doi: 10.17221/429/2015-CJFS

# Comparing Phenolic Composition of Cabernet Gernischet Wines between Rain-shelter Cultivation and Open-field Cultivation

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**Abstract**

He Y., Ning P., Yue T., Zhang Z. (2015): **Comparing phenolic composition of Cabernet Gernischet wines between rain-shelter cultivation and open-field cultivation**. Czech J. Food Sci., 34: 254–270.

The phenolic compounds of *Vitis vinifera* cv. Cabernet Gernischet wine in 2010 and 2011 vintage from rain-shelter cultivation and open-field cultivation were detected and compared by high performance liquid chromatography coupled with diode array detector and electrospray ionisation mass spectrometry. The results showed that rain-shelter cultivation increased the total anthocyanin and non-anthocyanin phenolic compound concentration. For the compositions, ratios of peonidin-derivative and malvidin-derivative pigments were similar in the two vintages, rain-shelter cultivation increased the proportion of these derivatives; ratios of delphinidin-derivative and petunidin-derivative pigments were different in the two vintages. In the wine of rain-shelter cultivation, the ratio of flavan-3-ol and hydroxybenzoic acids was increased, the ratio of flavonol was decreased and the composition of other compounds varied between the two vintages. To most of the phenolic compounds identified, their content in wine under rain-shelter cultivation was higher compared to those under open-field cultivation.

**Keywords**: anthocyanins; non-anthocyanins phenolic compounds; HPLC-MS

*Vitis* is a worldwide cultivated fruit, and with the Chinese wine industry developing in recent years, the grape planting area in China has increased rapidly. It reached 680 Mha in 2013, ranking the fourth in the world according to the OIV statistical report on world vitiviniculture (OIV 2013). Located on the Pacific Ocean West Bank, most of China enjoys a marked continental monsoonal climate, characterised by hot and rainy summers-autumns, cold and dry winters-springs. Such climatic conditions are unfavourable for grape growth, sugar accumulation, organic acid degradation, and phenolic compound formation, and they seriously hinder the development of the grape and wine industry in China, and improvement of China's wine quality.

To overcome the disadvantages caused by local climatic conditions installation cultivation was introduced into China in the 1980s; it is a fruit culture practice using an artificial microclimate that meets fruit growth requirements in some installations in the case of an unfavourable natural environment for fruit growth (He 1999). Rain-shelter cultivation is usually implemented in the production of table grape berries in the south of China (Meng *et al.* 2013). It has indicated that the incidence of some major diseases, such as downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), botrytis (*Botrytis cinerea*), rip rot (*Glomerella cingulata*), and sour rot (imperfect yeasts), can be effectively reduced by rain-shelter cultivation, by keeping rainwater away from leaves and fruits (Chavarria *et al.* 2007), which reduced the use of pesticides in vineyards (Cortell *et al.* 2007).

In wine, phenolic compounds are a part of the major quality factors which directly influence some important organoleptic characteristics such as col-

Supported by the National Technology System for Grape Industry, Project No. CARS-30-zp-9.

Yan-Nan He and Peng-Fei Ning equally contributed to this work.

doi: 10.17221/429/2015-CJFS

our, flavour, bitterness, and astringency (Garrido & Borges 2011). Most of the phenolic compounds in wine originate from the grape berry, and some come from microbial and oak sources (Kennedy 2008). Phenolic compounds are mainly found in grape skins and seeds, and they are extracted from grape skins and seeds into wines by maceration. Many factors such as grape variety, edaphoclimatic conditions and cultural and technological practices, may influence the phenolic composition of wines (Cynkar *et al*. 2009; Obreque-Slier *et al*. 2010; Puértolas *et al*. 2010; Meng *et al*. 2013). It has been reported that rain-shelter cultivation effectively delayed the maturation of grapes (Berli *et al*. 2011; Meng *et al*. 2013), slowed sugar accumulation by reducing photosynthetically active radiation (PAR) (Chavarria *et al*. 2008; Chavarria *et al*. 2009; Conceição & Marin 2009), increased berry and cluster weight, and improved economic returns (Tangolar *et al*. 2007). During the several last decades, although rain-shelter cultivation has been studied for its commercial value on table grapes and other fruits (Fanizza & Ricciardi 1991; Tangolar *et al*. 2007; Chavarria *et al*. 2011; Pedro Júnior *et al*. 2011), few studies have focused on wine grapes and wine. What is more, to date there is no published literature on the phenolic profiles in the wines of Cabernet Gernischet grape (*Vitis vinifera* L.) cultured under a rain-shelter model.

For all the above, the purpose of this study was to compare the phenolic profiles of Cabernet Gernischet wine whose grape berries were cultured under rain-shelter and open-field conditions. Based on the practical purpose, this study was conducted to provide sufficient experimental evidence for further application and expansion of rain-shelter cultivation. And makes it possible to cultivate Cabernet Gernischet grapes and makes the wine industry in an organic production system.

## MATERIAL AND METHODS

***Plant and wine material***. A two-year study (2010 and 2011) was conducted at the Grape Demonstration Base of the College of Oenology, Northwest A&F University, Jingyang County of Shaanxi Province, China (34°40'56''N, 108°38'53''E). The mesoclimate of the vineyard during the growing season (from April to September) of the two vintages is shown in Table 1. The own-rooted cv. Cabernet Gernischet (*Vitis vinifera* L.) were planted in 2006, and rows were north-south oriented on the flat terroir with sandy soil. Distances of vines and rows were spaced at 1.8 × 2.7 m and pruned to two buds per spur. Vines were trained to a bilateral cordon at 0.8 m above the ground, in which shoots were trained upwards and each vine carried *ca.* 20 grape clusters. The vertical shoot-positioned canopies were uniformly managed. All vines were divided into two groups. Group one was grown using the rain-shelter cultivation technology. Shelters were built along the vine rows before berry coloration (on June 30, 2010 and July 3, 2011) and were 2.2 m high, 1.7 m wide, and covered with colourless and transparent polyethylene film. The film 0.03 mm in thickness was bought from the agricultural materials company of Jingyang. Group two was a control and it was cultured on the open field (open-field cultivation).

