster 7 included total anthocyanin derivatives, total and all individual vitisin components (**Fig. S2G & Table S4**). During the initial fermentation process, vitisins showed a rapid increase followed by a decline after the SG1020 stage and then remained stable throughout the bottle storage period. The total anthocyanin derivatives increased by approximately 1.19-fold in the EM and BS stages for CA-treated wines compared to CK wines. However, the CM treatment did not have a significant impact on this type of compound (**Fig. S2G**). Cluster 1 included total acetaldehyde-bridged anthocyanin-flavan-3-ol (A-e-F) derivatives, comprising Mv-e-di(e)cat, Mv-e-(e)cat, MvC-e-(e)cat, and MvA-e-(e)cat. A-e-F derivatives displayed a consistent upward trend throughout the fermentation, followed by a slight decrease after four months of bottle storage. Compared with CK wines, seed addition significantly increased A-e-F levels from SG1.050 to BS stages (**Fig. S2A**). Total direct-connected anthocyanin-flavan-3-ol derivatives (F-A) and their two components ((E)cat-Mv and (E)cat-MvA) were found in Cluster 2. The concentrations of these compounds gradually increased

Table 1
The physical and chemical properties of wine samples at the end of alcoholic fermentation and malolactic fermentation.

Parameters	Calibration curve	EA-C	EA-CA	EA-CM	EM-C	EM-CA	EM-CM
Alcohol degree (%, v/v)		12.67 ± 0.19 a	12.64 ± 0.33 a	12.51 ± 0.27 a	12.52 ± 0.46 a	12.25 ± 0.24 a	12.23 ± 0.30 a
Total acidity (g/L)		6.78 ± 0.23a	6.57 ± 0.16a	6.92 ± 0.27a	4.73 ± 0.24a	4.56 ± 0.26a	4.79 ± 0.22a
Volatile acidity (g/L)		0.55 ± 0.03a	0.52 ± 0.05a	0.52 ± 0.02a	0.7 ± 0.06a	0.74 ± 0.05a	0.73 ± 0.1a
Residual sugar (g/L)		1.49 ± 0.10a	1.43 ± 0.07a	1.42 ± 0.03a	1.15 ± 0.3a	1.00 ± 0.14a	1.11 ± 0.06a
pH		3.93 ± 0.05a	3.91 ± 0.02a	3.93 ± 0.01a	4.14 ± 0.01b	4.19 ± 0.01a	4.13 ± 0.02b
Total phenol (g/L)	$y = 3.3277\times$ - 0.2184 ($R^2 = 0.9983$)	1.23 ± 0.02b	1.44 ± 0.18a	1.51 ± 0.07a	1.42 ± 0.14c	1.84 ± 0.05b	1.99 ± 0.03a

Note: The wine samples were marked as "sample point-treatment". The prefixs "EA" and "EM" indicated that the wines were sampled at the end of alcoholic fermentation and the end of malolactic fermentation, respectively. The suffixs "C", "CA", and "CM" indicated the wines of control group, the seed-treatment group which seeds were macerated until the end of alcoholic fermentation, and seed-treatment group which seeds were macerated until the end of malolactic fermentation, respectively. Data was expressed as "mean ± sd" ($n = 3$). Different lower-case letters indicated that there were significant differences between different treatment groups at the same sampling stage (*Duncan*, $p < 0.05$).

