The effects of regions and the wine aging periods on the condensed tannin profiles and the astringency perceptions of Cabernet Sauvignon wines

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A R T I C L E   I N F O

Keywords:
Condensed tannins
Wine
Region
Aging
Astringency

Chemical compounds:
(+)-catechin (PubChem CID9064)
(-)-epicatechin (PubChem CID72276)
(+)-gallocatechin (PubChem CID65084)
(-)-epigallocatechin (PubChem CID72277)
(-)-epicatechin gallate (PubChem CID107905)
(-)-epigallocatechin gallate (PubChem CID65064)

A B S T R A C T

This study sought to determine the effects of wine-producing regions and aging periods on the astringency and chemistry of condensed tannins of Cabernet Sauvignon dry red wines. A wine quality study was performed with 5 vintages of 32 Cabernet Sauvignon wines produced in four Chinese wine-producing regions, Hebei (H), Xinjiang (X), Inner Mongolia (NM), and Ningxia (NX). Condensed tannin profiles were assessed by high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD). The (-)-epicatechin as the terminal subunit (IEC) is the major differential component between regions. Correlation analysis revealed that condensed tannin concentration and composition significantly affected the sensory evaluation of astringency. Condensed tannin concentrations were significantly and negatively correlated with wine aging periods. However, no significant correlation was found between aging periods and condensed tannin subunits (as mole%) composition. The current findings enhance the understanding of condensed tannins’ chemical and astringency characteristics in Cabernet Sauvignon wines.

Introduction

Condensed tannins are plant secondary metabolites widely found in fruits, bark, leaves, and seeds. Condensed tannins from the grape berries enter the wine through maceration during the winemaking process. Condensed tannins, also known as proanthocyanidins, are polymers formed by the condensation of nucleophilic flavan-3-ols and electrophilic flavan-3,4-diols through a C 4 -O-C 6 bond (Type B), and connected by additional C 6 -O-C 7 bond (Type A). According to the number of hydroxyl groups on the B ring and the difference in the 3-hydroxyl group structure on the C ring in its constituent unit flavan-3-ol, tannins’ monomer can be divided into (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-gallocatechin gallate (GCG) (Table S1). The condensed tannins’ structural components depend on the extension and the terminal units of flavan-3-ols, the polymerization degree, the location and stereochemistry of the small molecule unit linkage, and the presence of 3-hydroxy modifications (Rousserie et al., 2019). Differences in building blocks, attachment positions, and conformations provide condensed tannin structural diversity, and the number of condensed tannin isomers increases significantly with the polymerization degree (Nakashima et al., 2012).

Wine’s unique flavor characteristics are influenced by the quality of the wine grapes, determined by the different geographical environments (including climate, soil environment, and varieties suitable for each region), thus giving the wine a strong terrestrial origin (Cortell et al., 2005). China has a very scattered distribution of wine regions with significantly different ecological conditions. Ningxia production area is in the alluvial plain at the eastern foot of the Helan Mountains, with a dry climate and a large temperature difference between day and night. Xinjiang production area is deep inland, with a large temperature difference between day and night. Hebei production area is close to the ocean, rich in heat, and abundant rainfall. Inner Mongolia’s production area is cold in winter, with low temperatures during the grape growing period.
Wine samples

Materials and methods

Wine samples

Thirty-two Cabernet Sauvignon red wines from Hebei (36°57′N, 119°45′E), Xinjiang (44°30′N, 86°21′E), Inner Mongolia (39°69′N, 106°82′E), and Ningxia (38°26′N, 106°04′E) wine-producing regions were selected (Fig. S1 and Table 1). Wines produced in five continuous vintages (2015–2019) were collected from four production regions, and the wines of different years were brewed by traditional brewing techniques. After brewing, they were stored in wine bottles (750 mL, amber, cork), and stowed in cellars at 15 ± 2 °C and 65 ± 5 % relative humidity. All analyses were conducted in fall 2020. Six bottles of each wine were collected, for 192 bottles. Three bottles of each wine were used for chemical measurements, and the other three bottles were used for sensory analysis. For different chemical measurements, three bottles of each wine were opened, immediately divided into centrifuge tubes, and stored in a refrigerator at −80 °C for subsequent chemical analysis.

Reagents and standards

The (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin gallate (+)-gallocatechin and (-)-epigallocatechin gallate, (purity > 98 %) were purchased from Shanghai Yuanye Biotechnology Co. Ltd (Shanghai, China). Methanol, acetone, acetic acid, formic acid, hydrochloric acid (HPLC grade), gallic acid, rutin, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Shanghai, China). Phloroglucinol and ascorbic acid were purchased from Shandong Xinya Chemistry Industry Ltd (Linyi, China). Sodium dodecyl sulfate (SDS), triethanolamine (TEA), and Folin-phenol were purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). Sodium carbonate, sodium hydroxide, aluminum chloride, sodium nitrite, and sodium acetate were purchased from Tianjin Bodi Chemical Co., Ltd (Tianjin, China). The C-18 SPE column was obtained from Tianjin Chengzhou Technology Co. Ltd (Tianjin, China). The deionized water was obtained from a Milli-Q system (Merck Millipore, Darmstadt, Germany).