Small-scale wine making was conducted using the following protocol: control and rain-shelter cultivation fruits (20 kg for each of the three replicates per treatment) were harvested at their physiological maturity, the harvest date was August 22 for open-field cultivation and September 18 for rain-shelter cultivation in 2010 vintage, and August 30 and September 15 for the two respective variants in 2011 vintage (Table 2). The grapes were crushed, destemmed, and placed separately in stainless steel tanks immediately

Table 1. Mesoclimate of vineyards during the growing season of the 2010 and 2011 vintages

| Parameter | April | May | June | July | August | September | April–September |
|---|---|---|---|---|---|---|---|
| **Vintage 2010** | | | | | | | |
| Average temperature (°C) | 13.2 | 19.9 | 25.4 | 27.2 | 24.7 | 21.3 | 22.0 |
| Rainfall (mm) | 41.0 | 42.7 | 23.8 | 77.0 | 145.1 | 85.9 | 415.5 |
| Sunlight hours | 170 | 150.5 | 219.6 | 158 | 145.4 | 152.1 | 995.6 |
| **Vintage 2011** | | | | | | | |
| Average temperature (°C) | 16.5 | 19.4 | 25.5 | 26.5 | 24.1 | 18.3 | 21.7 |
| Rainfall (mm) | 15.2 | 83.5 | 25.7 | 50.3 | 87.2 | 321.1 | 583.0 |
| Sunlight hours | 224.6 | 197.5 | 200.5 | 183.1 | 189.1 | 94.7 | 1089.5 |

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Table 2. Physicochemical parameters of the must and wine in different treatments at winemaking stages

| | Vintage | | Harvest date | Total sugar[#] (g/l) | Total acidity[$] (g/l) | pH | Ethanol (% vol) |
|---|---|---|---|---|---|---|---|
| Must after filling the tank | 2010 | T1 | 09–18 | 179.24 ± 6.35$^{Aa}$ | 4.67 ± 0.38$^{Aa}$ | – | – |
| | | T2 | 08–22 | 171.21 ± 5.61$^{Aa}$ | 4.23 ± 0.35$^{Aa}$ | – | – |
| | 2011 | T1 | 08–30 | 176.74 ± 7.25$^{Aa}$ | 3.56 ± 0.16$^{Ab}$ | – | – |
| | | T2 | 09–15 | 153.71 ± 5.31$^{Bb}$ | 3.89 ± 0.28$^{Aa}$ | – | |
| At the end of alcoholic fermentation | 2010 | T1 | – | 1.84 ± 0.18$^{Aa}$ | 5.60 ± 0.42$^{Aa}$ | 3.56 ± 0.15$^{Aa}$ | 12.60 ± 1.24$^{Aa}$ |
| | | T2 | – | 1.91 ± 0.21$^{Aa}$ | 6.40 ± 0.46$^{Aa}$ | 3.30 ± 0.12$^{Aa}$ | 10.05 ± 1.03$^{Aa}$ |
| | 2011 | T1 | – | 1.70 ± 0.35$^{Aa}$ | 4.82 ± 0.35$^{Bb}$ | 3.39 ± 0.14$^{Aa}$ | 11.3 ± 0.58$^{Aa}$ |
| | | T2 | – | 2.10 ± 0.31$^{Aa}$ | 5.88 ± 0.42$^{Aa}$ | 3.19 ± 0.09$^{Aa}$ | 11.2 ± 0.46$^{Aa}$ |

[#]concentration represented by glucose; [$]concentration presented by tartaric acid; T1 – rain- shelter cultivation; T2 – open-field cultivation; the same letter mean are not statistically different in different treatments ($P < 0.05$)

after their transportation to the laboratory. Fifty mg/l sulphur dioxide and 25 mg/l pectinase (LALLZYME EX; Lallemand Company, Bourgogne, France) were added to the musts and the contents were mixed by hand. After maceration of the musts for 24 h, 200 mg/l active dry wine yeast (*Saccharomyces cerevisiae* strain RC212; Lavlin, Bourgogne, France) was added to the musts according to commercial specifications. Alcoholic fermentation was carried out at 22–25°C to dryness (reducing sugar < 4 g/l), and this process lasted for about 10 days for each wine. Throughout this period, three times per day mass homogenisations were performed to dissolve the cap of the wine. Temperature and density were also recorded three times per day to evaluate fermentation arrests. At the end of alcoholic fermentation, the wines were separated from pomace, and 50 mg/l $SO_2$ were added. After fermentation, the wine samples were bottled and stored at 4°C prior to analysis (for physicochemical parameters see Table 2). The phenolic compounds were detected four months after the wines were well fermented each year (around 2011–02–20 and 2012–02–24).

***Chemicals and standards***. The standards of all phenolic compounds, including (+)-catechin, quercetin, gallic acid, caffeic acid, *trans*-resveratrol, and malvidin-3-*O*-glucoside, were bought from Sigma-Aldrich Co. (St. Louis, USA). The purities of all the six standards were > 97%. Methanol, formic acid, acetonitrile, and glacial acetic acid (HPLC grade) were obtained from Fisher Co. (Fairlawn, USA). Ethyl acetate (AR) was from Tianjin Bodi Chemical Reagent Co. Ltd. (Tianjin, China). All other chemicals used were analytical grade.

