Wine quality of grapevine ‘Cheongsoo’ and the related metabolites on proton nuclear magnetic resonance (NMR) spectroscopy at the different harvest times

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Abstract

Harvest time is major factor of affecting fruit and wine quality of grape. But quantification evaluation of wine quality according to harvest time is too difficult. We conducted this study to identify the metabolites, affecting the sensory, quality and fruit characters of grapevine ev. ‘Cheongsoo’ at the different harvest times, using proton nuclear magnetic resonance (NMR) spectroscopy. Our results showed that there was a clear separation of the spectrum data on the PCA plot at each harvest time. In addition, the S-plot of OPLS-DA was useful to identify the essential metabolites across the harvest times. Tartaric acid (84.47) was a main factor responsible for the titratable acidity and the acidity of the wine on the sensory test. In addition, both proline (62.37) and arginine (63.25) are nitrogen source during wine fermentation, and they were closely associated with the body and balance of the wine on the sensory test. The relative concentration of proline increased across the harvest times, and it was a positive factor that is associated with changes in the body and balance of the wine. In conclusion, our results indicate that the metabolic profiling based on proton NMR spectroscopy, combined with a multivariate analysis, is useful in identifying the metabolites that are responsible for the quality of grape and wine.

Keywords: Vitis labruscana; Proton NMR; Metabolic profiling; Multivariate data analysis; Berry ripening.

Abbreviations: PCA_principal component analysis; OPLS-DA_orthogonal projections to latent structures discriminant analysis; NMR_Nuclear magnetic resonance; TSP_trimethylsilyl propionic-2,2,3,3-d4 acid.

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Introduction

Generally, there are 10,000 cultivars of grapevine and they are classified as wine, table grape and raisin according to the use. Grape ripening is affected by some factors such as climate, irrigation, soil condition and pest controls (Creasy et al., 2009). It is generally known that it is very difficult to decide on the timing of harvesting the grapevine for wine making. This is because the quality of wine grape and its flavor should also be considered during the wine aging (Jackson, 2008). Moreover, the quality of grapevine cannot be heterogenous to make sure that the homogeneous features should be maintained (Smith, 2004). It has been reported that the timing of harvesting the grapevine is closely associated with the firmness, color and sugar/acid ratio of fruit (Weaver, 1976). But this does not apply to the quality of grapevine. Therefore, the optimal timing of harvesting the grapevine should be determined on the accumulated sensory test. Previous studies have been conducted to analyze the sugar, thus showing that the aroma and phenolic compound are responsible for grape ripening (Neira et al., 2004). The quality of wine is an integrated response to the sensory properties of the wine but it varies depending on the taster. Prior to the sensory test, it is possible to perform an analysis of the chemical constituents such as tannin, anthocyanin, and volatile acid and residual sugar. Thus, the quality of wine can be indirectly evaluated. There have been recent studies to quantify changes in the amount of the volatile compounds during grape ripening or winemaking (Rocha et al., 2010). Moreover, other studies have also been conducted to make a rapid determination of the quality of wine on near-infrared (NIR) spectroscopy (Le Moigne et al., 2008). Metabolic profiling is a useful technique to examine the clue to the variation (Sumner et al., 2003), and it can be done based on various methods of multivariate data analysis. Moreover, it is also useful to analyze the variations of metabolites using a large amount of samples such as random mutants of Arabidopsis (Fiehn et al., 2000). To date, many analytical techniques have been developed to perform a metabolic profiling experiment and these include infrared spectroscopy (IR), nuclear magnetic resonance (NMR), gas chromatography-mass spectroscopy (GC/MS), liquid chromatography-mass spectroscopy (LC/MS) and other types of coupled spectroscopy (Sumner et al., 2003). Of these, NMR methods can be identified chemical specificity for compounds containing elements with non-zero magnetic moments such as proton(H), carbon(C), nitrogen(N), and phosphorus(P) (Sumner et al., 2003). These elements are commonly found in most metabolites (Bligny and Bouce, 2001). NMR is useful to obtain a broad-spectrum of data about metabolites even with the simple preparation of samples. It can therefore be stated that the NMR is an ideal tool for rapidly analyzing overall metabolic changes. Some previous studies have reported that the NMR is useful to perform a metabolic profiling experiment for the evaluation of the quality of wine (Ali et al., 2012; Bligny and Douce 2001, Hong et al., 2012, Son et al., 2009). PCA is an unsupervised method which reduced the
dimensionality of the variances (Ericksson et al. 2006). It is possible to plotting in the multidimensional space for determine the similarities and differences between data in the treatments. OPLS-DA is a recent modification of the PLS method. This method concentrated predictive power into the first component, so provide more improving model for transparency and interpretability (Ericksson et al. 2006). Multivariate data analysis with NMR spectroscopy is very important to providing a significant clue. Grape cv. ‘Cheongsoo’ is a hybrid grape between ‘Seibel 9110’ and ‘Himrod Seedless’, and it was first bred in 1965 according to the grape-breeding program and then preliminarily selected in 1990. Moreover, it is a seedless white grape with a full fruit aroma and has been used for table and wine grape production (Lee et al., 1994).Given the above background, we conducted this study to evaluate the quality of grape cv. ‘Cheongsoo’ wine by analyzing its metabolites across the harvest times and the results of sensory test. Moreover, we also attempted to identify the correlation between the variability of metabolites and the harvest time on multivariate analysis. 

