Effects of Different Harvest Times on the Maturity of Polyphenols in Two Red Wine Grape Cultivars (*Vitis vinifera* L.) in Qingtongxia (China)

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Due to the special climate conditions in the Qingtongxia region, grapes are high in sugar and low in titratable acidity from the stages of ripening. Therefore, the common methods used for determining the maturity of grapes, which depend on the ratio of sugar and titratable acidity in other regions, are inappropriate in Qingtongxia. This research was done in order to seek for a simple and convenient method of determining the optimal harvest time of grapes, further providing some theoretical basis for improving the quality of wine in Qingtongxia. Phenolic contents and some basic physico-chemical parameters of Merlot and Pinot Noir were evaluated during different ripening stages. The results showed that a different harvest time significantly affects the phenolic contents and physico-chemical parameters of Merlot and Pinot Noir. The total contents of anthocyanins in skins and total contents of phenolic in seeds was screen out as two important indexes to evaluate the maturity of polyphenols, in order to better improve the quality of grape and wine.

INTRODUCTION

Grapes are cultivated globally, and the quality of the grape is a key factor for the quality of the wines, and the grape maturity significantly affects the quality of grape, thus it is vital to determine appropriate grape maturity during ripening (Kontoudakis et al., 2011; Myunghee et al., 2014; Magariño and José, 2006; Magariño and José, 2013). However, the optimal harvest time is controlled by endogenous numbers and environmental factors including varieties, viticultural technologies, soil, climatic characteristics as well as maturity of grapes (Chira *et al.*, 2009; Conde *et al.*, 2016; Cook and Wolkovich, 2016; Myunghee *et al.*, 2014).

Total soluble solids (°Brix) and titratable acidity, apart from phenolic content, are the best parameters to use in the evaluation of grape quality (Conde *et al.*, 2006; Kontoudakis *et al.*, 2011; Ribera-Fonseca *et al.*, 2016; Urraca *et al.*, 2015). However, the content of reduced sugar could not guarantee the best quality of grapes and wines (Conde *et al.*, 2006). The maturity of phenolic, namely ‘phenolic maturity’ in grapes at harvest time is one of the main factors that affect the quality of the wine (Kontoudakis *et al.*, 2011; Magariño and José, 2006; Rajha *et al.*, 2017). Phenolic compounds are large and complex compounds, which are mainly present in skins and seeds (Obreque-Slier *et al.*, 2010). According to the chemical structure, phenolic compounds can be divided into two groups: flavonoids (flavonols, anthocyanins, flavan-3-ols) and non-flavonoids (cinnamic acid, stilbenes). Therefore, the content of phenolic compounds are the most important quality parameters of grapes and wines (Gil *et al.*, 2012; Lasanta *et al.*, 2014). There was also significant evidence of phenolic compounds affecting the quality of wines and the organoleptic properties, such as the skeleton, structure, colour, the character and quality of wines (Chira *et al.*, 2009; Conde *et al.*, 2006; Garrido and Borges, 2011; Hufnagel and Hofmann, 2008; Marti *et al.*, 2015). However, factors including climate, soil, variety, growth condition, the winemaking process and stages of ripeness affect the content of phenolic compounds (Canals *et al.*, 2005; Cook and Wolkovich, 2016; Intrieri *et al.*, 2010; Jin *et al.*, 2009; Mattivi *et al.*, 2009; Obreque-Slier *et al.*, 2010; Obreque-Slier *et al.*, 2013; Romerocascales *et al.*, 2005; Vilanova *et al.*, 2010). O-Marques *et al.* (2005) found that ripeness strongly influences the phenolic composition of grape and wines. Previous research reported that insufficiently ripened grapes have a lower extractability of anthocyanins and proanthocyanidins from skins and a higher extractability of proanthocyanidins from seeds, which may produce more astringent and bitter wines (Canals *et al.*, 2005; Peyrot and Kennedy, 2003). However, there is little information available regarding the relationship between the local climatic conditions and phenolic maturity (Sadras and
Currently, the Northwest wine region of China is popular, and the Qintongxiao wine region is a very important part of it. It is located in arid and semi-arid areas with ideal weather conditions for the growth of grapes, with reasonable light and moderate temperature throughout the year. The soil is mainly sand gravel and grey calcareous clay. Moreover, it belongs to the Yellow River irrigation area, which also makes a big difference. A lot of famous red wine grape varieties including Cabernet Sauvignon, Merlot, and Pinot Noir are cultivated in Qintongxiao. Research has found that the harvest time is strongly related to the kind of wine to be made. In the Qintongxiao region, most of the grapes are used to make aged red wines. Series of studies have reported the relationship between the harvest time of grapes and the quality of wines, which provided a united method to judge the optimal harvest time. However, for the special climatic conditions in Northwest China (Li. *et al.*, 2011), the grapes are affected by several special factors in the process of maturation, with high reducing sugar and low acidity. Few studies have focused on a standard for ensuring the appropriate maturity of grapes in China. The confirmation of the relationship among these factors provided insight into production in this locality. The aim of this study was to find a new way to guide the production practice in the Qintongxiao region. Furthermore, we studied the changes of phenolic compounds in the two red wine grapes during maturation in the years of 2014 and 2015, to determine the optimal harvest time of Pinot Noir and Merlot in Qintongxiao.