***Determination of some basic physicochemical parameters***. Residual sugar, total acid, and alcohol content were quantified according to the National Standard of the People's Republic of China (GB/T15038:2006). The extraction and determination of total anthocyanins and total non-anthocyanins were based on available methods developed by the Centre for Viticulture and Oenology, China Agricultural University (He *et al*. 2010; Li *et al*. 2011).

***Extraction of phenolic compounds***. For non-anthocyanin phenolics (including flavan-3-ols, flavonols, hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes), 100 ml wine, 100 ml distilled water, and 80 ml ethyl acetate were added to a separatory funnel, thoroughly mixed and let stand for the mixer solution layering, the aqueous phase was separated, then 30 ml ethyl acetate were added, let stand for layering, the aqueous phase was separated again, the upper ester phase was removed to a 250 ml round bottom flask, extracted three times repeatedly. And the ester phase was subjected to rotary evaporation (SENCO-R series; Shanghai Shensheng Biotech Co. Ltd., Shanghai, China) at 35°C under vacuum to dry. Then the dryness was redissolved in methanol to 5 ml. This methanol solution was filtered through a 0.45-μm organic membrane and analysed by high-performance liquid chromatography (HPLC) coupled with diode array detector (DAD) and electrospray ionisation mass spectrometry (ESI-MS).

For anthocyanins, the wine was filtered through a 0.45-μm organic membrane. Finally, the resulting filtrates were used for qualitative and quantitative analyses by HPLC-DAD/ESI-MS.

***HPLC-DAD/ESI-MS analysis of phenolic compounds***. An Agilent 1200 series LC-MSD trap XCT system (Agilent Corporation, Santa Clara, USA) equipped with a G1322A degasser, G1312B bin pump, G1367C HiP-ALS autosampler, G1316B TCC (ther-

doi: 10.17221/429/2015-CJFS

mostated column compartment), G1314C VWD (variable wavelength detector) and a reversed phase column (ZORBAX SB-C18, 3 × 50 mm, 1.8 μm) was used for non-anthocyanin detection. The mobile phase consisted of (A) 1% acetic acid in water solution and (B) 1% acetic acid in acetonitrile solution. The elution profile had the following proportions (v/v) of solvent B: 0–5 min, 5–8%; 5–7 min, 8–12%; 7–12 min, 12–18%; 12–17 min, 18–22%; 17–19 min, 22–35%; 19–21 min, 35–100%; 21–25 min, 100%; 25–27 min, 100–105%. The column was held at 25°C and was flushed at a flow rate of 1.0 ml/min. The injection volume was 2 μl and analyses were detected at 280 nm. MS conditions were as follows: electrospray ionisation (ESI) interface, negative ion model, nebuliser pressure 35 psi, dry gas flow rate 10 ml/min, dry gas temperature 325°C, and scans between *m/z* 100 and 1000 (Li *et al*. 2011).

An Agilent 1100 series LC-MSD trap VL system (Agilent, Santa Clara, USA), equipped with a G1379A degasser, G1312BA QuatPump, G1313A ALS, G1316A column, G1315A DAD, and a Kromasil 100-5-C18 column (250 × 4.6 mm, 5 μm) was used for anthocyanin detection. The mobile phase consisted of (A) 6% (v/v) acetonitrile containing 2% (v/v) formic acid and (B) 54% (v/v) acetonitrile containing 2% (v/v) formic acid. The elution profile had the following proportions (v/v) of solvent B: 0–1 min, 10%; 1–18 min, 10–25%; 18–20 min, 25%; 20–30 min, 25–40%; 30–35 min, 40–70%; 35–40 min, 70–100%; 40–45 min, 100–10%. The column was held at 50°C and was flushed at a flow rate of 1.0 ml/minutes. The injection volume was 30 μl. Diode array detection was performed from 200 nm to 900 nm and quantification was carried out by peak area measurements at 525 nm. MS conditions were as follows: electrospray ionisation (ESI) interface, positive ion model, nebuliser pressure 35 psi, dry gas flow rate 10 ml/min, dry gas temperature 325°C, and scans between *m/z* 100 and 1000.

All phenolic compounds were identified by comparison of their order of elution and retention time with those of standards and the weight of molecular ion and the fragment ion was compared with standards and references (de Villiers *et al*. 2004; Peña-Neira *et al*. 2007; Han *et al*. 2008; Fanzone *et al*. 2012; Zhu *et al*. 2012). Quantitative determinations were done by using the external standard method with the commercial standards. The calibration curves were obtained by injection of standard solutions under the same conditions as for the samples analysed, over the range of concentrations observed. The compounds for which no standards were available were quantified with the curves of quercetin (flavonols and dihydroflavonols), *trans*-resveratrol (stilbenes), gallic acid (hydroxybenzoic acids), caffeic acid (hydroxycinnamic acids), (+)-catechin (flavan-3-ols), and malvidin-3-*O*-glucoside (anthocyanins). Therefore, flavonols, flavan-3-ols, hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes were respectively expressed as quercetin equivalence (QE), (+)-catechin equivalence (CE), gallic acid equivalence (GAE), caffeic acid equivalence (CAE), *trans*-resveratrol equivalence (RE), and malvidin-3-*O*-glucoside equivalence (ME) per ml. All of the analyses were performed in duplicate.