Results and Discussion

**Fruit characters**

In this study, the fruit characters of ‘Cheongsoo’ were significant differences in the length, width and weight of the fruit between the harvest times (Table 1). Their approximate values were 16.4-17.4 mm, 15.8-16.9 mm and 2.9-3.2 g in the corresponding order, thus reaching harvest maturity at 99 DAF, set at the harvest time when the fruit growth was completed. In white grapes, it is difficult to examine their veraison on gross examination. But it can be evaluated based on changes in their firmness. The firmness of the fruit was decreased after the harvest time I.

**Wine characters**

We evaluated the quality of wine across the four harvest times. The pH of wine 3.14 at the harvest time I and it was increased as the harvest time was prolonged (Table 2). In addition, it was 3.46 at the harvest time IV. But there was no significant difference in the pH of wine between the harvest time II (September) and IV. It is generally known that the pH of wine is subject to the fermentation process, the degree of maturation and contamination during storage and the storage conditions (Jackson, 2008). The optimal pH of grape juice prior to fermentation process is 3.2-3.6 and that of the final wine is 3.2-3.3. If the pH of wine is higher than 3.6, this would cause bacterial contamination during storage. But if it is lower than 3.2, this would decrease the quality of wine because of the strong acidity (Park et al., 2002). There was a significant decrease in the titratable acidity across the harvest times. The percentage of acid content of the grape should fall in a range between 0.65 and 0.85. The optimal percentage of acid content of the white wine should be 0.65% (w/v) (Conde et al., 2007).

There was a gradual decrease in the total amount of polyphenol across the harvest times, but there was no significant difference in it between the harvest times III and IV. In addition, the amount of tannin reached the maximum at the harvest time III. It has been reported that if there is an excess of polyphenol or tannin in the white wine, this would cause a bitter taste. Therefore, a high amount of polyphenol or tannin would be undesirable (Fischer et al., 1994). The concentration of volatile acid was changed from 307 to 355 mg/L. This strongly indicates that it was increased across the harvest times. The quality of the grape is a key factor for winemaking process. It is generally known that the quality of wine is decreased when there is an excess of the volatile acid content.