Table 1: Cabernet Sauvignon wine samples list.

| Wine Year | Region | Vineyard |
|-----------|--------|---------|
| H1 2015   | Hebei  | MOUTAI WINE CO., ltd |
| H2 2016   | Hebei  | MOUTAI WINE CO., ltd |
| H3 2017   | Hebei  | MOUTAI WINE CO., ltd |
| H4 2018   | Hebei  | MOUTAI WINE CO., ltd |
| H5 2015   | Hebei  | COFCO HUAXIA GREATWALL WINE CO., ltd |
| H6 2016   | Hebei  | COFCO HUAXIA GREATWALL WINE CO., ltd |
| H7 2017   | Hebei  | COFCO HUAXIA GREATWALL WINE CO., ltd |
| H8 2018   | Hebei  | COFCO HUAXIA GREATWALL WINE CO., ltd |
| H9 2019   | Hebei  | COFCO HUAXIA GREATWALL WINE CO., ltd |
| X1 2017   | Xinjiang | XINJIANG SUNYARD WINE Co., ltd |
| X2 2018   | Xinjiang | XINJIANG SUNYARD WINE Co., ltd |
| X3 2019   | Xinjiang | XINJIANG SUNYARD WINE Co., ltd |
| X4 2015   | Xinjiang | CITIC GUOAN WINE Co., ltd |
| X5 2016   | Xinjiang | CITIC GUOAN WINE Co., ltd |
| X6 2017   | Xinjiang | CITIC GUOAN WINE Co., ltd |
| X7 2018   | Xinjiang | CITIC GUOAN WINE Co., ltd |
| X8 2019   | Xinjiang | CITIC GUOAN WINE Co., ltd |
| NM1 2015  | Inner Mongolia | SUNSHINE TIANYU WINE Co., ltd |
| NM2 2016  | Inner Mongolia | SUNSHINE TIANYU WINE Co., ltd |
| NM3 2017  | Inner Mongolia | SUNSHINE TIANYU WINE Co., ltd |
| NM4 2018  | Inner Mongolia | SUNSHINE TIANYU WINE Co., ltd |
| NM5 2019  | Inner Mongolia | SUNSHINE TIANYU WINE Co., ltd |
| NX1 2015  | Ningxia | IMPERIAL HORSE |
| NX2 2016  | Ningxia | IMPERIAL HORSE |
| NX3 2017  | Ningxia | IMPERIAL HORSE |
| NX4 2018  | Ningxia | IMPERIAL HORSE |
| NX5 2019  | Ningxia | IMPERIAL HORSE |
| NX6 2015  | Ningxia | XIXIAKING WINERY (GROUP) CO., ltd |
| NX7 2016  | Ningxia | XIXIAKING WINERY (GROUP) CO., ltd |
| NX8 2017  | Ningxia | XIXIAKING WINERY (GROUP) CO., ltd |
| NX9 2018  | Ningxia | XIXIAKING WINERY (GROUP) CO., ltd |
| NX10 2019 | Ningxia | XIXIAKING WINERY (GROUP) CO., ltd |

H: samples collected in Hebei region; X: samples collected in Xinjiang region; NM: sample collected in Inner Mongolia region; NX: sample collected in Ningxia region.

Analysis of physicochemical parameters of wines

A fully automated wine analyzer measured free sulfur and total sugars, acids, and sulfur in wine samples (Y15, BioSystems, Spain). Ethanol was measured using a biosensing analyzer (SBA-40D, Zhongxing Weiyi Instruments Co., Ltd., China). The pH was measured using a pH meter. The total phenolic contents of the wines were determined using the Folin–Ciocalteu method (Jayaprakasha et al., 2001), and the
results are expressed as the equivalent value of gallic acid (mg/L). Total flavan was determined by the p-dimethylamino cinnamaldehyde method (Li et al., 1996), and the result is expressed as the equivalent value of catechin (mg/L). The total flavonoid was determined according to the method of Peinado (Peinado et al., 2009), and the result is expressed as rutin equal value (mg/L). The total anthocyanin content was quantified according to the color variation as a function of pH, and the result is expressed as mg/L of malvidin-3-O-glucoside (Meng et al., 2012). All measurements were performed in triplicate.