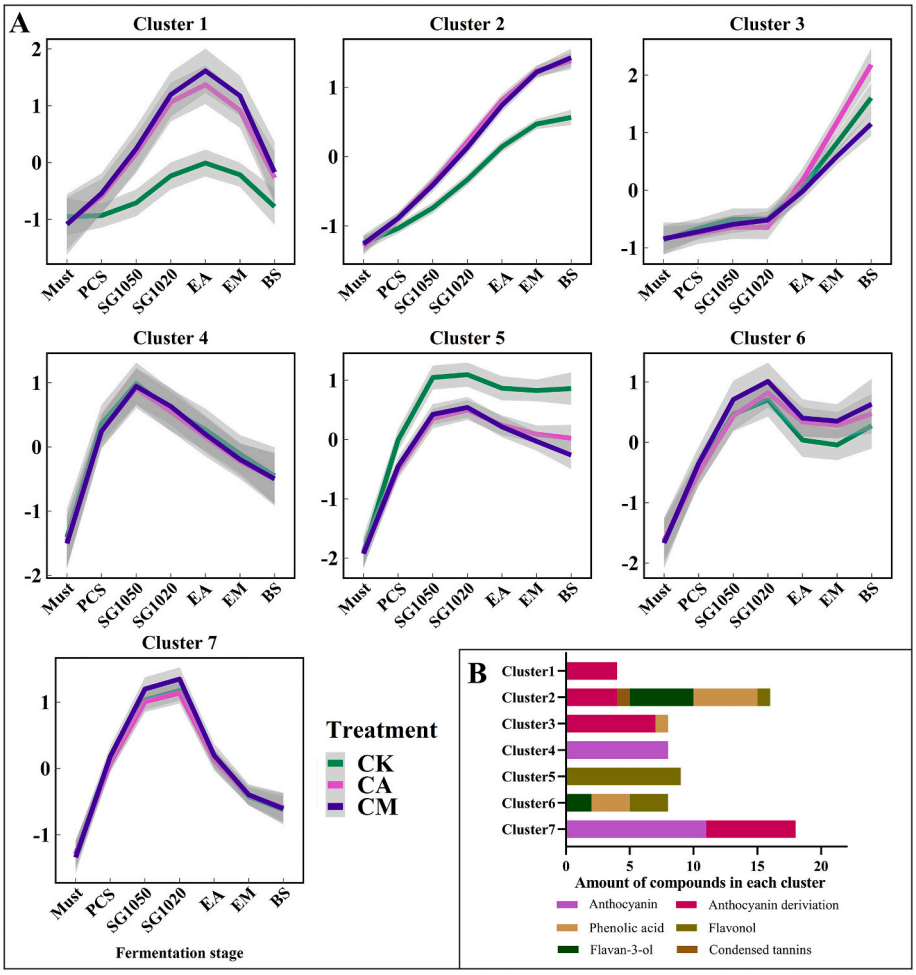

Fig. 2. (A) *K*-means analysis of the phenolic compounds in wines during the fermentation; The y-axis shows the normalized concentrations. **(B)** Amount of compounds in each cluster.

during the period from alcoholic fermentation to the malolactic fermentation process and slightly decreased during the BS stage. Moreover, in seed-adding wines, their concentrations were observed to be higher at least one stage after alcoholic fermentation compared to the control group (**Fig. S2B and Table S4**). Six pinotin compounds and total pinotins were all grouped into Cluster 3, exhibiting a continuous increasing trend in their concentrations. The CA treatment significantly reduced both MvA-vpol and total pinotin concentration at the SG1020 stage. Nevertheless, at the EM and BS stages, the CA treatment significantly enhanced the total concentration of pinotins (**Fig. S2C**).

3.4.2. *Non-anthocyanin phenolic compounds*

A total of six flavan-3-ol components, six phenolic acid components, and eight flavonol components were detected in the wines. Compounds in Clusters 5 and 6 displayed a rapidly increasing trend until the middle phase of alcohol fermentation, followed by a slight decrease or relatively stable levels (**Fig. S2 E&F**). Cluster 5 contained three flavonols of quercetin-type flavonols, isorhamnetin-3-*O*-glucoside, and kaempferol-3-*O*-glucoside. These compounds were quickly extracted during cold soak. However, the addition of seeds impeded the leaching process from grape skins, leading to decreased flavonol levels in the wines (**Fig. S2E & Table S3&S4**). Two flavonols of myricetin-type were categorized under Cluster 6, and their total concentration was also reduced in the CA-treated and CM-treated wines at the EM stage compared with CK wines. Caffeic acid, the most prominent hydroxycinnamic acid, was assigned to Cluster 6 and higher in seed addition wines than CK wines. Especially for the CM-treated wines, this elevation at sample points from SG1050 to BS was significant (**Table S4**). Meanwhile, components in Cluster 2 showed increased levels until the EM stage, with seed-added wines having a more pronounced increase than CK wines (**Fig. S2B**). These components included gallic acid (the most prominent hydroxybenzoic acid), and two prominent flavan-3-ols ((+) -catechin and (−)-epicatechin), along with procyanidin B2 and procyanidin C1 (**Table S4).** They were likely leached from both the added seeds and the CS grapes.