***Statistical analysis.*** Data were reported as mean ± standard deviation (SD) values of triplicate experiments, and were analysed using SPSS 18.0 (2013). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the significance of the difference between samples, with a significance level of 0.05.

## RESULTS AND DISCUSSION

***Physicochemical parameters***. As the raw material of wine production, the quality of grape directly affects wine composition and quality (Gil *et al*. 2013). Some families from grape are well known to be closely related to sensory attributes of wine such as colour and tasting perceptions like bitterness, astringency, and mouthfeel (He *et al*. 2012a). The oenological parameters (Table 2) show that good ripeness and uniformity were achieved in the grapes. While it should be noted, in 2011 vintage, to overcome the diseases caused in grape, the treatment under open-field cultivation was harvested earlier than the other. Except for the total sugar content of open-field cultivation in 2011 vintage, there were no significant differences in the must total sugar, total acidity before fermentation and in the alcohol content of wines for all treatments (Table 2). As reported in the previous studies (Considine & Kriedemann 1972; Gladstones 2005; Meng *et al*. 2013) rain-shelter cultivation reduced the berry diseases such as downy mildew and botrytis rots. As a result, a longer and better environment for the grape growing under rain-shelter cultivation was achieved. In our study, in the two years, grapes under rain-shelter cultivation were harvested 27 and 15 days later than those under open-field cultivation, respectively. So that grapes

doi: 10.17221/429/2015-CJFS

under rain-shelter cultivation have a higher sugar content. And in the corresponding wines, higher alcohol content, higher residual sugar content, and lower acidity content.

***Effect of rain shelter on phenolic compounds of wine***. In red wine the phenolic compounds are important because they contribute to the following properties: colour, astringency, bitterness, oxidation reactions, interactions with proteins, and aging behaviour. Anthocyanins and other derived pigments mainly contributed to the colour (He *et al*. 2012b); however, other phenolic compounds, such as phenolic acids, flavonols and flavanols, which exert a copigmentation effect will also have the influence on the wine colour (Boulton 2001). Flavanols and their polymers (proanthocyanidins) are also related to the bitterness and astringency of wine (Peleg *et al*. 1999), and some relations have been established between these perceptions and the proanthocyanidin structures (De Freitas & Mateus 2001).

A total of 34 phenolic compounds were detected in all samples, with anthocyanins and non-anthocyanins being the two categories. Tables 3 and 4 list all the phenolic compounds identified and their quantitative results.

***Anthocyanins***. In grapes and young wines, anthocyanins are mainly responsible for colour (Ferrandino & Guidoni 2010; Zhu *et al*. 2012). They are mostly present in grape skins and are transferred to wine through maceration during alcoholic fermentation (Kennedy 2008). In this study, a total of 18 anthocyanins were detected in wines of two vintages. There were 16 and 17 anthocyanins identified in rain-shelter cultivation (T1) and open-field cultivation (T2) wines, respectively, in 2010 vintage; while 10 and 11 in 2011 vintage. Dephinidin-3-*O*-(6-*O*-acetyl)-glucoside was found only in rain-shelter cultivation, peonidin-3-*O*-(6-*O*-acetyl)-glucoside and peonidin-3-*O*-glucoside-4-vinylphenol were detected only in open-field cultivation in 2010 vintage. In 2011 vintage, trans-malvidin-3-*O*-(6-*O*-coumaryl)-glucoside was found only in open-field cultivation (Table 3). The main compounds making difference were delphinidin-3-*O*-monoglucoside, petunidin-3-*O*-monoglucoside, petunidin-3-acetylglucoside, malvidin-3-*O*-monoglucoside, malvidin-3-*O*-monoglucoside-acetaldehyde,

Table 3. Concentrations (mg/l) of anthocyanin compounds in different treatment wines at the end of alcoholic fermentation (AF) in two vintages