**MATERIALS AND METHODS**

**Experimental design and sample collection**

Pinot Noir and Merlot (*Vitis vinifera* L. cv.) grape berries were sampled from the experimental vineyard belonging to *Yuma* in Qintongxiao, Ningxia, China (38.02° N, 106.07° E), at different ripeness stages during 2014 and 2015 vintages. In 2014 and 2015, the highest temperature in Qintongxiao was 35.8°C and 35.7°C, with an average annual temperature of 10.3°C and 10.1°C (lower than the national average of 14.4°C and 14.6°C). An annual total precipitation of 178.5 mm and 184.6 mm was found (lower than the national average of 913.6 mm and 1011.6 mm), an annual total sunshine duration of 3086.1 h and 3181.7 h (higher than the national average of 1991.9 h and 2408.2 h), and lastly, an annual temperature of 14.4°C and 14.6°C (lower than the national average of 10.3°C and 10.1°C). An annual total precipitation of 10.3°C and 10.1°C. An annual total precipitation of 10.3°C and 10.1°C. The soil in the region is part of it. It is located in arid and semi-arid areas with ideal weather conditions for the growth of grapes, with reasonable light and moderate temperature throughout the year. The soil is mainly sand gravel and grey calcareous clay. Moreover, it belongs to the Yellow River irrigation area, which also makes a big difference. A lot of famous red wine grape varieties including Cabernet Sauvignon, Merlot, and Pinot Noir are cultivated in Qintongxiao. Research has found that the harvest time is strongly related to the kind of wine to be made. In the Qintongxiao region, most of the grapes are used to make aged red wines. Series of studies have reported the relationship between the harvest time of grapes and the quality of wines, which provided a united method to judge the optimal harvest time. However, for the special climatic conditions in Northwest China (Li. *et al.*, 2011), the grapes are affected by several special factors in the process of maturation, with high reducing sugar and low acidity. Few studies have focused on a standard for ensuring the appropriate maturity of grapes in China. The confirmation of the relationship among these factors provided insight into production in this locality. The aim of this study was to find a new way to guide the production practice in the Qintongxiao region. Furthermore, we studied the changes of phenolic compounds in the two red wine grapes during maturation in the years of 2014 and 2015, to determine the optimal harvest time of Pinot Noir and Merlot in Qintongxiao.

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The grapes were harvested at five levels of ripeness, and the first harvest was one week after veraison. The second to fifth harvests were carried out every week form two to five weeks after veraison. The experimental design used the ‘Z’ method to gather samples. Each sample consisted of 300 berries randomly collected in terms of sun exposure and backlight, the inside and the outside of the cluster, the top, the bottom, and the middle of the cluster. Moreover, it was done one day a week from the beginning of ripeness until harvest. In the last three sampling times, we have harvested another sample of grapes (20 kg) used for making wines. These samples were stored at -20°C before use.

Phenolic compounds of grapes in skins and seeds were extracted according to the methods proposed by Di *et al.* (1991), with minor modifications. They were comprised three independent replicates and each replicate consisted of 30 berries, of which grape skins and seeds were carefully removed using razor blades. Then water and residue on the surface of the grapes were removed and weighed. It was added to 30 mL of buffer solution (12% (v/v) ethanol + 600 mg/L sodium metabisulphite + 5 g/L tartaric acid, 1 M NaOH to adjust pH to 3.20), and put in a swing bed (100 r/min, 25°C). Extraction took place for three days, finally collecting the supernatant, which was also placed in -20°C stored away from light before use.

**Determination of the physicochemical indexes of grape berry**

Grape juice was collected and used for assaying reducing sugar and titratable acids, which were analysed according to the methods proposed by OIV (2012).

The tests for total phenolic content (TPC) and total tannin content (TTC) were performed as described by Harbertson *et al.* (2003) with minor modifications. All buffer solutions were prepared before the experiment. Buffer A was the washing buffer of 200 mM acetate acid and 170 mM sodium chloride, pH adjusted to 4.9 with sodium hydroxide. Buffer B was a model wine (5.0 g/L potassium bitartrate and 12% (v/v) ethanol, pH adjusted to 3.3 with HCl). Buffer C was a resuspension buffer consisting of 5% (v/v) triethanolamine and 5% (w/v) sodium dodecyl sulfate, pH adjusted to 9.4 with HCl. The ferric chloride reagent was 0.01 M HCl and 10 mM ferric chloride.

For TTC determination, a protein solution for tannin precipitation was prepared by dissolving Bovine serum albumin (BSA) into buffer A, in order to give a final protein concentration of 1.0 mg/mL. The skin extract was diluted with buffer B, 1.0 mL of the protein solution and 500 μL diluted extract of sample A in a 1.5 mL microfuge tube. After being incubated for 15 minutes with slow agitation at room temperature, the mixture was centrifuged at 14,000 g for 5 minutes at 4°C. After the supernatant was poured out, the residue was washed with buffer A three times and then resolubilised in 875 μL of buffer C. The absorbance of the ferric chloride reagent was added and shaken for 10 minutes. The absorbance of the solution was read at 510 nm for tannin background (*A*510). Then, 125 μL of the ferric chloride reagent was added and shook for 10 minutes. The solution was read at 510 nm for tannin final (*A*510). Buffer C was used as a blank and read at 510 nm for tannin initial (*A*510). After the incubation period, the absorbance at 510 nm was determined in Shimadzu 640 spectrophotometer using the TEA buffer as a blank. TTC values are reported in catechin equivalents (C.E.) as described here (Harbertson *et al.*, 2003).

For TPC, 20.0 μL of wine sample and 855 μL of buffer C were mixed. After incubated for 10 minutes, the mixture was read at 510 nm (total phenolic background *A*510). Then, 855 μL of ferric chloride reagent was added into the reaction system. The absorbance was read at 510 nm (total phenolic final *A*510). TPC values are reported in catechin equivalents.
(C.E.) as described below.

The absorbance for TTC = [(tannin final A₅₁₀)-(tannin initial A₂₃₀)] – (tannin background A₁₀₀) × 0.875. The absorbance for TPC = [(total phenolic final A₅₁₀)-(tannin initial A₂₃₀)] – (total phenolic background A₁₀₀) × 0.875.

Total flavonoid content (TFC) was determined according to the method of Peinado et al. (2009) with minor modification. In a centrifuge tube, 0.2 mL of grape extract was added, then, methyl alcohol up until 1.0 mL, then, 2.7 mL 30% methyl alcohol, 0.3 mL of NaNO₂ (0.5 M) and 0.3 mL of AlCl₃ (0.3 M) in this sequence. After 5 minutes, 1.0 mL of NaOH (1.0 M) was added to the reaction system. The absorbance was measured against the blank at 510 nm. Results were expressed as rutin equivalents (RE).