3.5. *Effects on wine color evolution*

Significant impact of seed additives on the L^*, a^*, and C^*_{ab} values of wines was observed at the EM and BS stages. The wines with seed additives displayed lower L^* and higher a^* and C^*_{ab} compared to the control wines. Furthermore, prolonged seed maceration time had a more pronounced effect on L^* and a^* values at the EM stage. The difference between CM and CA wines disappeared after four months of bottle storage. From the SG1020 stage onwards, seed additions significantly increased the b^* value in comparison to CK. The results indicated that CA- and CM-treated wines had a darker brick-red hue (**Fig. S3A)**. There was no notable variation in the h^*_{ab} value between different treatments throughout the process. However, during sensory evaluation, human subjects could not discriminate the color difference between different wine groups at the BS stage (**Fig. S3B**).

The partial least squares regression (PLSR) model was utilized to investigate the contribution of anthocyanins to wine color based on the data at the EM and BS stages, given that pre-fermentative seed additions

altered the color parameters of wines at the two sampling points (Fig. 3B). The anthocyanins were set as independent variables, while the CIELab color parameters served as dependent variables. The first two principal components yielded a model quality with an R2X(cum) of 0.683, an R2Y(cum) of 0.963, and a Q2(cum)of 0.919, and explained 42.2% and 32% of the total variance, respectively. The seed-treated and control wines were mainly distributed in the positive and negative halves of the Y-axis. The red hue a^* value was distributed in the positive half-axis of Y, and the L^* value in the negative half-axis of Y, respectively. The results show that anthocyanin derivatives of A-e-F and F-A types had a strong positive correlation with the a^* value, and a strong negative correlation with the L^* value. This is in agreement with higher concentrations of these compounds in CA- and CM-treated wines compared with the control (Fig. 2 & **Table S4**). Meanwhile, h^*_{ab} and b^* were co-distributed in the negative half-axis of the X-axis, indicating a close association with pinotins and vitisins.

Different anthocyanin compounds may have varying resistances to SO_2 bleaching (Alcalde-Eon et al., 2019; Alcalde-Eon et al., 2023). To examine the effect of seed addition on the resistance against SO_2, we calculated the change ratios of wine color parameters at the EM and BS stages after SO_2 bleaching (Fig. 3C). The addition of seeds reduced the increase rate of h^*_{ab} and C^*_{ab} and decreased the reduction rate of a^*, indicating that the wine treated with seeds had increased resistance to SO_2.

3.6. Effects on wine astringency

The artificial saliva precipitation index (ASPI), electronic tongue, and sensory evaluation were used to assess the intensity of wine astringency. ASPI results revealed a significant increase in astringency intensity by 1.67 times for CA-treated wines and 1.76 times for CM-treated wines at the EM stage compared to CK wines (Fig. 4A). Notably, the difference between the CM treatment and control groups was sustained even after four months of bottle storage. As illustrated in the results of the electronic tongue (Fig. 4B), the addition of seeds resulted in a notable elevation in astringency and astringency aftertaste in the wines at the BS stage, in comparison to the control wines. Correspondingly, during the sensory evaluation (Fig. 4C), the seed-added wines demonstrated higher scores for taste structure, wine body satiation, and tannin intensity when compared to control wines at the BS stage. However, in accordance with the findings of the electronic tongue, the addition of seeds did not affect the intensity of bitterness, which was evaluated as one aspect of tannin texture. There was no significant difference found in the sensory evaluation between the wines of the CA and CM groups in regard to these aspects.