| Anthocyanins | 2010 | | 2011 | |
|---|---|---|---|---|
| | T1 | T2 | T1 | T2 |
| Delphinidin-3-*O*-monoglucoside | 2.78 ± 0.03$^{Aa}$ | 1.36 ± 0.31$^{Bb}$ | 4.51 ± 0.33$^{Aa}$ | 4.18 ± 0.25$^{Aa}$ |
| Delphinidin-3-acetylglucoside | 1.26 ± 0.04 | nd | nd | nd |
| Petunidin-3-*O*-monoglucoside | 2.91 ± 0.05$^{Aa}$ | 2.03 ± 0.33$^{Ab}$ | 2.90 ± 0.28$^{Aa}$ | 2.22 ± 0.18$^{Bb}$ |
| Petunidin-3-acetylglucoside | 3.49 ± 0.02$^{Aa}$ | 2.45 ± 0.75$^{Ab}$ | 2.28 ± 0.41$^{Aa}$ | 2.66 ± 0.26$^{Aa}$ |
| Petunidin-3-coumaroylglucoside | 3.90 ± 0.1$^{Aa}$ | 2.43 ± 1.56$^{Aa}$ | nd | nd |
| Peonidin-3-*O*-monoglucoside | 1.79 ± 0.01$^{Aa}$ | 1.68 ± 0.12$^{Aa}$ | 1.42 ± 0.05$^{Aa}$ | 1.44 ± 0.08$^{Aa}$ |
| Peonidin-3-acetylglucoside | nd | 1.76 ± 0.62 | 3.89 ± 0.88$^{Aa}$ | 2.16 ± 0.26$^{Ab}$ |
| Peonidin-3-coumaroylglucoside | 2.66 ± 0.2$^{Aa}$ | 2.01 ± 0.44$^{Aa}$ | nd | nd |
| Peonidin-3-*O*-monoglucoside-pyruvid acid | nd | 1.49 ± 0.48 | nd | nd |
| Malvidin-3-*O*-monoglucoside | 77.05 ± 1.22$^{Aa}$ | 55.67 ± 6.44$^{Bb}$ | 95.10 ± 33.56$^{Aa}$ | 58.47 ± 25.36$^{Aa}$ |
| Malvidin-3-*O*-monoglucoside-acetaldehyde | 39.96 ± 0.56$^{Aa}$ | 29.31 ± 3.56$^{Ab}$ | 45.09 ± 6.34$^{Aa}$ | 31.11 ± 5.58$^{Ab}$ |
| Malvidin-3-coumaroylglucoside | 14.49 ± 1.05$^{Aa}$ | 7.98 ± 1.12$^{Bb}$ | nd | 1,16 |
| Malvidin-3-caffeoylglucoside | 3.45 ± 0.33$^{Aa}$ | 2.78 ± 0.21$^{Ab}$ | nd | nd |
| Malvidin-3-*O*-acetylglucoside | 1.33 ± 0.48$^{Aa}$ | 1.89 ± 0.44$^{Aa}$ | 1.26 ± 1.35$^{Aa}$ | 3.03 ± 1.25$^{Aa}$ |
| Malvidin-3-*O*-monoglucoside-pyruvid acid | 3.18 ± 1.21$^{Bb}$ | 6.23 ± 1.12$^{Aa}$ | 5.93 ± 0.5$^{Aa}$ | 5.86 ± 0.35$^{Aa}$ |
| Malvidin-3-*O*-acetylglucoside-acetaldehyde | 2.86 ± 0.26$^{Aa}$ | 3.05 ± 0.13$^{Aa}$ | nd | nd |
| Malvidin-3-*O*-acetylglucoside-pyruvid acid | 2.77 ± 0.66$^{Ab}$ | 4.73 ± 0.36$^{Aa}$ | nd | nd |
| Malvidin-3-*O*-monoglucoside-ethyl-(epi)catechin | 2.15 ± 0.31$^{Aa}$ | 2.41 ± 0.22$^{Aa}$ | 1.51 ± 0.22$^{Ab}$ | 4.17 ± 1.21$^{Aa}$ |

T1 – rain-shelter cultivation; T2 – open-field cultivation; nd – not detected; different letters indicate significant differences according to Duncan's test in different treatments ($P < 0.05$)

doi: 10.17221/429/2015-CJFS

Table 4. Concentrations (mg/l) of the non-anthocyanin phenolic compounds in different treatment wines at the end of alcoholic fermentation (AF) in two vintages

| Nonanthocyanins phenolic compounds | 2010 | | 2011 | |
|---|---|---|---|---|
| | T1 | T2 | T1 | T2 |
| **Flavan-3-ols** | | | | |
| Catechin | 49.79 ± 5.33$^{Aa}$ | 39.51 ± 4.62$^{Ab}$ | 30.07 ± 10.26$^{Aa}$ | 18.17 ± 8.75$^{Aa}$ |
| Epicatechin | 72.30 ± 15.26$^{Aa}$ | 42.30 ± 12.13$^{Bb}$ | 26.37 ± 6.33$^{Aa}$ | 14.96 ± 5.64$^{Aa}$ |
| Procyanidin dimer 2 | 57.88 ± 5.12$^{Aa}$ | 52.91 ± 6.42$^{Aa}$ | 28.38 ± 3.86$^{Aa}$ | 9.28 ± 1.25$^{Ab}$ |
| Procyanidin trimer 1 | 21.36 ± 3.46$^{Aa}$ | 17.30 ± 1.52$^{Aa}$ | 13.74 ± 1.63$^{Ab}$ | 23.53 ± 4.25$^{Aa}$ |
| Gallocatechin | nd | nd | 1.09 ± 0.03$^{Aa}$ | 1.04 ± 0.02$^{Aa}$ |
| **Flavonols** | | | | |
| Quercetin-3-*O*-glucoside | 3.65 ± 0.23$^{Aa}$ | 3.48 ± 0.28$^{Aa}$ | 1.48 ± 0.08$^{Aa}$ | 1.50 ± 0.05$^{Aa}$ |
| Quercetin-3-*O*-glucuronide | 2.24 ± 0.15$^{Aa}$ | 1.93 ± 0.23$^{Aa}$ | 2.34 ± 0.36$^{Aa}$ | 1.88 ± 0.15$^{Aa}$ |
| Quercetin-3-*O*-galactoside | 3.07 ± 0.11$^{Aa}$ | 1.82 ± 0.04$^{Bb}$ | 2.18 ± 0.16$^{Aa}$ | 2.15 ± 0.12$^{Aa}$ |
| Dihydroquercetin-*O*-hexoside | 5.72 ± 0.25$^{Aa}$ | 4.05 ± 0.31$^{Ab}$ | 3.37 ± 0.22$^{Aa}$ | 3.20 ± 0.15$^{Aa}$ |
| Laricitrin-3-*O*-glucoside | 3.17 ± 0.26$^{Aa}$ | 3.63 ± 0.35$^{Aa}$ | 3.88 ± 0.33$^{Aa}$ | 3.03 ± 0.35$^{Aa}$ |
| Myricetin-3-*O*-glucoside | 4.95 ± 0.34$^{Aa}$ | 5.55 ± 0.26$^{Aa}$ | 2.48 ± 0.65$^{Aa}$ | 1.59 ± 0.34$^{Aa}$ |
| Kaempferol-3-*O*-galactoside | 5.02 ± 1.25$^{Aa}$ | 2.72 ± 0.28$^{Ab}$ | 1.14 ± 0.12$^{Aa}$ | 1.29 ± 0.08$^{Aa}$ |
| **Hydroxybenzoic acids** | | | | |
| Dimer (*epi*)gallocatechin-(*epi*)catechin | 0.15 ± 0.04$^{Aa}$ | 0.07 ± 0.02$^{Ab}$ | 0.06 ± 0.01$^{Aa}$ | 0.04 ± 0.01$^{Aa}$ |
| Syringetin-3-*O*-glucoside | 0.45 ± 0.05$^{Aa}$ | 0.34 ± 0.02$^{Ab}$ | 0.81 ± 0.03$^{Aa}$ | 0.78 ± 0.02$^{Aa}$ |
| **Hydroxycinnamic acids** | | | | |
| Ferulic acid | 1.01 ± 0.12$^{Ab}$ | 1.64 ± 0.11$^{Aa}$ | 1.91 ± 0.22$^{Aa}$ | 1.60 ± 0.13$^{Aa}$ |
| **Stilbenes** | | | | |
| Resveratrol | 0.82 ± 0.03$^{Aa}$ | 0.79 ± 0.01$^{Aa}$ | 1.18 ± 0.12$^{Aa}$ | 0.93 ± 0.05$^{Aa}$ |