Total anthocyanin content (TAC) was estimated using the pH differential method with minor modification (Lee et al., 2005). Each grape and wine extract was diluted 40 times with buffers at pH 1.0 and 4.5 to attain the same dilution. The absorbance was measured at 520 and 700 nm in both pH 1.0 and 4.5 buffers. The TAC (expressed in terms of cyanidin-3-glucoside) was calculated using the following formula:

\[
TAC = A \times DF \times MW \times 1000 / (ε \times C)
\]

where MW is the molecular weight of cyanidin-3-glucoside (449 g/mol), DF is the dilution factor, ε is the molar extinction coefficient of cyanidin-3-O-glucoside (29,600), and C is the concentration of extracted volume.

Total flavanol content was determined according to the method of Li et al. (1996) with minor modification. The grape extract of skins and seeds, including the skin grape extract undiluted and the seed grape extract diluted 5 times, was added with 0.2 mL of grape extract to the centrifuge tube respectively. Then, mixed with 3 mL p-DMACA solution, after 10 minutes, the absorbance was measured at 640 nm. Results were expressed as catechin equivalents (CE).

**Determination the content of monomer anthocyanins**

The chromatographic analyses of anthocyanins were performed using LC-20AT HPLC system (Shimadzu Corporation) equipped with a reversed phase column (Synergi Hydro-RP C18, 250 × 4.6 mm, 4 μm). The mobile phase was ultrapure water, acetonitrile and methanoic acid (800:100:25) as solvent A; and ultrapure water, acetonitrile and methanoic acid (400:500:25) as solvent B. The elution profile had the following proportions (v/v) of solvent B: 0.00-15 min, 0%-10%; 15-30 min, 10%-20%; 30-45 min, 20-35%; 45-46 min, 35%-100%; 46.00-50.00 min, 100%. The column was held at 35°C and was flushed at a flow rate of 0.00-15 min, 0%-10%; 15-30 min, 10%-20%; 30-45 min, 20-35%; 45-46 min, 35%-100%; 46.00-50.00 min, 100%. The absorbance was measured against the blank at 510 nm. The injection volume was 20 µL and analyses were detected at 520 nm.

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 compared to standards and references. Quantitative determinations were made by using the external standard method compared to the commercial standards. The calibration curves were obtained by injection of standard solutions under the same conditions of the samples analysed. Anthocyanins and flavan-3-ols were expressed respectively as micrograms of malvidin-3-O-glucoside (ME) and catechin equivalence (CE)/L of grape skins.

**Sensory analysis of wines**

The last three samples were used for making wines. A sensory tasting team was created, made up of 12 people who were trained wine panelists from the College of Enology, Northwest A & F University (7 females and 5 males, 23–28 years of age). Appearance, aroma, flavor and the overall balance were evaluated according to the tasting table. Finally, statistical analysis was based on the tasting table.

**Statistics analysis**

Data were reported as mean ± standard deviation (SD) values of the triplicate experiments and were analysed using SPSS 24.0 software (IBM Corporation, Armonk, NY, USA). One-way analysis of variance (ANOVA) and Duncan’s multiple range tests (MRT) were used to determine the significance of differences among the means at each sampling time at the significance level of 0.05. The figures were drawn using the Microsoft Excel 2010.

**RESULTS AND DISCUSSION**

**The basic indexes of grapes**

Indexes such as 100 berries’ weight, pH and soluble solids content (SSC) are defined as the technological ripeness of grapes. Table 1 and Table 2 shows the basic indexes of Merlot and Pinot Noir respectively. As expected, most of these indexes presented a rising trend during ripening and showed significant differences between one another (p≤0.05). The two tables also showed that the 100 berries’ weight presented rising trends at first, and then turned into a downtrend (p≤0.05). The SSC, reducing sugar, TA (titratable acidity) and sugar/TA ratio increased regularly during ripening and there was a significant difference among them (p≤0.05). These changes kept in line with what Nedomová et al. (2017) previously reported. However, a special phenomenon was that the content of sugar was higher than the standard level, while the content of TA was lower than the standard level during grape ripening, which was different from other reports. It has been found that the climatic characteristics have an innate impact on the harvest time and the quality of the grape, and it can also determine the particular style of the wines in local areas (Cook and Wolkovich, 2016). We inferred that this special phenomenon is caused by the local climate.

The content of reducing sugar varied from 154.62±2.76 g/L, 182.17±0.76 g/L to 235.15±2.73 g/L, 238.87±1.13 g/L; the sugar/TA varied from 16.94±0.31, 19.82±0.31 to 40.30±1.36, 42.67±0.99; the content of SSC varied from 16.27±0.15°Brix, 19.07±0.12°Brix to 22.20±0.01°Brix, 235.15±2.73 g/L, 238.87±1.13°Brix; the sugar/TA ratio increased regularly during ripening and there was a significant difference among them (p≤0.05). These changes kept in line with what Nedomová et al. (2017) previously reported. However, a special phenomenon was that the content of sugar was higher than the standard level, while the content of TA was lower than the standard level during grape ripening, which was different from other reports. It has been found that the climatic characteristics have an innate impact on the harvest time and the quality of the grape, and it can also determine the particular style of the wines in local areas (Cook and Wolkovich, 2016). We inferred that this special phenomenon is caused by the local climate.

The content of reducing sugar varied from 143.65±3.10 g/L, 179.57±0.74 g/L to 232.0±0.01 g/L, 240.38±0.66 g/L; the sugar/TA varied from 13.48±0.18, 17.94±0.15 to 28.89±0.01, 33.47±0.42; the content of SSC varied from
TABLE 1
Basic phyto-chemical indexes of Merlot grapes from different harvest times.