4. Discussion

4.1. Seed addition inhibits the leaching of phenolics from wine grapes to different extent

This study revealed that the pre-fermentative addition of seeds had varying inhibitory effects on the extraction of various phenolic compounds from CS grape skins and seeds into the wine. Flavan-3-ols presented the most significant inhibition, followed by flavonols, phenolic acids, and anthocyanins. Free flavan-3-ols, as well as terminal and extension units of proanthocyanidins, were leached at a significantly reduced level from the skins and seeds of CS.

Anthocyanins predominantly occur in the vacuoles of grape skin cells, and are easily extracted from the skins into wines upon crushing owing to their water solubility and the application of pectinolytic enzymes (Bimpilas et al., 2015). Flavonols are co-located with anthocyanins and share a similar pattern of leaching from the skin (Casassa & Harbertson, 2014). Flavan-3-ols are mainly situated in the thick-walled inner cells of the hypodermis of grape skins and in the thin-walled cells that exist between the external hydrophobic cuticle and the inner

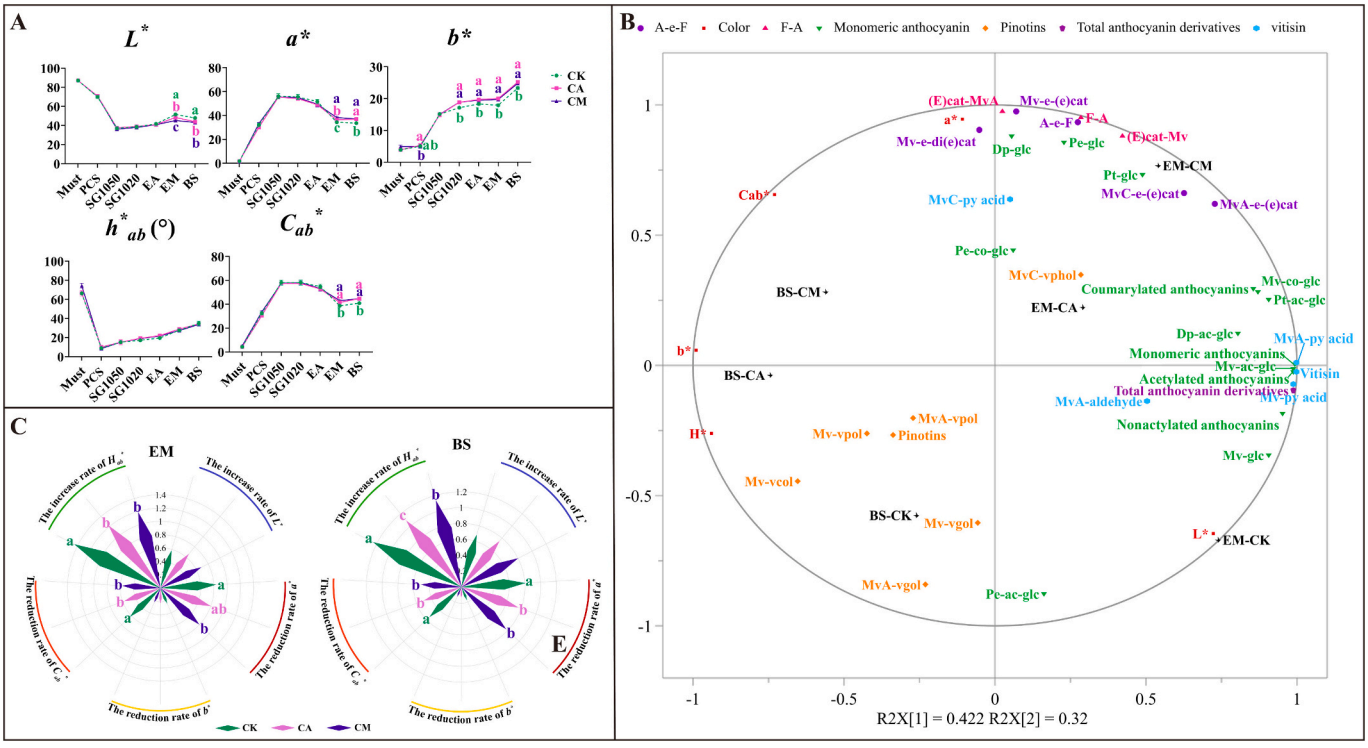