T1 – rain-shelter cultivation; T2 – open-field cultivation; nd – not detected; different letters indicate significant differences according to Duncan's test in different treatments ($P < 0.05$)

malvidin-3-coumaroylglucoside, malvidin-3-caffeoylglucoside, peonidin-3-acetylglucoside, and malvidin-3-*O*-monoglucoside-ethyl-(*epi*)catechin, in which the content of delphinidin-3-*O*-monoglucoside, petunidin-3-*O*-monoglucoside, petunidin-3-acetylglucoside, malvidin-3-*O*-monoglucoside, malvidin-3-*O*-monoglucoside-acetaldehyde, malvidin-3-coumaroylglucoside, malvidin-3-caffeoylglucoside was observably higher in rain-shelter cultivation (T1) in 2010 vintage; in 2011 vintage, malvidin-3-*O*-monoglucoside-acetaldehyde, petunidin-3-acetylglucoside, and peonidin-3-acetylglucoside1-pentanol were higher in rain-shelter cultivation (T1), while malvidin-3-*O*-monoglucoside-ethyl-(*epi*)catechin was higher in open-field cultivation (T2) and the difference was remarkable. In all the anthocyanins detected in the two years, malvidin monoglucoside and its derivatives were dominant anthocyanins in the two vintages of wine and accounted for an average of 88–90% of the total anthocyanin compounds identified, followed by the petunidin and peonidin class, while the delphinidin class was the least, and the cyanidin class was not detected in any wine (Figure 1).

According to the difference in substituents on the B-ring of the anthocyanin, anthocyanins can be divided into two groups: 3'-substituted anthocyanins and 3',5'-substituted anthocyanins. The former include cyanidin and peonidin monoglycosides and their acylated derivatives, and the latter consist of glycosylated forms of delphinidin, petunidin, and malvidin and their acylated derivatives (Boss *et al.* 1996). The biosynthetic pathways of 3'- and 3', 5'-substituted anthocyanins in grapes are different, the former coming from dihydroquercetin and the latter from dihydromyricetin. Tanaka *et al.* (2008) confirmed that the ratio of 3'-substituted anthocyanins to 3', 5'-substituted anthocyanins depends upon the transcriptional levels of the flavonoid 3'-hydroxylase (F3'H) and the flavonoid 3', 5'-hydroxylase (F3'5'H). F3'H and F3'5'H can convert dihydrokaempferol into