**Sensory test**

Sensory attributes of ‘Cheongsoo’ wine are represented in Table 3. Its clarity was evaluated as high and then given a score of 4.5-4.8 out of 5 points. Moreover, there was no significant correlation between the clarity of ‘Cheongsoo’ wine and the harvest time. Moreover, its color was the darkest at the harvest time I, but it was given a score of > 4.3 points at the other harvest times. The golden wine had the highest color score. All the wines were given a score of > 3.0-4.1 points for their aroma and bouquet and 2.1-3.3 points for the intensity of aroma. But there were no significant differences in these two parameters between the harvest times. At the harvest time, the degree of aroma and bouquet and that of fruit flavor were the lowest. Moreover, ‘Cheongsoo’ wine had the highest degree of aroma and sweet flavor, as shown in other well-ripened fruits such as pineapple, melon, peach and apple. There was a significant correlation between the acidity of the wine and the harvest time. The degree of acidity was the highest at the harvest time I and then given a score of 3.0 points. Overall, however, it was moderate at the harvest times III and IV and then was given a score of 1.6 and 1.1 points, respectively. The acidity of wine is closely associated with tartaric, malic and lactic acids (Jackson, 2008). Our results also showed that there was a significant correlation between the body of the wine and the harvest time. The body of wine had a score of 1.9 points at the harvest time I, being the lowest, and 3.3 points at the harvest time IV, being the highest. These results indicate that the body of wine had a higher quality at later harvest time. In white wine, if there is an excess of tannin, this would cause a bitter taste (Fisher et al., 1994; Fontoin et al., 2008). The body of wine is associated with sugar and alcohol, but this is not of great significance (Jackson, 2008). The overall balance of the wine showed the highest score of 3.5 points at the harvest time III. Previous studies have shown, however, that proline level may also be the only indicator that affects the body of wine (Skogerson et al., 2009).

**Metabolic profiling**

There was an overlap of signals in the NMR spectra of such constituents as sugar. But most of them remained unidentified. *J*-resolved NMR spectroscopy is one of the methods for identifying metabolites from the overlapped signals. Because the coupling constant (*J*) associated with each multiplet is also a valuable aid to structure elucidation (Fan 1996). A sum of spectra is obtained and then compared with libraries of spectra corresponding to pure standard and *J*-resolved data. Binmed 1H NMR data of the grape harvested at four different times was obtained and then plotted with principal component analysis (PCA) (Fig. 1). The NMR signal was plotted based on the eigenvector scores for the three different groups. Two principal components (PCs) accounted for 83.1% of total variations; PC1 did 52.0% and PC2 did 31.1%. The samples from the harvest times I and II were clustered on the positive side of PC1 and PC2, respectively. In addition, they were also individually analyzed, but those from the harvest times III and IV were analyzed together. The results of PCA were described based on two variables: R2 and Q2 values. Our results revealed an R2 of
That is, the 
7 and δ3.81, 
δ3.77 s III 
easts cannot typically 
onditions 
time factors include only a few were entered in our database. 
metabolites associated with the positive and negative factors, 
negative factors include 
(Jung et al. 2010) 
correlation 
al. 
find markers of metabolites in factors interacting with each other, whose results are useful to 
OPLS showing no significant difference on the PCA plot, for the 
did not use the data obtained at the 
two 
showed the 
such as 
model 
The 
significant 
PCA is an unsupervised method that attempt to 
Factors interacting with each other, whose results are useful to 
OPLS analysis, we 
did not use the data obtained at the 
harvest times (Fig. 
In addition, we also identified the negative factors during the 
same period and these include δ2.77, δ2.85, δ2.89, δ3.53, δ3.57, δ3.73, δ3.77, δ3.81 and δ4.45. 
In addition, we also identified the negative factors during the 
same period and these include δ2.57, δ2.61, δ4.37 and δ4.41. 
The positive factors include 
proline (δ2.37), citric acid (δ2.65, δ2.77), malic acid (δ2.73), asparagines (δ2.89), glycine (δ3.57), 
glutamic acid (δ3.73) and arginine (δ3.77) and the negative ones include tartaric acid (δ4.37) (Fig. 2D). 
These results suggest that the amount of proline (δ2.37) was increased and that of tartaric acid (δ4.47) and arginine (δ3.25) was decreased after the harvest time II (Fig. 3). 
Nitrogenous compounds are the second most essential nutrient for yeast in the wine fermentation. The optimal concentration of nitrogenous compounds ranges between 330 and 530 mg/L, which varies depending on yeast strain and sugar content of the must (Stines et al., 2000). It is generally known that yeasts cannot typically use proline in anaerobic conditions. Therefore, the concentration of proline is maintained as high. Moreover, yeasts use other amino acids, including arginine in particular, as a nitrogen source (Ough, 1968; Rochfort, 2010). Proline has been reported to be an essential metabolite contained in the body of wine (Skogerson, 2009). In the current study, the sensory test showed that the body and balance of the wine had a high amount of proline at the harvest times III and IV. Previous studies have shown that the amount of proline may be an indicator that solely affects the body of wine (Skogerson et al., 2009). With the increased amount of proline, the viscosity

Table 1. Fruit characters of grape cv. ‘Cheongsoo’ at the four different harvest times.