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</thead>
<tbody>
<tr>
<td>100-berries weight</td>
<td>125.55±1.18e</td>
<td>159.81±1.53ab</td>
<td>126.29±1.37d</td>
<td>135.74±0.97c</td>
<td>164.79±4.51a</td>
<td>144.73±0.68a</td>
<td>158.41±1.81b</td>
<td>158.41±1.81b</td>
<td>151.37±0.88c</td>
<td></td>
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<tr>
<td>pH</td>
<td>3.14±0.01e</td>
<td>3.24±0.01e</td>
<td>3.41±0.01c</td>
<td>3.39±0.01d</td>
<td>3.39±0.01d</td>
<td>3.56±0.01b</td>
<td>3.66±0.01b</td>
<td>3.58±0.01a</td>
<td>3.73±0.01a</td>
<td></td>
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<tr>
<td>SSC (Brix)</td>
<td>16.27±0.15e</td>
<td>19.07±0.12e</td>
<td>19.10±0.001d</td>
<td>21.87±0.23d</td>
<td>21.87±0.12c</td>
<td>22.61±0.02c</td>
<td>21.97±0.06b</td>
<td>23.39±0.10b</td>
<td>22.20±0.001a</td>
<td>24.03±0.05a</td>
</tr>
<tr>
<td>Reducing sugars (g/L)</td>
<td>154.62±2.76e</td>
<td>182.17±0.76e</td>
<td>191.45±7.60d</td>
<td>214.17±1.05d</td>
<td>214.07±5.20b</td>
<td>221.38±0.57c</td>
<td>209.72±6.64c</td>
<td>230.49±1.21b</td>
<td>235.15±2.73a</td>
<td>238.87±1.13a</td>
</tr>
<tr>
<td>TA (tartaric acid g/L)</td>
<td>9.13±0.11a</td>
<td>9.19±0.10a</td>
<td>7.85±0.11b</td>
<td>8.40±0.00b</td>
<td>6.62±0.19d</td>
<td>7.37±0.10c</td>
<td>7.11±0.22e</td>
<td>6.36±0.05d</td>
<td>5.84±0.18e</td>
<td>5.60±0.10e</td>
</tr>
<tr>
<td>sugars/TA</td>
<td>16.94±0.51e</td>
<td>19.82±0.31e</td>
<td>24.41±0.92d</td>
<td>25.49±0.13d</td>
<td>32.39±0.68b</td>
<td>30.04±0.46c</td>
<td>29.52±0.30c</td>
<td>36.24±0.43b</td>
<td>40.30±1.36a</td>
<td>42.67±0.99a</td>
</tr>
</tbody>
</table>

Notes: SSC: soluble solids content, TA: titratable acidity. Each value represents mean of three replicates ± SD (standard deviation). Different letters (a, b, c, d) within the same row for each sampling time indicate significant difference at \( P \leq 0.05 \) by Duncan’s multiple range test.

TABLE 2
Basic phyto-chemical indexes of Pinot Noir grapes from different harvest times.

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</thead>
<tbody>
<tr>
<td>100-berries weight</td>
<td>160.11±0.76d</td>
<td>177.56±0.92b</td>
<td>161.56±0.80c</td>
<td>173.20±2.67bc</td>
<td>169.28±0.50b</td>
<td>187.34±1.32a</td>
<td>154.91±0.62e</td>
<td>184.86±3.34a</td>
<td>173.01±0.54a</td>
<td>169.46±1.91c</td>
</tr>
<tr>
<td>pH</td>
<td>3.13±0.01e</td>
<td>3.11±0.02d</td>
<td>3.25±0.01d</td>
<td>3.21±0.01c</td>
<td>3.35±0.01c</td>
<td>3.47±0.01b</td>
<td>3.40±0.01b</td>
<td>3.46±0.01b</td>
<td>3.42±0.01a</td>
<td>3.61±0.01a</td>
</tr>
<tr>
<td>SSC (Brix)</td>
<td>15.67±0.42e</td>
<td>18.45±0.38e</td>
<td>17.90±0.10d</td>
<td>21.24±0.27d</td>
<td>20.83±0.15c</td>
<td>22.98±0.08e</td>
<td>22.17±0.06b</td>
<td>23.61±0.04b</td>
<td>22.13±0.06b</td>
<td>24.35±0.26a</td>
</tr>
<tr>
<td>Reducing sugars (g/L)</td>
<td>143.65±3.10e</td>
<td>179.57±0.74e</td>
<td>194.92±2.14d</td>
<td>207.13±0.98d</td>
<td>200.05±3.93c</td>
<td>223.08±0.12c</td>
<td>223.38±2.85c</td>
<td>232.26±1.07b</td>
<td>232.0±0.01a</td>
<td>240.38±0.66a</td>
</tr>
<tr>
<td>TA (tartaric acid g/L)</td>
<td>10.67±0.37b</td>
<td>10.01±0.05a</td>
<td>10.74±0.19a</td>
<td>9.25±0.10b</td>
<td>8.32±0.19c</td>
<td>8.52±0.10c</td>
<td>7.73±0.01e</td>
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<td>24.07±0.89c</td>
<td>26.18±0.33c</td>
<td>28.88±0.37b</td>
<td>30.77±0.31b</td>
<td>28.89±0.01a</td>
<td>33.47±0.42a</td>
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</table>
15.67±0.42 °Brix, 18.45±0.38 °Brix to 22.13±0.06 °Brix, 24.35±0.26 °Brix, and pH varied from 3.13±0.01,3.11±0.02 to 3.42±0.01, 3.61±0.01 in the grapes of Pinot Noir in 2014 and 2015 respectively. The reducing sugars were slightly higher than that of other wine regions and the TA was seriously under the normal standard level. This phenomenon is common in Northwest China, while it is detrimental to the grape production. Furthermore, the phenomenon influences the quality of wines to some extent (Mota et al., 2011). Therefore, increasing the content of TA becomes a crucial technology during vinification. However, there is very little research available about the factors influenced by the harvest time in Northwest China.