doi: 10.17221/429/2015-CJFS

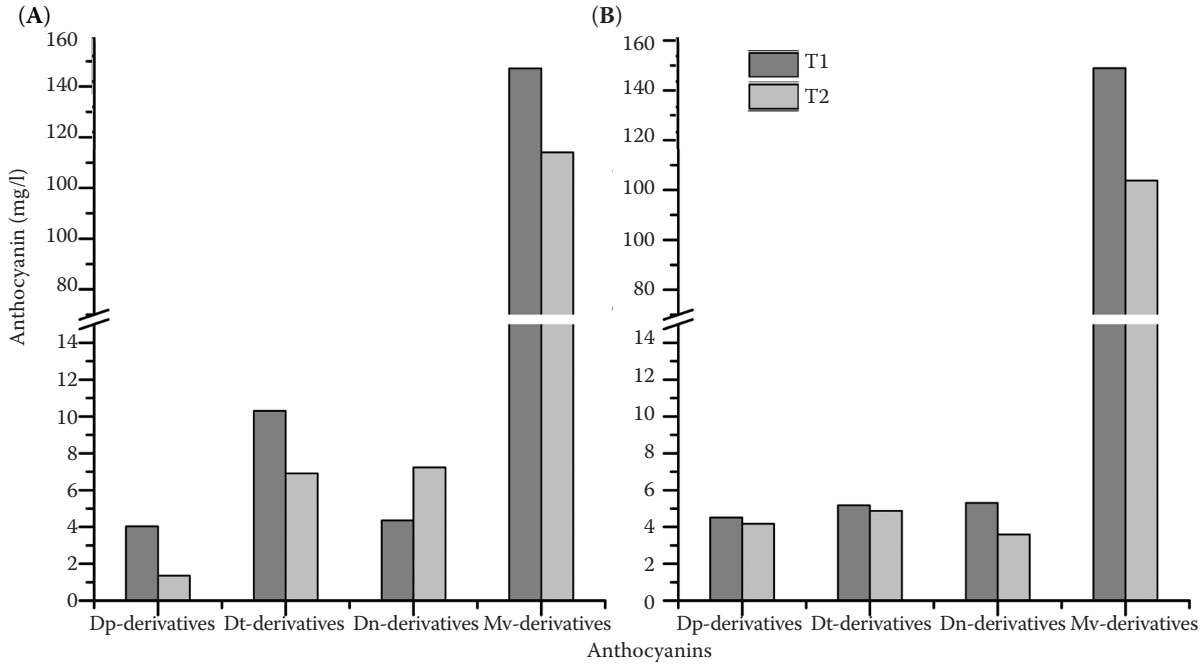


Figure 1. Concentrations of anthocyanin compounds in wines in 2010 (**A**) and 2011 (**B**)

The breaks were all from 15 to 50; T1 – rain-shelter cultivation; T2 – open-field cultivation; Dp – Delphinidin; Pt – Petunidin; Pn – Peonidin; Mv – Malvidin

dihydroquercetin and dihydromyricetin, as well as their derivatives, respectively. They are considered as two parallel branch pathways. In the downstream pathways of these two branches, cyanidin-derived anthocyanins and delphinidin-derived anthocyanins are synthesised from dihydroquercetin in the F3'H branch pathway and dihydromyricetin in the F3'5'H branch pathway, respectively (Mattivi *et al*. 2006). Therefore, the high concentrations found for malvidin derivatives (Figures 1 and 2) could be explained by the fact that the F3'5'H has a higher activity than F3'H in the grape berry maturation. In the two pathways that anthocyanins synthesise (Figure 2), the content of F3'5'H branch pathway was higher in rain-shelter cultivation, but the F3'H has different expression in two vintages; the ratio of F3'5'H branch pathway was higher in rain-shelter cultivation in 2010 vintage, while it was opposite in 2011 vintage as malvidins were the most stable structure of the anthocyanins in grape. The higher proportion of malvidins makes the wine under rain-shelter cultivation more stable in colour.

***Flavan-3-ol profiles***. Flavan-3-ols are the main phenolic compounds related to the astringency, bit-

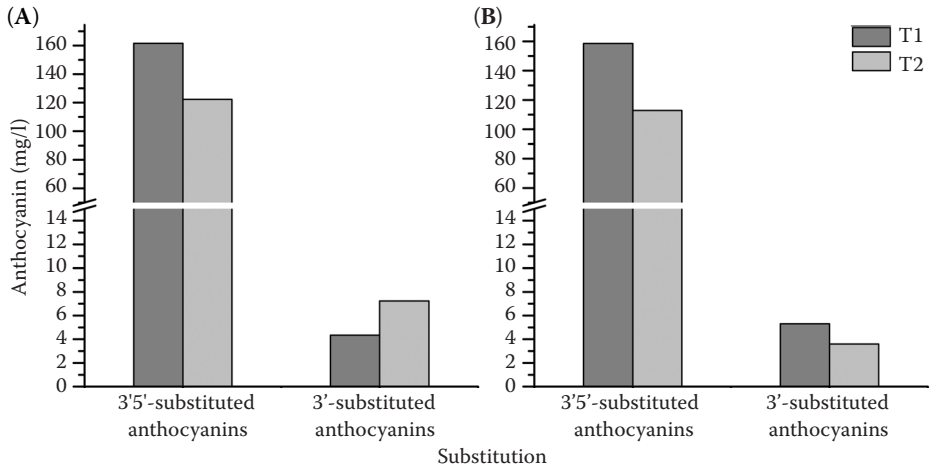


Figure 2. Concentrations of anthocyanin compounds distribution by substituents in wines in 2010 (**A**) and 2011 (**B**)

The breaks were all from 15 to 50; T1 – rain-shelter cultivation; T2 – open-field cultivation