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Berry length (mm)</th>
<th>Berry width (mm)</th>
<th>Berry weight (g)</th>
<th>Firmness (kgf⁻¹·cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13.2±1.02</td>
<td>12.8±1.12</td>
<td>1.5±0.38</td>
<td>1.0±0.23</td>
</tr>
<tr>
<td>II</td>
<td>16.4±0.13</td>
<td>15.8±0.08</td>
<td>2.9±0.08</td>
<td>0.4±0.03</td>
</tr>
<tr>
<td>III</td>
<td>16.6±0.18</td>
<td>16.3±0.10</td>
<td>3.0±0.12</td>
<td>0.4±0.03</td>
</tr>
<tr>
<td>IV</td>
<td>17.4±0.83</td>
<td>16.9±0.73</td>
<td>3.2±0.47</td>
<td>0.3±0.05</td>
</tr>
</tbody>
</table>

I is DAF85 (29 Jul.), II is DAF99 (8 Sep.), III is DAF113 (19 Sep.), and IV (29 Sep.) is DAF120. ²Length, width, berry weight and firmness are measured from 30 berry grains. Total polyphenol and total anthocyanin in the skin are measured from triplicates. Means with the same letter are not significantly different at the 5% by Tukey’s HSD.

Fig 1. PC1 scores vs PC2 scores of Principal component analysis (PCA) score plot of four different harvested wine The numbers indicate the different harvest times; 1 is DAF85 (29 Jul.), 2 is DAF99 (8 Sep.), 3 is DAF113 (19 Sep.), and 4 (29 Sep.) is DAF120. ²Length, width, berry weight and firmness are measured from 30 berry grains. Total polyphenol and total anthocyanin in the skin are measured from triplicates. Means with the same letter are not significantly different at the 5% by Tukey’s HSD.
Table 2. Wine characters of grape cv. ‘Cheongsoo’ at the different harvest time.

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>pH</th>
<th>Titeratable acidity (% w/v)</th>
<th>Total sugar (% w/v)</th>
<th>Total polyphenol (mg·L⁻¹)</th>
<th>Tannin (mg·L⁻¹)</th>
<th>Volatile acid (mg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.14 b</td>
<td>0.86 a</td>
<td>0.11 a</td>
<td>147 c</td>
<td>313 b</td>
<td>309 b</td>
</tr>
<tr>
<td>II</td>
<td>3.26 b</td>
<td>0.68 b</td>
<td>0.10 a</td>
<td>239 b</td>
<td>421 ab</td>
<td>307 b</td>
</tr>
<tr>
<td>III</td>
<td>3.47 c</td>
<td>0.59 c</td>
<td>0.11 a</td>
<td>286 a</td>
<td>449 a</td>
<td>355 a</td>
</tr>
<tr>
<td>IV</td>
<td>3.46 a</td>
<td>0.55 d</td>
<td>0.10 a</td>
<td>298 a</td>
<td>414 ab</td>
<td>352 a</td>
</tr>
</tbody>
</table>

* Means with the same letter are not significantly different at the 5% by Tukey’s HSD.

Fig 2. OPLS-DA analysis on the differential harvested grapevine cv. ‘Cheongsoo’. A and C plot is score plot and S plot of OPLS-DA between harvest time I and II. C and D plot is score and S plot of OPLS-DA between harvest time III and IV. Dash line in the plot A and B is Hotelling’s T² range with 95% confidence. Open triangle in the plot C and D is significantly positive or negative related metabolites between harvest times ($p ≥ 0.05$ and $p$ (corr) $≥ 0.5$).