According to the OIV, grapes are considered to be ripened when SSC reached the content of 220.00 g/L. However, as can be seen in Table 1 and Table 2, between three and four weeks after veraison, the SSC content did not show significant difference and reached above of 220.00 g/L both for Merlot and Pinot Noir. Therefore, it was difficult to determine the certain harvest time in Northwest China. Meanwhile, due to more sunlight (262.9 h and 316.2 h in August of 2014 and 2015, respectively) and less rainfall (45.9 mm and 19.9 mm in August of 2014 and 2015, respectively) during the early ripening in 2015 compared with 2014, higher levels of the basic indexes was found in 2015 than in 2014. However, the relationship between the maturity of polyphenols and the harvest time has not been fully elucidated in Northwest China. Therefore, we concluded that not only the basic indexes should be considered (SSC, reducing sugar and sugar/TA), but also other factors, such as phenolic compounds that affect the quality of wines (Ribera-Fonseca et al., 2016), when determining the most appropriate harvest time.

Notes: SSC: soluble solids content, TA: titratable acidity. Each value represents mean of three replicates ± SD (standard deviation). Different letters (a, b, c, d) within the same row for each sampling time indicate significant difference at \( P<0.05 \) by Duncan’s multiple range test.

**Phenolic compounds of grape berries**

Total contents of polyphenols in the skins and seeds of grapes were determined in Merlot and Pinot Noir at different ripening degrees. As can be seen from Tables 3, 4 and Figures 1, 2, 3 and 4, the different stages of maturity significantly influenced the content of phenolic compounds from different parts.

As shown in Table 3, 4 and Figure 1, 2, 3 and 4, the content of phenolic compounds reached the maximum five weeks after veraison in 2014. However, the content of phenolic compounds attained the maximum three weeks after veraison in 2015. Generally, the content of phenolic compounds in the skins of grapes showed a rising trend during ripening, while these showed a downtrend in the seeds of grapes. However, the content of phenolic compounds showed a similar trend both in skins and seeds of grapes during ripening (Table 3 and 4) five weeks after veraison in 2014. In 2015, three weeks after veraison the content of phenolic compounds were higher compared to before or after this stage. The content of total phenolic compounds, the anthocyanin and the flavonoids in the skins were significantly higher during ripening (Allegro et al., 2016; Fournand et al., 2006). The content of phenolic compounds in the skins of grapes were significantly influenced by the sampling time. Similarly, the total phenolic contents, the anthocyanin and the flavonoids in the seeds showed a downtrend in the continuous two years, entirely. In 2015, the content of total phenolic compounds reached the highest value (69.34±1.43 mg/g), the content of anthocyanin reached 7.80±0.14 mg/g on August 28 in the skins of Pinot Noir. In Merlot, the content of total phenolic compounds reached the highest value (32.52±0.76 mg/g) in the skins and in the seeds (77.95±2.30 mg/g) after three and five weeks of veraison, respectively. Furthermore, these indexes showed a higher level in 2015 than in 2014. This trend might be related to the local climate characterised by more rainfall during ripening in 2014, which would result in the decreased biosynthesis of phenolic compounds. Our results relate well to this trend (Gil et al., 2012; Li et al., 2014; Lorrain et al., 1991).

According to the content of phenolic compounds, including the phenolic compounds in the skins and in the seeds, optimal harvest time could be performed five weeks after veraison in 2014 and three weeks after veraison in 2015. This time the quality of grapes and wines was best, and parts of the sensory analysis of the wines also confirmed the conclusion. A series of literature reported that the content of phenolic compounds and the maturity of the grape have a significant correlation (Bordiga et al., 2011; Obreque-Slier et al., 2013).

Table 3 and 4 showed the content of phenolic compounds, which were medium compared to other wine grape cultivars from different regions (Li et al., 2011). Principal component analysis (PCA) and correlation analysis displayed that when reducing sugar-acid ratio in order to reach the requirements of harvest, the contents of anthocyanins in skins and the total content of phenolic compounds in seeds were vital for the quality of grapes and wines (Chira et al., 2009; Hernándezhierro et al., 2014).

**Determination of the content of monomer anthocyanins in wines**

The anthocyanins in wine are mainly macerated from the peel of grapes, which is crucial for the colour of wines (Bindon et al., 2013; Gil et al., 2012; Magariño and José, 2006; Magariño and José, 2013; Romerocascales et al., 2005). Table 5 and 6 showed the kinds and contents of monomer anthocyanins at different sampling times in Pinot Noir and Merlot. Five kinds of non-acylated anthocyanins were detected in Pinot Noir at different harvest times, and five kinds of non-acylated anthocyanins and four kinds of acylation anthocyanins were detected in Merlot. The anthocyanin content was 63.99%~71.41% in wines. During ripening, there was a variation of monomer anthocyanins in the skins of Pinot Noir and Merlot, which was a significant difference \( (P<0.05) \). In 2014, the content of monomer anthocyanins reached a maximum in the wines of Pinot Noir and Merlot five weeks after veraison, however, these kinds of phenomenon appeared after three weeks of the veraison in 2015. Nine kinds of monomer anthocyanins were detected, in which the content of malvidin-3-O-glucoside are most...
### TABLE 3
Polyphenol contents of Merlot grapes from different harvest times.