doi: 10.17221/429/2015-CJFS

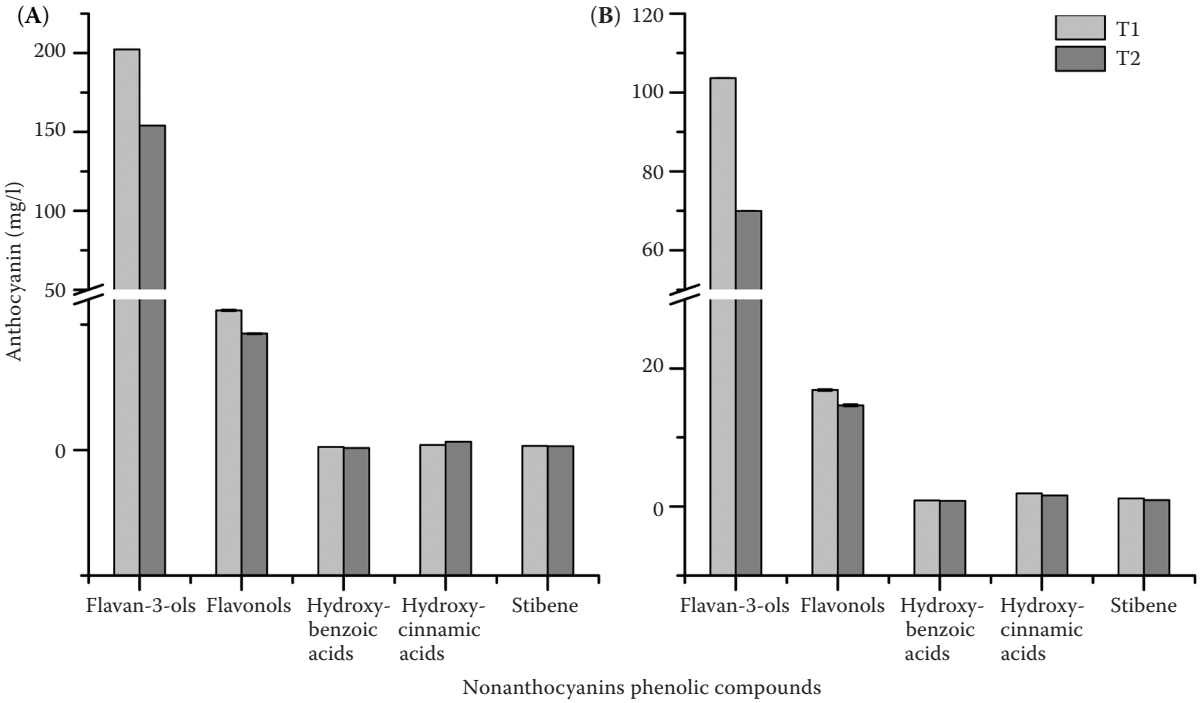


Figure 3. Concentrations of non-anthocyanin compounds in wines in 2010 (**A**) and 2011 (**B**)

The breaks were all from 30 to 50; T1 – rain-shelter cultivation; T2 – open-field cultivation

terness, and structure of wines, and also an important factor in stabilising the colour of aging wines as anthocyanin copigments (Gawel 1998; Hufnagel & Hofmann 2008; Obreque-Slier *et al*. 2010; Zhu *et al*. 2012). Flavan-3-ols are the major types of phenolic compounds present in the Cabernet Gernischet wine samples. Five and four flavan-3-ols were observed by HPLC-MS/MS in 2010 and 2011 vintage, respectively, and including three monomers, one dimer and one trimer. Gallocatechin was not found in wine from 2010 vintage (Table 4). The content of flavan-3-ols was higher in rain-shelter cultivation wine in the two vintages (Figure 3). The main compounds making difference were catechin, epicatechin, procyanidin dimer, and procyanidin trimer, the content of catechin and epicatechin was higher in rain-shelter cultivation in the two vintages, the difference was significant; while in 2011 vintage, the content of procyanidin dimer was higher in rain-shelter cultivation and procyanidin trimer was higher in open-field cultivation, the difference was remarkable.

***Flavonol profiles***. Flavonols contribute to bitterness and colour (Obreque-Slier *et al*. 2010) and they originate from the berry skins of grapes, and are transferred to wine during the process of wine-making. They vary in colour from white to yellow, closely related in structure to the flavones (Makris *et al*. 2006). They also contribute to the colour stabilisation of red wines by reinforcing the pigmentation due to anthocyanins, a phenomenon known as copigmentation (Boulton 2001). Flavonols are the second major types of phenolic compounds present in the Cabernet Gernischet wine samples (Figure 3). In this study, there were seven flavonols detected in wines under two treatments in the two vintages. In the wine of two vintages, the total content of flavonols was higher in rain-shelter cultivation, and the difference was not significant.

***Phenolic acid profiles***. There are two groups of phenolic acids in wine, hydroxybenzoic acids, and hydroxycinnamic acids. Hydroxybenzoic acids are derived from benzoic acid. In this study, two hydroxybenzoic acids, including dimer (*epi*)gallocatechin-(*epi*)catechin and syringic acid were identified in the wines. And the concentration of the two hydroxybenzoic acids was higher in rain-shelter cultivation, while the difference was significant only in 2010 vintage. There was one hydroxycinnamic acid, ferulic acid in wines of two treatments in the two vintages, and it was a dominant phenolic acid in the wines. This result is in agreement with most vinifera wines (Ferrandino & Guidoni 2010; Garrido & Borges 2011; Fanzone *et al*. 2012).

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***Stilbene profiles***. Among non-flavonoids, stilbenes (resveratrol and its analogues) are important compounds due to their putative protective effects against cardiovascular diseases and a remarkable inhibitory potential of various stages of tumour development (Saiko *et al*. 2008). It was suggested that the concentrations of these compounds in wines vary from values of < 1–30 mg/l, depending on many factors such as grape variety, fungal infections, winemaking procedures, and weather conditions (Vitrac *et al*. 2002). In this study, only *trans*-resveratrol was identified. And the content of *trans*-resveratrol was higher in rain-shelter cultivation in the two vintages, while the difference was not significant.

## CONCLUSIONS

Phenolic characterisation of Cabernet Gernischet wine under rain-shelter cultivation was detected and compared with that under open-field cultivation. Our results indicate that rain-shelter cultivation increased the content of anthocyanin and non-anthocyanin phenolic profiles significantly. Anthocyanins contribute to more steady structures in rain-shelter cultivation, which makes the wine more steady in colour. Meanwhile, rain-shelter cultivation made a long growing duration for grape, and then better-quality raw material for wine making was produced. It suggests that the rain-shelter cultivation makes the cultivation of cv. Cabernet Gernischet grape possible in the organic production system, for reducing the application of chemical pesticides in grape and wine industry.

***Acknowledgements***. The authors would like to thank the Centre for Viticulture and Oenology, China Agricultural University, for technical assistance in the completion of the HPLC/MS experiments.

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Received: 2015–08–31
Accepted after corrections: 2016–05–12
Published online: 2016–06–09

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