**Determination of condensed tannin concentration**

The condensed tannin content was determined by the protein precipitation method (Harbertson et al., 2003). A 2 mL wine sample was placed in a 10 mL centrifuge tube with 4 mL 1 g/L BSA buffer (BSA was added to buffer A containing 0.2 mol/L acetic acid and 0.17 mol/L NaCl adjusted to pH 4.9 with 10 % NaOH), mixed well, and permitted to react for 15 min. The mixture was centrifuged for 5 min at 15,000 g. After centrifugation, the supernatant was removed, and the protein-tannin pellet was mixed with 3.5 mL of buffer C (5 % TEA (v/v) and 5 % SDS (w/v) was adjusted to pH 9.4 with 2 mol/L HCl). The tube was vortexed to completely dissolve the protein-tannin precipitate. The solution was allowed to stand at room temperature for 10 min, and then the absorbance at 510 nm was determined. After zeroing with buffer C, 0.875 mL of the solution was aspirated, and placed on a cuvette to determine the absorbance value A1 at 510 nm. Then 0.25 mL of FeCl3 (0.01 mol/L, FeCl3 in 0.01 mol/L HCl) solution was added to the cuvette and incubated in the dark for 10 min. The absorbance value A2 was determined at 510 nm. The tannin absorbance value of the sample was calculated as A = A2-A1 and expressed in catechin equivalents (mg/L) compared with a standard curve prepared with catechin. Each sample was measured 3 times.

**Determination of constitutive subunits composition and mDP**

Condensed tannin composition was determined according to the method of Kennedy (Kennedy & Jones, 2001). Briefly, a 10 mL wine sample was decompressed, concentrated to 4 mL at 40 °C to remove alcohol, and diluted to 20 mL with water. The SPE-C18 column was activated with 25 mL methanol and 25 mL distilled water. Then the diluted was passed through the SPE-C18 column, followed by a wash with 50 mL distilled water, and finally dried for 15 min. Fifty milliliters of methanol were added to remove the methanol from the filling to obtain a tannin eluate. The tannin eluate was concentrated to dryness by rotary evaporation at 30 °C. The residues were dissolved with 2 mL chromatographic methanol. Two hundred microliters of tannins extracts were added to the same volume of phloroglucinol reagent solution (0.1 mol/L hydrochloric acid in methanol, containing 50 g/L phloroglucinol and 10 g/L ascorbic acid). The mixtures were placed at 50°C for 20 min, and the reaction was terminated by 5 volumes of 0.04 mol/L sodium acetate solution. Before injection, the solution was filtered through a 0.22 μm organic filter, and constitutive subunit composition and the mean degree of polymerization (mDP) of condensed tannin were analyzed by HPLC (Ju et al., 2021). HPLC chromatographic conditions: Synergi Hydro-RP C18 chromatographic column (250 mm × 4.6 mm, 4 μm) was used. Mobile phase A: water/formic acid (98:2, v/v), mobile phase B: acetonitrile/solvent A (80:20 v/v). Gradient elution program: 0–5 min, 0 % B; 5–35 min, 0–10 % B; 35–65 min, 10 % B; 65–70 min, 20–0 % B; 70–75 min, 0 % B. Flow rate: 0.8 mL/min; column temperature: 30 °C; detection wavelength: 280 nm; injection volume: 10 μL. A chromatogram that illustrates the peaks of monomeric flavan-3-ols is shown in Fig. S2. In order to calculate the percentages of terminal and extension subunits, the calibration curves of the flavan-3-ols-the C, EC, ECG, EGc, GC and ECGG were prepared.