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>TPCsk</td>
<td>22.38±0.13d</td>
<td>21.65±0.41e</td>
<td>18.15±0.23e</td>
<td>29.38±0.57c</td>
<td>24.40±1.09c</td>
<td>32.52±0.76a</td>
<td>28.87±2.13b</td>
<td>31.75±1.35b</td>
<td>35.95±2.65a</td>
<td>27.96±0.75d</td>
</tr>
<tr>
<td>TACsk</td>
<td>7.28±0.14d</td>
<td>7.85±0.12e</td>
<td>7.33±0.05c</td>
<td>12.83±0.19c</td>
<td>5.92±0.02e</td>
<td>15.71±0.24a</td>
<td>14.71±0.20a</td>
<td>14.6±0.03b</td>
<td>14.39±0.02a</td>
<td>12.58±0.35d</td>
</tr>
<tr>
<td>TFLCsk</td>
<td>24.22±0.11c</td>
<td>23.55±0.46e</td>
<td>20.74±0.23e</td>
<td>31.87±0.21b</td>
<td>21.10±0.89d</td>
<td>36.07±1.54a</td>
<td>29.01±0.27b</td>
<td>30.24±1.34c</td>
<td>36.55±0.61a</td>
<td>29.15±0.64d</td>
</tr>
<tr>
<td>TFCsk</td>
<td>3.37±0.15e</td>
<td>2.01±0.19c</td>
<td>3.39±0.12d</td>
<td>2.09±0.05b</td>
<td>1.13±0.02e</td>
<td>2.25±0.13a</td>
<td>2.25±0.03b</td>
<td>2.07±0.06c</td>
<td>4.3±0.13a</td>
<td>1.85±0.09d</td>
</tr>
<tr>
<td>TTCsk</td>
<td>36.96±1.08b</td>
<td>87.98±0.27a</td>
<td>83.68±0.29d</td>
<td>76.96±0.77c</td>
<td>26.55±0.78e</td>
<td>72.96±1.66d</td>
<td>34.15±2.29c</td>
<td>77.95±2.30b</td>
<td>37.74±0.64a</td>
<td>55.78±1.79e</td>
</tr>
<tr>
<td>TPCs</td>
<td>24.40±1.09c</td>
<td>32.52±0.76a</td>
<td>28.87±2.13b</td>
<td>31.75±1.35b</td>
<td>35.95±2.65a</td>
<td>27.96±0.75d</td>
<td>20.60±0.11b</td>
<td>19.72±0.34e</td>
<td>22.44±0.23b</td>
<td>19.72±0.34e</td>
</tr>
<tr>
<td>TFLCs</td>
<td>15.14±0.10b</td>
<td>14.86±0.21a</td>
<td>14.00±0.07c</td>
<td>12.63±0.10b</td>
<td>11.58±0.06c</td>
<td>8.16±0.13a</td>
<td>10.35±0.36d</td>
<td>6.26±0.06c</td>
<td>7.99±0.18e</td>
<td>8.16±0.13a</td>
</tr>
<tr>
<td>TFCs</td>
<td>56.83±1.70a</td>
<td>96.54±0.5a</td>
<td>37.92±0.51e</td>
<td>87.54±1.59c</td>
<td>56.07±0.65b</td>
<td>79.48±2.46d</td>
<td>56.21±0.16c</td>
<td>61.26±1.93e</td>
<td>56.21±0.16c</td>
<td>61.26±1.93e</td>
</tr>
</tbody>
</table>

Notes: TPC: Total phenolic content; TAC: Total anthocyanin content; TFLC: Total flavanol content; TFC: Total flavonoid content; TTC: Total tannin content; sk: the skin of grapes; s: the seeds of grapes. Each value represents the mean of three replicates ± SD (standard deviation). Different letters (a, b, c, d) within the same row for each sampling time indicate significant difference at $P \leq 0.05$ by Duncan’s multiple range test.

### TABLE 4
Polyphenol contents of Pinot Noir grapes from different harvest times.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TPCsk</td>
<td>22.14±0.48a</td>
<td>21.20±1.17d</td>
<td>13.46±0.08e</td>
<td>22.25±0.36c</td>
<td>16.62±0.28d</td>
<td>23.49±0.30a</td>
<td>17.94±0.39c</td>
<td>22.61±0.45b</td>
<td>20.60±0.11b</td>
<td>19.72±0.34e</td>
</tr>
<tr>
<td>TACsk</td>
<td>5.40±0.02c</td>
<td>6.15±0.22d</td>
<td>5.21±0.09d</td>
<td>6.67±0.04c</td>
<td>3.46±0.02e</td>
<td>7.80±0.14a</td>
<td>6.69±0.03b</td>
<td>6.89±0.31b</td>
<td>7.09±0.03a</td>
<td>5.27±0.04e</td>
</tr>
<tr>
<td>TFLCsk</td>
<td>1.32±0.01c</td>
<td>1.46±0.02e</td>
<td>1.19±0.02d</td>
<td>2.09±0.05b</td>
<td>0.82±0.07e</td>
<td>2.25±0.13a</td>
<td>1.63±0.02b</td>
<td>2.07±0.06c</td>
<td>2.24±0.06a</td>
<td>1.85±0.09d</td>
</tr>
<tr>
<td>TFCsk</td>
<td>29.05±0.22b</td>
<td>25.34±0.38c</td>
<td>19.26±0.31e</td>
<td>25.99±0.72b</td>
<td>22.15±0.99c</td>
<td>26.03±0.65a</td>
<td>21.01±0.39d</td>
<td>22.56±0.27d</td>
<td>29.95±0.28a</td>
<td>22.28±0.24e</td>
</tr>
<tr>
<td>TTCsk</td>
<td>4.63±0.07a</td>
<td>2.17±0.05c</td>
<td>3.58±0.11c</td>
<td>2.22±0.05b</td>
<td>3.34±0.11d</td>
<td>3.32±0.10a</td>
<td>3.14±0.14e</td>
<td>1.67±0.13d</td>
<td>3.89±0.11b</td>
<td>1.55±0.07e</td>
</tr>
<tr>
<td>TPCs</td>
<td>37.55±1.48a</td>
<td>81.52±1.02a</td>
<td>31.67±0.41b</td>
<td>75.53±0.54b</td>
<td>69.34±1.43d</td>
<td>26.80±0.75d</td>
<td>67.09±0.90e</td>
<td>27.85±0.83c</td>
<td>69.55±2.86c</td>
<td>69.55±2.86c</td>
</tr>
<tr>
<td>TFLCs</td>
<td>8.61±0.02a</td>
<td>18.39±0.14a</td>
<td>7.34±0.02d</td>
<td>17.68±2.20b</td>
<td>7.58±0.03c</td>
<td>14.53±0.19e</td>
<td>8.16±0.10b</td>
<td>14.66±0.28d</td>
<td>6.73±0.03e</td>
<td>15.51±0.29c</td>
</tr>
<tr>
<td>TFCs</td>
<td>62.37±0.99a</td>
<td>107.34±2.29a</td>
<td>56.77±0.99b</td>
<td>102.24±1.56b</td>
<td>44.04±0.51e</td>
<td>93.77±2.22c</td>
<td>52.59±1.16c</td>
<td>83.79±3.38e</td>
<td>52.02±0.13d</td>
<td>91.67±1.04d</td>
</tr>
<tr>
<td>TTCs</td>
<td>12.67±1.07a</td>
<td>10.65±0.12a</td>
<td>7.98±0.11b</td>
<td>9.66±0.17b</td>
<td>7.77±0.14e</td>
<td>9.01±0.16d</td>
<td>6.40±0.37d</td>
<td>9.10±0.32d</td>
<td>4.41±0.1e</td>
<td>9.54±0.17c</td>
</tr>
</tbody>
</table>