Fig. 3. **(A)** The effects of fermentation with additional grape seeds on CIELab parameters. The y-axis shows the normalized concentrations. **(B)** The partial least squares regression (PLSR) model of CIELab parameters and anthocyanins at the end of malolactic fermentation (EM) and after four-month bottle storage (BS). **(C)** The effect of adding seeds on the change in CIELab parameters of wines at EM and BS stages after bleaching with SO_2. Different lower-case letters represent a statistical difference between different treatment groups (*Duncan*, $p < 0.05$). No lower-case letters refer to no statistical difference.

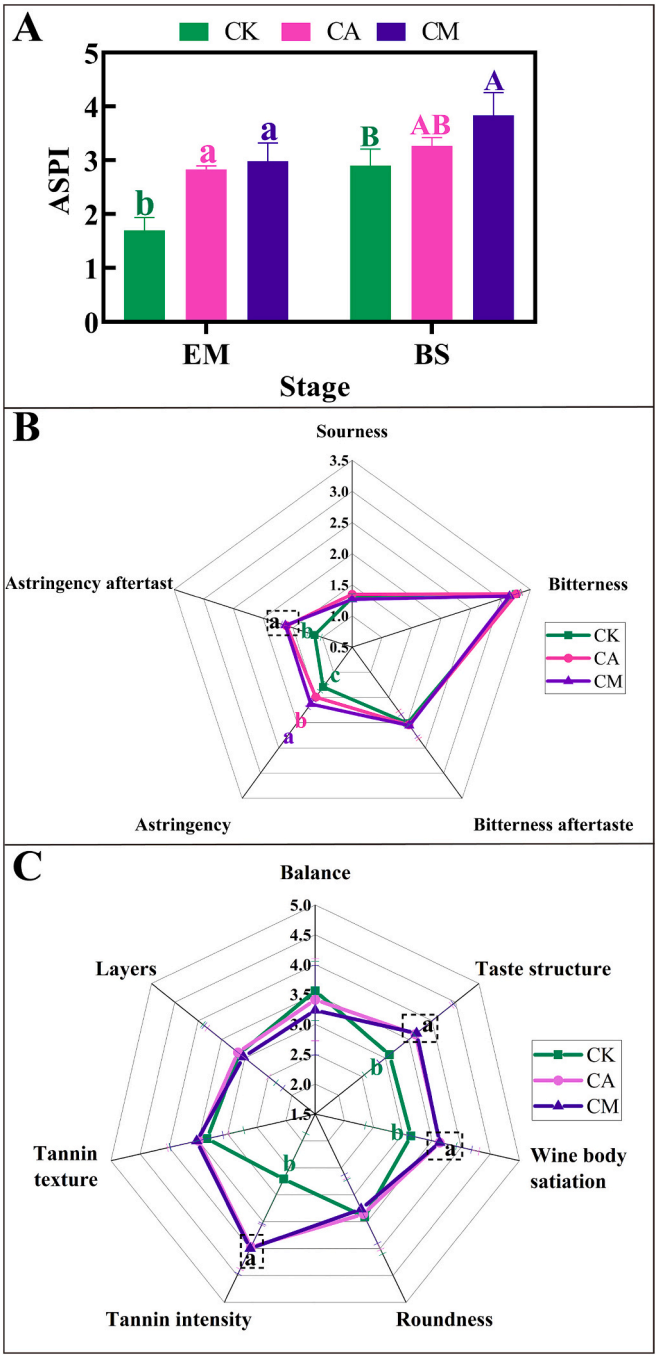

Fig. 4. (A) Effects of fermentation with additional grape seeds on artificial saliva precipitation index (ASPI) of wines at the end of malolactic fermentation (EM) and after four-month bottle storage (BS). Different lowercase letters and capital letters represent statistical differences between different treatment groups at the EM stage and BS stage, respectively (*Duncan*, $p < 0.05$). **(B)** The taste profile of BS wines was measured using an electronic tongue. Different lower-case letters represent statistical differences between different treatment groups (*Duncan*, $p < 0.05$). No lower-case letters indicate no statistical difference. **(C)** The sensory evaluation on the taste of BS wines. Different lower-case letters represent statistical differences between different treatment groups (*Duncan*, $p < 0.05$). No lower-case letters indicate no statistical difference.