**Sensory evaluation of wine**

The sensory panel did the wine sensory analysis following the sensory analysis process by Heras-Roger (Heras-Roger et al., 2016). Sensory evaluation of wine samples was performed by 11 professionally trained tasters (5 males and 6 females) aged between 21 and 27 years old, all with a solid foundation in wine tasting theory and professional sensory training. To ensure that all panelists exercised similar criteria in evaluating the sensory properties of the wines, we equipped the panelists with sensory evaluation training solutions. To train the panelists for distinguishing astringent, bitter, sweet, and sour tastes, 3 g/L commercial tannins, 15 mg/L quinine sulfate, 15 g/L sucrose, and 3 g/L tartaric acid were selected, respectively. The tasters were trained on the astringency intensity with 0 g/L, 1 g/L, 2 g/L, 3 g/L, 4 g/L, and 5 g/L tannin solutions. The sensory analyses of wines were performed in a standard wine sensory laboratory. The laboratory was equipped with a separate compartment at ambient temperature (20 ± 2 °C), free of noise or other interferences. Each panelist evaluated 30 mL of each wine sample. The panelist covered his or her tongue with the wine and gently stirred the wine with it. After 3 ~ 5 s, the panelist took a breath, held it for 3 s, and expectorated the wine. Upon completing the sensory analysis of one wine sample, the panelists were allowed to rinse their mouths twice with deionized water, chew soda crackers to recover their taste buds, and wait for at least 30 s before evaluating the subsequent sample. Each panelist completed the sensory evaluation of each wine sample within 3 min. When evaluating the astringency of wine samples, astringency quality was evaluated using descriptors such as silky, velvety, coarse, grainy, dry, and puckeriness (Table S2). Subsequently, the intensities of the drying astringency descriptors (puckeriness, coarse, graininess, and dry) and the intensities of the velvety astringency descriptors (silky and velvety) were evaluated by 5-point scales (1: no perception, 2: slight perception, 3: moderate perception, 4: strong perception, 5: very strong perception) (Campo et al., 2005; Loira et al., 2013). After the two astringency descriptors were selected, the intensity of drying astringency or the intensity of velvety astringency, and modified frequency (MF%), a mixture of intensity and frequency of detection were calculated. The quantitative intensity value was calculated as MF% = √(F(%)/I(%) / F), where F denotes the frequency of a particular astringent feature word, and I denotes the average intensity rate. Each convergent descriptor’s average intensity was calculated based on the intensity of the descriptors stated by all tasters. Modified frequency (MF%) was calculated according to the National Standard of the People’s Republic of China (GB/T 16861-1997, 1997). All wine samples were analyzed in duplicate.

**Statistical analysis**

Data are reported as mean ± standard deviation (SD, n = 3) values for the triplicate experiments. Duncan’s tests with a 0.05 significance level were used to analyze the data using SPSS 26.0 (IBM, USA). Sensory radar plots, and correlation heat maps were created using Origin 2021 (Origin Lab Corporation, USA). The condensed tannin content and condensed tannin profiles data were normalized by Z-score and subjected to hierarchical cluster analysis (HCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) by Umetrics SIMCA V14.1 (Umetrics, Sweden).

**Results and discussion**

**Wine physicochemical parameters**

Cabernet Sauvignon wines’ basic physicochemical parameters follow the International Vine and Wine Organization (OIV) standards (Table S3). The total acid was significantly higher in the NX region than in the NM region. With the aging increase, the total acid showed a downward trend, and the total acid of the wine aged for 1 year was...
significantly higher than that of wine aged for 5 years. A previous study reported that the wine’s total acidity determined its ultimate pH and affected its color and stability (Meng et al., 2012), thus playing an important role in the sensory quality of the wine. Total sugar, pH, and alcohol were significantly different between some regions, but the differences were not significant with aging periods. Flavanols and total anthocyanins changed during aging. There were no significant differences in flavanols in wines aged 1 to 3 years. However, the flavanol content in wines aged 1 to 3 years differed significantly from those aged 4 to 5 years. The total anthocyanin content in Cabernet Sauvignon wines aged for 1 year was 155.73 mg/L, and decreased to 35.34 mg/L after 5 years of aging. The longer the aging time, the lower the total anthocyanins content in wine. Although total anthocyanins form a series of more stable pigments during wine aging, these pigments may not be likely to accumulate (Zhang et al., 2021). The total phenols and flavonoid contents in the X region were significantly higher than those in the other three regions, and the content of the H producing area was the lowest.

Condensed tannin concentration in wines from different regions and aging periods

Condensed tannin entered the wine through maceration during fermentation. The condensed tannin content in the berries is not representative of that in wine (Romero-Cascales et al., 2005; Harbertson et al., 2003). Condensed tannin content and wine composition depend on the grape variety, age, and wine-producing regions (Bautista-Ortin et al., 2004). Condensed tannin concentration differs by production region (Fig. 1). In the NX region, condensed tannin concentration is significantly higher than in the H production area. This is probably due to the unique climatic, soil, and cultivation conditions of the NX region. During the growing season, the large temperature difference between day and night and the long sunlight hours helps the grapes photosynthesize, aiding sugar accumulation. The higher the ethanol content during wine fermentation, the more condensed tannins can be extracted from the skins and seeds (Gambuti et al., 2009). With the increase in aging time, condensed tannin concentration in Cabernet Sauvignon wines from the four regions showed an overall downward trend, which might be due to tannin precipitation after their association with other components or to the formation of pigmented tannin (Li & Sun, 2019). The condensed tannin concentration of the X and NM regions gradually decreases with aging, while the H and NX regions do not show this gradual decrease. A previous report found that climatic factors in the year of harvest led to higher levels of condensed tannins. In addition, relatively dry growing conditions also increase tannin concentrations in the grape skin (Koundouras et al., 2006).