Notes: TPC: Total phenolic content; TAC: Total anthocyanin content; TFLC: Total flavanol content; TFC: Total flavonoid content; TTC: Total tannin content; sk: the skin of grape; s: the seeds of grape. Each value represents the mean of three replicates ± SD (standard deviation). Different letters (a, b, c, d) within the same row for each sampling time indicate significant difference at $P \leq 0.05$ by Duncan’s multiple range test.
Effects of Harvest Time on the Maturity of Grape Polyphenols

Polyphenol indexes of skins and seeds of Merlot grapes at different sampling times in 2014. TPC: Total phenolic content; TAC: Total anthocyanin content; TFLC: Total flavanol content; TFC: Total flavonoid content; TTC: Total tannin content; sk: the skin of grapes; s: the seeds of grapes. Each value represents the mean of three replicates ± SD (standard deviation). Within each sampling time, the bars with different letters (a, b, c, d) are significantly different at P<0.05 (Duncan’s multiple range test).

FIGURE 1

Polyphenol indexes of skins and seeds of Merlot grapes at different sampling times in 2015. TPC: Total phenolic content; TAC: Total anthocyanin content; TFLC: Total flavanol content; TFC: Total flavonoid content; TTC: Total tannin content; sk: the skin of grapes; s: the seeds of grapes. Each value represents the mean of three replicates ± SD (standard deviation). Within each sampling time, the bars with different letters (a, b, c, d) are significantly different at P<0.05 (Duncan’s multiple range test).

FIGURE 2

The content of monomeric anthocyanins in wines decreased markedly during ripening in 2015, contrary to 2014. The anthocyanin synthesis and content of monomeric anthocyanins were affected by the temperatures, for example, in the cold year (2014), levels were significantly higher than in the hot and dry year (Liang et al., 2012). As indicated in Table 5, the contents of malvidin-3-O-glucoside were highest in MW-3. MW-3 and PW-3 was 122.98 mg/g, 114.86 mg/g respectively, and content of malvidin-3-O-glucoside was relatively low in MW-1 and PW-1, as it was 69.72 mg/g and 86.22 mg/g respectively in 2014.

Contents of malvidin-3-O-glucoside varied from 69.72 mg/L~122.98 mg/L in Merlot, which accounted for 57.3%~62.3% of the total anthocyanins. The contents of total anthocyanins ranged from 121.61 to 197.41 mg/L, in which non-acylated anthocyanins accounted for 76.9 % of total anthocyanins. In MW-3, acylated anthocyanins accounted for 20% of the wines. In Pinot Noir, the monomeric anthocyanins...
Effects of Harvest Time on the Maturity of Grape Polyphenols

Polyphenol indexes of skins and seeds of Pinot Noir grapes at different sampling times in 2014. TPC: Total phenolic content; TAC: Total anthocyanin content; TFLC: Total flavanol content; TFC: Total flavonoid content; TTC: Total tannin content; sk: the skin of grapes; s: the seeds of grapes. Each value represents the mean of three replicates ± SD (standard deviation). Within each sampling time, the bars with different letters (a, b, c, d) are significantly different at P<0.05 (Duncan’s multiple range test).

Polyphenol indexes of skins and seeds of Pinot Noir grapes at different sampling times in 2015. TPC: Total phenolic content; TAC: Total anthocyanin content; TFLC: Total flavanol content; TFC: Total flavonoid content; TTC: Total tannin content; sk: the skin of grapes; s: the seeds of grapes. Each value represents the mean of three replicates ± SD (standard deviation). Within each sampling time, the bars with different letters (a, b, c, d) are significantly different at P<0.05 (Duncan’s multiple range test).

were non-acylated anthocyanins, and the contents of malvidin-3-O-glucoside were also the maximum, accounting for 90% of total anthocyanins. As can be seen from Table 5 and 6, the malvidin-3-O-glucoside was the most abundant monomeric anthocyanin, reaching more than 52%, followed by malvidin-3-O-(6-O-Acetyl)-glucoside, of which the content accounted for 79.47%–82.26% and 82.49%–85.49% of the total content of monomer anthocyanins, respectively. This indicated that both malvidin-3-O-glucoside and malvidin-3-O-(6-O-Acetyl)-glucoside play pivotal roles in the anthocyanin, which makes the colour of the wine (Bindon et al., 2014; Magariño and José, 2013). Furthermore, it was crucially important to control the maturity of the phenolic compounds for the quality of grapes and wines according to the results (Bindon et al., 2013; Bindon et al., 2014; Magariño and José, 2006). Also, the monomer composition and content of anthocyanins were related to the grape varieties (Liang et al., 2008; Segade et al., 2009).

Sensory analysis of wine

After homogenisation of the tasting data, the data were conducted with Quantitative descriptive analysis (QDA). The results demonstrated that the wines from grapes on the third harvesting times (PW-3) are the best in the continuous two years (Fig.5, 6).

The organoleptic properties of PW-3 wines, including the clarity, flavour preferences and overall balance were the best, followed by PW-2 and PW-1. However, the colour and aroma intensity of wines from PW-2 were the best, 0.94 and 0.90 respectively, followed by PW-3 and PW-1 in 2015. Previous studies have shown that the compounds related to colour and aroma are very important for determining the optimal harvest time (Cadot et al., 2012; Chang et al., 2014). The colour and aroma intensity of the wines from PW-2 was
TABLE 5
Contents of anthocyanins in wine from different harvest times in 2014.