lignified layers in grape seeds (Casassa & Harbertson, 2014; Rousserie et al., 2019; Zhao et al., 2023). Their extraction into wines transpires through intricate dissolutive and diffusive kinetics (Casassa et al., 2019). The phenolic compounds introduced by white grape seeds can affect the solid/liquid partition of these phenolic compounds in the fermentation broth, impeding their diffusion from red grapes to wine. This causes the compounds to reach their solubility limit and reduces the leaching ratio. According to Setford et al. (2017), the concentration-dependent diffusion of proanthocyanidins and the extraction of a substantial portion of flavan-3-ols from the supplemental grape seeds not only complement the reduced leaching of flavan-3-ols from CS grapes, but also results in higher levels of flavan-3-ols in the wines treated with pre-fermentative addition of seeds compared to control wines. This elucidates the reason for the greater suppression of the leaching ratio of flavan-3-ols following seed addition treatment, in contrast to other phenolic compounds in the skins of CS grapes.

We found that the pre-fermentative addition of white grape seeds had a stronger inhibitory effect on the leaching ratio of flavan-3-ols from the skins of the CS grapes than from their seeds. Generally, grape seeds contain a considerably higher amount of flavan-3-ols than skins (González-Manzano et al., 2004). Although flavan-3-ols are more difficult to extract from grape seeds than from the skins due to the high proportion of gallic-esterified components, a majority of flavan-3-ols in wine come from grape seeds rather than skins after alcoholic fermentation (González-Manzano et al., 2004). On the other hand, extracted flavan-3-ols and proanthocyanidins can be re-absorbed into grape pomace via a non-covalent interaction (Ruiz-Garcia et al., 2014). Therefore, it is understandable that the extraction of flavan-3-ol compounds is lower in the CS skins as compared to the CS seeds.

Adding white grape seeds had a pronounced dampening effect on the accumulation of flavonol compounds in the CS wines, which was consistent with the observation of Alcalde-Eon et al. (2019), which could resulted from the inhibitory impact on the leaching of these compounds in CS skins. However, other research described that the addition of overripe seeds had minor effects on the concentrations of flavonols (Gordillo et al., 2021; Rivero et al., 2017), suggesting the controversial effects of adding seeds on flavonols levels.

The concentration of phenolic acids, predominantly gallic acid, was significantly higher in the seeds-treated wines compared to control wines in this study. The finding is consistent with earlier reports by Gordillo et al. (2021) and Rivero et al. (2019), as well as the identification of phenolic acids in 'Gewürztraminer' seeds (Sochorova et al., 2020). This suggests that this kind of compound extracted from the added seeds may have contributed to the observed increase in phenolic acids in red wines.

Previous studies have reported conflicting effects of adding seeds on monomeric anthocyanins in wine production. Gordillo et al. (2021) and Rivero et al. (2019) found that adding seeds after fermentation reduced anthocyanin levels in the wine, while Rivero et al. (2017) observed the opposite effect. In our study, we found that the pre-fermentative addition of seeds did not affect the levels of grape-derived monomeric and acylated anthocyanins in the wines. This lack of effect could be attributed to the minor impact of adding seeds on the leaching ratio of these anthocyanins from wine grapes. Although there was a significant increase in the formation of A-e-F and F-A type pigments in the seed-treated wines compared to the control wines, the concentrations of total anthocyanin derivatives in the wines did not differ significantly between the treatment groups. This is because the two types of stable pigments presented low levels in contrast to other anthocyanins in the wines.