Condensed tannin profiles in wines from different regions and wine aging periods

During wine aging, condensed tannins undergo structural rearrangements, including the fracture and formation of fusion bonds and precipitation reactions. Changes in the mDP of tannins reflect the changes in condensed tannins composition (Curko et al., 2021). As shown in Table 2, the mDP of Cabernet Sauvignon wines showed an overall decreasing trend from 2015 to 2019. The mDP of wines aged 1 year was significantly higher than wines aged 2 to 5 years. There was no significant difference in mDP for wines aged 2 to 4 years. This study found that the mDP of condensed tannins varied more in the 1st year of aging, followed by an increase in mDP of condensed tannins at 4 years of aging and a decrease in mDP of wines at the 5th year. It has been shown that the tannins’ mDP increases in the early stages of wine aging (Cano-Lopez et al., 2008). The mDP of condensed tannins usually decreases with aging (Chira et al., 2012). The mDP of Cabernet Sauvignon wines differs by region, with values NM > NX > X > H, and the mDP of the NM
| Wines | Age | tC | tEC | tEGC | eGC | eC | eEG | %PC | %PD | %G | mDP |
|-------|-----|----|-----|------|-----|----|-----|-----|-----|----|-----|
| H1    | 5   | 9.14 ± 36.5 | 4.96 ± 8.43 | 12.98 ± 11.44 | 3.09 ± 0.02A | 0.02A | 0.02A | 36.5 ± 4.96 | 8.43 ± 12.98 | 11.44 ± 3.09 |
| H2    | 5   | 16.58 ± 29.3 | 2.68 ± 3.99 | 80.19 ± 7.41 | 2.21 ± 12.4 | 0.02C | 0.02B | 29.3 ± 2.68 | 3.99 ± 80.19 | 7.41 ± 2.21 |
| X4    | 5   | 17.34 ± 13.0 | 5.37 ± 3.82 | 97.64 ± 9.6 | 2.63 ± 1.6 | 0.03C | 0.03B | 13.0 ± 5.37 | 3.82 ± 97.64 | 9.6 ± 2.63 |
| NM2   | 5   | 13.1 ± 14.1 | 3.27 ± 11.15 | 6.87 ± 6.36 | 2.51 ± 6.9 | 0.03A | 0.03B | 14.1 ± 3.27 | 11.15 ± 6.87 | 6.36 ± 2.51 |
| NX3   | 5   | 18.32 ± 18.1 | 3.2 ± 2.75 | 0.03A | 0.03B | 0.03B | 18.1 ± 3.2 | 2.75 ± 0.03 |
| NX6   | 5   | 14.3 ± 14.3 | 2.68 ± 3.99 | 80.19 ± 7.41 | 2.21 ± 12.4 | 0.02C | 0.02B | 14.3 ± 2.68 | 3.99 ± 80.19 | 7.41 ± 2.21 |
| H1    | 4   | 11.63 ± 26.8 | 4.61 ± 7.83 | 64.19 ± 22.89 | 3.52 ± 12.92 | 0.02B | 0.02A | 26.8 ± 4.61 | 7.83 ± 64.19 | 22.89 ± 3.52 |
| H6    | 4   | 15.67 ± 18.0 | 3.88 ± 7.39 | 67.02 ± 8.62 | 3.04 ± 6.9 | 0.02B | 0.02A | 18.0 ± 3.88 | 7.39 ± 67.02 | 8.62 ± 3.04 |
| X5    | 4   | 14.58 ± 6.7 | 5.02 ± 4.05 | 95.61 ± 2.79 | 2.5 ± 1.6 | 0.02A | 0.02B | 6.7 ± 5.02 | 4.05 ± 95.61 | 2.79 ± 2.5 |
| X1    | 3   | 13.91 ± 23.1 | 20.82 ± 29.0 | 90.52 ± 7.4 | 10.11 ± 2.08 | 0.02A | 0.02B | 23.1 ± 20.82 | 29.0 ± 90.52 | 7.4 ± 10.11 |
| X6    | 3   | 14.88 ± 16.9 | 5.07 ± 2.81 | 1.67 ± 1.78 | 9.25 ± 1.6 | 0.01A | 0.01B | 16.9 ± 5.07 | 2.81 ± 1.67 | 1.78 ± 9.25 |
| X3    | 3   | 5.22 ± 6.3 | 2.17 ± 2.08 | 90.52 ± 7.4 | 10.11 ± 2.08 | 0.02A | 0.02B | 6.3 ± 2.17 | 2.08 ± 90.52 | 7.4 ± 10.11 |
| X8    | 3   | 13.84 ± 14.5 | 3.19 ± 3.92 | 11.44 ± 18.23 | 2.6 ± 12.28 | 0.01A | 0.01B | 14.5 ± 3.19 | 3.92 ± 11.44 | 18.23 ± 2.6 |
| H4    | 2   | 6.88 ± 48.9 | 27.22 ± 5.14 | 44.38 ± 25.32 | 3.96 ± 30.3 | 0.02B | 0.02A | 48.9 ± 27.22 | 5.14 ± 44.38 | 25.32 ± 3.96 |
| H8    | 2   | 14.11 ± 22.5 | 4.4 ± 5.76 | 85.02 ± 8.62 | 2.04 ± 10.12 | 0.02A | 0.02B | 22.5 ± 4.4 | 5.76 ± 85.02 | 8.62 ± 2.04 |
| H2    | 2   | 15.54 ± 13.9 | 2.62 ± 20.4 | 74.91 ± 14.57 | 2.69 ± 10.53 | 0.03A | 0.03B | 13.9 ± 2.62 | 20.4 ± 74.91 | 14.57 ± 2.69 |
| X7    | 2   | 12.34 ± 19.1 | 5.17 ± 9.47 | 91.04 ± 8.55 | 2.73 ± 2.08 | 0.03A | 0.03B | 19.1 ± 5.17 | 9.47 ± 91.04 | 8.55 ± 2.73 |
| H9    | 3   | 17.2 ± 23.4 | 3.59 | 43.85 | 53.67 | 14.59 | 0.02A | 0.02B | 23.4 ± 3.59 | 8.53 ± 43.85 | 14.59 ± 53.67 |
| X3    | 1   | 0.11A ± 0.01B | 0.01A ± 0.01B | 0.01A ± 0.01B | 0.01A ± 0.01B | 0.01A ± 0.01B | 0.01B ± 0.01A | 0.01B ± 0.01A |
| X8    | 1   | 0.02A ± 0.02B | 0.02A ± 0.02B | 0.02A ± 0.02B | 0.02A ± 0.02B | 0.02A ± 0.02B | 0.02B ± 0.02A | 0.02B ± 0.02A |
| X10   | 1   | 10.11 ± 36.7 | 2.03 ± 27.88 | 67.69 ± 11.21 | 2.51 ± 22.11 | 0.02A | 0.02B | 36.7 ± 2.03 | 27.88 ± 67.69 | 11.21 ± 2.51 |