<table>
<thead>
<tr>
<th>Wine simples</th>
<th>Free anthocyanins contents in skins(mg/g)</th>
<th>Total contents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dp</td>
<td>Cy</td>
</tr>
<tr>
<td>Merlot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MW-1</td>
<td>7.41</td>
<td>1.18</td>
</tr>
<tr>
<td>MW-2</td>
<td>7.88</td>
<td>0.93</td>
</tr>
<tr>
<td>MW-3</td>
<td>9.83</td>
<td>1.23</td>
</tr>
<tr>
<td>Pinot Noir</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PW-1</td>
<td>1.22</td>
<td>0.51</td>
</tr>
<tr>
<td>PW-2</td>
<td>1.58</td>
<td>0.26</td>
</tr>
<tr>
<td>PW-3</td>
<td>1.92</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Notes: Dp: Delphinidin-3-O-glucoside; Cy: Cyanidin-3-O-glucoside; Pt: Petunidin-3-O-glucoside; Pn: Peonidin-3-O-glucoside; Mv: Malvidin-3-O-glucoside; Pn-Ac: Peonidin-3-O-(6-O-Acetyl)-glucoside; Mv-Ac: Malvidin-3-O-(6-O-Acetyl)-glucoside; Pt-Co: Petunidin-3-O-(6-O-Coumaryl)-glucoside; Mv-Co: Malvidin-3-O-(6-O-Coumaryl)-glucoside; “—”: shows did not check out; MW-1, 2, 3: the wines of Merlot made of grapes harvested on the three, four, and five weeks after veraison, respectively; PW-1, 2, 3: the wines of Pinot Noir made of the grapes harvested on three, four, and five weeks after veraison, respectively.

TABLE 6
Contents of monomer anthocyanins in wines from different harvest times in 2015.

<table>
<thead>
<tr>
<th>Wine simples</th>
<th>Free anthocyanins contents in skins(mg/g)</th>
<th>Total contents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dp</td>
<td>Cy</td>
</tr>
<tr>
<td>Merlot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MW-1</td>
<td>4.60</td>
<td>0.75</td>
</tr>
<tr>
<td>MW-2</td>
<td>4.98</td>
<td>0.87</td>
</tr>
<tr>
<td>MW-3</td>
<td>4.87</td>
<td>0.83</td>
</tr>
<tr>
<td>Pinot Noir</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PW-1</td>
<td>0.61</td>
<td>0.40</td>
</tr>
<tr>
<td>PW-2</td>
<td>0.42</td>
<td>0.41</td>
</tr>
<tr>
<td>PW-3</td>
<td>0.38</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Notes: Dp: Delphinidin-3-O-glucoside; Cy: Cyanidin-3-O-glucoside; Pt: Petunidin-3-O-glucoside; Pn: Peonidin-3-O-glucoside; Mv: Malvidin-3-O-glucoside; Pn-Ac: Peonidin-3-O-(6-O-Acetyl)-glucoside; Mv-Ac: Malvidin-3-O-(6-O-Acetyl)-glucoside; Pt-Co: Petunidin-3-O-(6-O-Coumaryl)-glucoside; Mv-Co: Malvidin-3-O-(6-O-Coumaryl)-glucoside; “—”: shows did not check out; MW-1, 2, 3: the wines of Merlot made of grapes harvested on the three, four, and five weeks after veraison, respectively; PW-1, 2, 3: the wines of Pinot Noir made of the grapes harvested on three, four, and five weeks after veraison, respectively.

As for the quality of Merlot, the quality of wines from MW-3 was the best, therefore, the optimal harvest time for Merlot is five weeks after veraison. However, the taste scores of MW-2 were the highest followed by MW-3 and MW-1, while, in addition to the taste, the other sensory indicators of MW-3 wines were the highest, followed by MW-1 and MW-2.

CONCLUSIONS
By studying the grapes and wines from different sampling times, a significant relationship was observed between the harvest time and the content of phenolic compounds. Also, at difference sampling times, the basic indexes and the content lowest, followed by PW-3 and PW-1. Due to the climate changes in 2014, the result was opposite in 2015. The aroma compounds in the grapes are affected by the degree of maturity, climate, variety and other factors (Coelho et al., 2007; Magariño and José, 2006). Only when the grapes reached high-level maturity, we could evaluate the quality of aroma (Coelho et al., 2007; Vilanova et al., 2010). According to the scored points of wines, PW-3 was the best, followed by PW-2 and PW-1.

As for the quality of Merlot, the quality of wines from MW-3 was the best, therefore, the optimal harvest time for
Effects of Harvest Time on the Maturity of Grape Polyphenols

The organoleptic properties of wines at different sampling times in 2014. MW-1, 2, 3: the wines made of Merlot grapes harvested on three, four, and five weeks after veraison, respectively; PW-1, 2, 3: the wines made of Pinot Noir grapes harvested on three, four, and five weeks after veraison, respectively.

The organoleptic properties of wines at different sampling times in 2015. MW-1, 2, 3: the wines made of Merlot grapes harvested on three, four and five weeks after veraison, respectively; PW-1, 2, 3: the wines made of Pinot Noir grapes harvested on three, four, and five weeks after veraison, respectively.

of phenolic compounds of grapes had significant differences. Hence, our data provide support for ensuring the best harvest time. Three and five weeks after veraison of 2014 and 2015, respectively, could be the optimal harvest time from looking at the content of phenolic compounds and from the sensory analysis of wines. Further studies about the relationship between the harvest time and the content of monomer anthocyanins in wines, as well as more sensory analysis of wines will be of greater benefit to determine the optimal harvest time, further to obtain the best quality of wines. On the basis of our findings from this study, we proposed two indexes in order to simplify the practice of winery. The content of anthocyanins in skins and total content of phenolic compounds in seeds are seen as the principal index when the reducing sugar-acid ratio reaches the requirements of harvest. This was done in order to illustrate the optimal harvest time and to ensure the best quality of grapes and wines in the locality. For the special climatic conditions in Northwest China, our conclusion would be a benefit to the quality of wines produced in the locality.

LITERATURE CITED


Effects of Harvest Time on the Maturity of Grape Polyphenols