4.2. *Seed addition promotes the production of polymeric pigments in wine*

In comparison to CK wines, the seed-treated wines exhibited higher levels of flavan-3-ol and anthocyanin condensation products (A-e-F and F-A types) at the EM and BS stages. This finding aligns with previous studies (Alcalde-Eon et al., 2019; Gordillo et al., 2021; Rivero et al., 2019), and may be attributed to the increased levels of precursor compounds, such as flavan-3-ols and condensed tannins, introduced by the white grape seeds. According to Zhang et al., 2022, anthocyanin derivatives of F-A and A-e-F types have a higher maximum absorption

wavelength than their precursor anthocyanins. These derivatives are characterized by a red-violet hue and a slightly pale red-purple hue, respectively. In terms of CIELab, the seed-treated wines presented higher red hue and saturation, as well as deeper color. The seed-treated wines showed a higher b^* value compared to the control wines, which can be attributed to the increased levels of pinotins and flavan-3-ols. Pinotins are known to exhibit an orange-red hue (Zhang et al., 2022). Additionally, Zhao et al. (2022) observed that the model wines exhibited a higher yellow hue after storage with the addition of flavan-3-ols. Previous research also reported a positive effect on wine colors when seeds were added. The addition of oenological tannin during alcoholic fermentation led to lower lightness and higher chroma of 'Tempranillo' wines (Alcalde-Eon et al., 2014). Moreover, the additional of grape seeds during the fermentation (Rivero et al., 2017) or during post-fermentation maceration (Alcalde-Eon et al., 2019; Gordillo et al., 2021; Rivero et al., 2019) resulted in a decrease in L^* and an increase in C^*_{ab} of 'Syrah' wines, leading to a more vivid and red-bluish color.

Our research also indicates that the wines treated with added seeds have greater resistance to SO_2 bleaching, consistent with the findings of Alcalde-Eon et al. (2019). As is well-known, anthocyanin derivatives such as pinotins and vitisin A are highly resistant to SO_2 bleaching (Alcalde-Eon et al., 2019; Alcalde-Eon et al., 2023; Zhang et al., 2022). However, there was no significant change in the level of vitisin A between the seed-treated and control wines. Additionally, there was no consistent effect on the production of pinotins between the wines of CA and CM treatment groups. It can be inferred that the increase in resistance to SO_2 bleaching for the seed-treated wines may be related to the enhanced formation of A-e-F type pigment derivatives. In this study, the addition of seeds significantly increased the levels of A-e-F and F-A type pigments (refer to **Fig. S2A&B** and **Table S4**). For A-e-F type pigments, such as Mv-e-di(e)cat, their folding conformation resulting from the intramolecular interactions effectively protects the C4 position of the anthocyanin molecule from the nucleophilic attack of bisulfite (Zhang et al., 2022). This improves the resistance to SO_2 bleaching. But for F-A type anthocyanins (e.g., (E)cat-Mv), the C4 position of the anthocyanin molecule is not protected by the incorporated flavan-3-ol chain, and cannot resist bisulfite discoloration (Zhang et al., 2022). In addition, the presence of high levels of flavan-3-ols in the seed-treated wines may benefit the stabilization of anthocyanin color through non-covalent copigmentation. Furthermore, previous studies have reported that adding grape seeds can enhance the stability of wine color. Kovac et al. (1995) reported that adding grape seeds a day after the beginning of fermentation slightly increased the color intensity value and the free anthocyanins levels of wines. Rivero et al. (2017) observed less color modification in wines treated with overripe seeds compared to control wines after being stored in bottles. The addition of overripe grape seeds twice led to a more stable wine color after post-fermentative maceration, as reported by Rivero et al. (2019). Gordillo et al. (2021) found that the color intensity of wines with overripe grape seed addition was higher and lasted longer than that of control wines after post-fermentation maceration.