Note: tC: (+)-catechin terminal subunit, tEC: (-)-epicatechin terminal subunit, tEGC: (-)-epicatechin gallate terminal subunit, eGC: (+)-catechin extension subunit, eC: (-)-epicatechin gallate extension subunit, eEG: (-)-epigallocatechin terminal subunit, eEGC: (-)-epigallocatechin gallate extension subunit, %PC: the percentage of procyanidins, %PD: the percentage of prodelphinidins, %G: the percentage of galloylated flavonols, mDP: mean degree of polymerization.

Values are the mean ± standard deviation of the three experiments, and table containing lowercase letters indicate significant differences by regions (p < 0.05). The table containing different uppercase letters indicate significant differences by aging periods (p < 0.05). H: samples collected in Hebei region; X: samples collected in Xinjiang region; NM: sample collected in Inner Mongolia region; NX: sample collected in Ningxia region.
region is significantly higher than the other three regions.

Condensed tannins are mainly composed of the extension and terminal subunits of C, EC, ECG, and EGC. There were differences in the proportions of condensed tannins constitutive subunits (as mole%) among regions (Table 2). In the H region, ECG as the terminal subunits (tECG) was the most abundant subunit, followed by EC as the extension and terminal subunits (eEC and tEC). The ECG and EGC as the extension subunits (eECG and eEGC) were the least abundant in the H region. The eEC was the most abundant subunit in the X, NM, and NX regions. The eEC had the highest proportion of all the extension units in the four regions, consistent with previous studies (Watrelot et al., 2017). Ju et al. (2021) investigated the differences in the condensed tannins profiles of 15 red spine wines. They showed that eEC in red spine wines were higher than those of Baboso Negro, Negramoll and Tintilla red wines (from Spanish). The X region’s eECG, tEC and C as terminal subunits (tC) were the same. The C as extension subunits (eC) content was higher in the NM region than in other regions, and the contents of other subunits were not much different in the NX region except for eEC and eEGG. The NM region has the highest proportions of total extended units among the four wine-producing regions, followed by the NX and X regions, and the lowest in the H region. Light exposure affects grapes’ condensed tannins composition. When exposure increases, it increases the fruit’s EGC concentration, while decreasing the ECG content (Cortell & Kennedy, 2006). Visible light also affects the condensed tannins biosynthesis and increases the B-ring hydroxylation level in eEC, thus promoting EGC synthesis (Koyama et al., 2012). The H, X, and NM regions had the highest percentage of procyanidins (%PC), while the NX region had the highest percentage of prodelphinidin (%PD). The highest percentage of galloylated flavanols (%G) was found in the X region, followed by H and NX, and the lowest in the NM region. The %PD in the NM region is significantly lower than that of 1 ~ 2 years old.

Analysis of condensed tannins profiles in wines by multivariate statistical method

Condensed tannin content and profiles were normalized by Z-score, and samples that did not fall within the 95% confidence interval were screened using SMICA 14.1. As shown in Fig. 2, hierarchical cluster analysis (HCA) classified these samples into three main categories. It indicates that the condensed tannin subunit composition can be used to distinguish some wine-producing regions. Different aging periods’ samples are not well differentiated. On this basis, orthogonal partial least squares-discriminant analysis (OPLS-DA) was used to screen the major differential components in different regions. In the OPLS-DA model, three multivariate analysis methods, VIP, S diagram, and the loading diagram, are important to find labeled compounds. We selected components with VIP > 1 as marker components to distinguish Cabernet Sauvignon wines from different regions. This model utilized three major classes divided into hierarchical clusters as classification Y variables, and different tannin structure components and total condensed tannin content were used as X variables. In the OPLS-DA model ($R^2 = 0.981; Q^2 = 0.793; Q^2 = 0.621$), cross-validation of 200 replacement trials showed the model’s reliability. The results showed that the condensed tannin components with VIP > 1 were tEC, eECG, tC, %PC, eEC, eC, %PD. Compared to the condensed tannin content, the tannins components varied more among the different regions. The tEC was the main differential constitutive subunit, followed by eECG and tC. EC and C are isomers with different astringency intensities (Scharbert & Hofmann, 2005), and EC is more astringent at the same concentration (Xu et al., 2018). GC is much more astringent than C (Zhang et al., 2016). EGC showed the strongest astringency. This indicates that the differences in tannin profiles may cause differences in wine astringency. The astringency intensity of wine correlates with condensed tannins and has a strong positive relationship with the condensed tannin composition and mDP (Ma et al., 2014).

Astringency perceptions of Cabernet Sauvignon wines

The astringency of the wines was classified as dry or velvety astringencies. Sensory descriptors, such as coarse, grainy, dryness, and

![Fig. 2. Multivariate statistical analysis diagrams of different wine samples. Note: A, cluster analysis; B, orthogonal partial least square data analysis (OPLS-DA); C, VIP diagram; D, OPLS-DA replacement test result.](image-url)
Overall, astringency intensity of the four regions, but scored similarly to the other regions in terms of dryness astringency. The velvety astringency differed in their astringency. The NX region had the lowest astringency intensity was the greatest in the H region and the least in the NX region. The silky taste intensity was the greatest in the H appellation. The NM region wine sample showed a more pronounced rough taste than the other three regions. This may be related to the concentration of condensed tannins in the wines of different regions, which is positively correlated with dryness and puckery (Saenz-Navajas et al., 2020).

Similar to the wine-producing region, wine age periods impact astringency (Fig. 3B). Cabernet Sauvignon wines aged for 1 year felt more puckery than those aged 2, 3, and 5 years. Coarse, grainy, dryness, silky, and velvety astringency changed little in 1 ~ 4 years, but changed greatly in the fifth year of aging, manifested by the decrease of astringent intensity. As previously reported, the ruffling sensation gradually diminishes during aging (Ma et al., 2014). In addition, reactions between tannins and anthocyanins are the main phenolic reactions that diminish during aging (Ma et al., 2014). In addition, reactions between tannins and anthocyanins are the main phenolic reactions that diminish during aging.

**Correlation analysis between condensed tannins and astringency in different regions and aging periods**

Condensed tannins play an important role in the sensory aspects of red wines, especially for the astringency and bitterness (Kyraleou et al., 2020). As shown in Fig. 4, drying astringency was significantly and positively correlated with condensed tannin concentration, consistent with previous studies (Kyraleou et al., 2016; Rinaldi et al., 2012). However, other researchers found no statistically significant correlation between astringency and total tannin concentration (Quijada-Morín et al., 2012). Velvety astringency was significantly and negatively correlated with condensed tannin concentration, which may be related to anthocyanins in the wine. During wine aging, anthocyanins reacted with tannin to form polymeric pigments or pigmented tannin, which have different protein-binding properties than the tannins themselves and therefore help to reduce astringency and make the astringency more subdued.