4.3. Seed addition enhances the astringency of wine

The evaluation of astringency is a crucial sensory characteristic in wine research. In this study, we examined wine's astringency using the ASPI method that was established by Qi et al. (2023), electronic tongue, and sensory evaluation. The results indicate that the seed-added wines had stronger astringency than the control wines (Fig. 4). This suggested that the astringency enhancement could be due to the elevation of flavan-3-ols and condensed tannins in the seed-treated wines. Flavan-3-ols and condensed tannins are known to cause astringency by interacting with proteins in human whole saliva (Casassa & Harbertson, 2014; González-Muñoz et al., 2022; Qi et al., 2023). Grape seeds are a rich source of flavan-3-ols and condensed tannins, which can be used to address phenolic deficiencies in wine production. Studies have shown that adding grape seeds either before or after fermentation significantly increases the concentrations of flavan-3-ols and condensed tannins in wines (Gordillo et al., 2021; Kovac et al., 1995; Rivero et al., 2017; Rivero et al., 2019). During maceration, the 'Gewürztraminer' seeds released most of their soluble tannins and flavan-3-ols into the red wine (Fig. 1 **& Table S1**), increasing the concentration of these compounds in the wine. This increase was particularly noticeable between the SG1050 and BS stages (**Fig. S2B & Table S4**). Canals et al. (2008) observed that adding seeds significantly intensified the astringency of 'Cabernet Sauvignon' wines. Alcalde-Eon et al. (2019) also reported a stronger intensity of astringency resulting from the post-fermentative addition of overripe seed extract. This suggests that these compounds were stacked into the copigmentation product, reducing their interaction with the proteins. Furthermore, some researchers have observed that removing seeds significantly reduced astringency and bitterness (Canals et al., 2008; Jagatić Korenika et al., 2023). However, Canals et al. (2008) found that adding seeds did not increase bitterness, which is consistent with our observation.

5. Conclusion

This study evaluates the evolution of anthocyanins and non-anthocyanin phenolic compounds in 'Cabernet Sauvignon' wines. It also demonstrates how white wine grape seeds can potentially enhance the color and astringency of red wines by modifying the phenolic profile. The addition of seeds before fermentation significantly inhibits the leaching of flavan-3-ols from the CS skins and free-form flavan-3-ols from the CS seeds. However, this treatment has a minor impact on the leaching of monomeric and acylated anthocyanins from the CS skins. As a result, it does not affect the levels of these anthocyanins in the wines during the fermentation process in contrast to the control wine. The addition of seeds does increase the levels of copigments, such as flavan-3-ols and phenolic acids. This ultimately facilitates the production of anthocyanin derivatives. The levels of A-e-F and F-A type derivatives have increased, increasing in a^* and C^*_{ab} values, as well as a decrease in L^* values in the seed-treated wines. This enhances the wine's resistance to SO_2 bleaching. Additionally, the addition of seeds substantially increases the concentration of condensed tannins in the wines, resulting in a stronger astringency, without causing an increase in bitterness. The comparison between CA and CM treatments shows no significant differences in the concentrations of most phenolic compounds, color parameters, and astringency intensity. Considering the cost of wine production, CA treatment, which involves removing both grape pomace and added seeds from the wine at the end of alcohol fermentation, could be an effective strategy to improve the astringency and color stability of red wine. Meanwhile, grape seeds must be stored in vacuum-sealed bags at low temperatures before application to prevent oxidation of fat and tannin. Considering the time gap for ripening between white and red grape varieties, it may be more feasible to include seeds from late-maturing white grape varieties in the fermentation process of early-maturing red grapes. In summary, this study explores the potential for valorizing white wine grape seeds and proposes a feasible method for improving the color and astringency of red wine. This can aid wine-makers in determining the vinification process based on their available resources.

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CRediT authorship contribution statement

Nong-Yu Xia: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Ao-Yi Liu:** Investigation, Data curation. **Meng-Yao Qi:** Methodology. **Hua-Lin Zhang:** Investigation. **Yong-Ce Huang:** Investigation. **Fei He:** Resources. **Chang-Qing Duan:** Supervision. **Qiu-Hong Pan:** Writing – review & editing, Supervision, Project administration, 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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101700.

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