Wine astringency is related to the condensed tannins concentration and strongly positively correlates with the condensed tannin profiles (Ma et al., 2014). The present study showed that drying and velvety astringency were positively correlated with mDP, and mDP significantly affected velvety astringency. However, the effect of mDP on astringency is currently controversial. It has been shown that there is a significant positive correlation between mDP and astringency (McRae et al., 2013). However, Quijada-Morín et al. (2012) observed no correlation between mDP and astringency. Moreover, for low mDP values (below 1.4), convergence increases with increasing mDP. For high mDP values (above 1.4), convergence decreases with increasing mDP. In contrast, the effect of mDP on convergence is related to their hydrophobicity (Ma et al., 2014).

Drying astringency was significantly negatively correlated with %G and significantly positively correlated with eEGC. Velvety astringency was not significantly correlated with %G. However, the literature has no agreement regarding the correlation between %G and astringency. Chira et al. (2011) observed a positive correlation between %G and astringency. Chira et al. (2012) showed that %G values were negatively correlated with the astringency of grape seed extracts. Conformational differences in tannin may explain this contradiction. However, measuring the tannin conformation in wines is currently challenging. Velvety astringency was significantly and negatively correlated with TC, and a highly significant positive correlation existed between velvety astringency and eEGC and %PD. Some studies illustrated the effect of %PC on astringency, including a strong negative linear relationship for %PC < 68%, a moderately strong positive linear relationship for 68% < %PC < 76%, and no relationship for %PC > 76% (Saenz-Navajas et al., 2020). The %PC in this study was below 68% in all production regions. There was a highly significant negative correlation between velvet astringency and %PC.

There was a highly significant negative correlation between aging periods and condensed tannin concentration, but no significant correlation between aging periods and condensed tannin subunits. This suggests that condensed tannins mainly precipitate with proteins and other substances with increasing aging rather than undergoing structural rearrangement. Previous studies have also shown that the chemical composition of wine may affect the changes in tannin properties during aging; long-term aging weakens this effect (Curko et al., 2021). Drying astringency negatively correlated with aging periods, and velvety

![Astringency radar map of different producing areas and aging periods. Note: H: samples collected in Hebei region; X: samples collected in Xinjiang region; NM: sample collected in Inner Mongolia region; NX: sample collected in Ningxia region.](image-url)
astringency positively correlated with aging periods. In general, large amounts of condensed tannins bind tightly to oral proteins and enhance astringency (Ma et al., 2014). As the aging time increases, condensed tannin characteristics change, and the astringency of the wine tends to be much velvety rather than drying astringency.

Conclusions

The results showed differences in condensed tannin concentration in different regions, and the concentration of condensed tannin in the NX region was significantly higher than that in the H region. With the increase in the aging period, the condensed tannin content in Cabernet Sauvignon wines from the four regions generally showed a downward trend. The eEC is the most common subunit of all the extension units in Cabernet Sauvignon wines from the four regions. The tEC was the main differential subunit in the four regions, followed by eECG and tC. During the aging process, the eEC proportion decreased gradually, and the eEC of the wine aged 5 years was significantly lower than that aged 1 ~ 2 years. The tEC of wine aged 5 years was significantly higher than those aged 1 and 3 years. Aging periods impacted astringency, and all kinds of astringency intensity decreased in the fifth year of aging. In addition, condensed tannin subunit composition was a more critical factor affecting astringency than condensed tannin concentration. Drying astringency is significantly and negatively correlated with %G. Velvety astringency is highly significantly and negatively correlated with %PC, and highly significantly positively correlated with %PD. There was a highly significant negative correlation between aging periods and condensed tannin concentration, but no significant correlation between aging periods and condensed tannin subunits. This study contributes to understanding the relationship between tannin characteristics and wine astringency in Cabernet Sauvignon wines from different regions of China and different aging periods.

CRediT authorship contribution statement

Qian Tu: Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Visualization. Shuzhen Liu: Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Visualization. Yuyu Li: Validation, Investigation, Writing – review & editing, Supervision. Lin Zhang: Validation, Investigation, Writing – review & editing, Supervision. Zhaoxiang Wang: Validation, Investigation, Writing – review & editing, Supervision. Chunlong Yuan: Conceptualization, Methodology, Investigation, Writing – review & editing, Funding acquisition, Writing – original draft, Supervision, Project administration.

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.

Acknowledgments

We are thankful to MOUTAI WINE CO., ltd, COFCO HUAXIA GREATWALL WINE CO., ltd, XINJIANG SUNYARD Wine CO., ltd, CITIC GUOAN WINE CO., Ltd, SUNSHINE TIANYU WINE CO., Ltd, IMPERIAL HORSE and XIXIAKING WINERY (GROUP) CO., Ltd for providing the wine of this work. This work was supported by the National Key Research and Development Project (item no. 2019YFD1002500-04); National key research and development program of Ningxia (2021BF02016) and The Key Industry Innovation Chain (Group), Agriculture of Shaanxi Province, China (no. 2020ZDLNY05-05).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100409.