and density of wine are increased. Our results were also in agreement with previous reports in this series (Fig. 3). During grape ripening, arginine is a major form of nitrogen compound transported in the phloem. Its relative concentration may vary up to 20-fold (Stines et al., 2000). Meanwhile, its relative peak area was lowered across the harvest times. It has been reported that the accumulation of glycine begins with the onset of **veraison** on the S-plot during grape ripening (Fig. 2C). Moreover, it is closely associated with the increased amount of sugar (Adams and Liyanage, 1993). In the current study, however, there was no significant difference in the amount of glycine between the harvest times II and III (Fig. 2D). **Grape ripening** begins in August and it lasts about 45 days of the **veraison**. Then, many metabolites are converted and then accumulated before harvest. Both tartaric and malic acid are a major organic acid that accounts for about 70-90% of the total acid contents (Kliwer, 1966; Jackson, 2008). Tartaric acid is not commonly present in a higher plant. It appears that the grape is the only widespread commercial fruit with it (Rufner, 1982). Moreover, it is a major acid constituent that is greatly responsible for the tart taste of wine. Furthermore, it contributes to both the biological stability and the longevity of wine (Conde et al., 2007). Our results showed that it is one of the essential negative factors and it showed a significant difference between the harvest times II and III (Fig. 2D). Its relative peak area was continuously decreased across the harvest times (Fig. 3). Our results showed that there were significant differences in the acidity and balance of the wine on the sensory test between the harvest times II and III. These findings are due to the pH, the titratable acidity of wine and its acidity on the sensory test. Malic acid is accumulated in green berries, and its concentration varies up to 15 mg/g fresh weight (FW). It is known that the concentration of malic acid is rapidly decreased 2-3 mg/g FW after the onset of grape ripening (Rufner, 1982). It plays a role as a nutrient reserve to replace glucose following **veraison**. Therefore, the concentration of malic acid is significantly decreased during grape ripening to a higher extent as compared with tartaric acid (Jackson, 2008). During grape ripening, it is converted to fructose and glucose or may also be used as a carbon and energy source for respiration before **veraison** (Conde et al., 2007). Our results showed that its relative peak area was correlated with the harvest times. In addition, it was also shown that it is a negative factor on the S-plot between the harvest times II and III (Fig. 2D). We do not assign to enter many significant factors in our database (marked as asterisk on the plot C and D). But we could identify changes in the metabolites at the different harvest times. We also detected various types of metabolites through metabolic profiling
Table 3. Sensory test results of ‘Cheongsoo’ wine at the different harvest time.

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Clarity</th>
<th>Color</th>
<th>Aroma Bouquet</th>
<th>Aroma intensity</th>
<th>Acidity</th>
<th>Sweetness</th>
<th>Body</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4.5 a</td>
<td>2.8 b</td>
<td>3.0 a</td>
<td>2.1 a</td>
<td>3.0 a</td>
<td>1.1 a</td>
<td>1.9 b</td>
<td>2.3 b</td>
</tr>
<tr>
<td>II</td>
<td>4.5 a</td>
<td>4.3 a</td>
<td>3.5 a</td>
<td>3.0 a</td>
<td>2.8 ab</td>
<td>1.4 a</td>
<td>2.6 ab</td>
<td>2.4 b</td>
</tr>
<tr>
<td>III</td>
<td>4.8 a</td>
<td>4.6 a</td>
<td>4.1 a</td>
<td>3.3 a</td>
<td>1.6 bc</td>
<td>1.5 a</td>
<td>3.3 a</td>
<td>3.5 a</td>
</tr>
<tr>
<td>IV</td>
<td>4.8 a</td>
<td>4.8 a</td>
<td>3.4 a</td>
<td>2.8 a</td>
<td>1.1 c</td>
<td>1.8 a</td>
<td>2.5 ab</td>
<td>2.8 ab</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at the 5% by Tukey’s HSD.

Fig 3. The relative peak area in the spectrum of influenced metabolites at the late harvested grapevine cv. ‘Cheongsoo’. A: Proline (δ2.37), B: Tartaric acid (δ4.47), C: Arginine (δ3.25).

Table 4. Proton NMR chemical shifts of wine metabolites identified by J-resolved and reference.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Chemical Shifts (ppm) and coupling constants (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>δ 1.94 (s)</td>
</tr>
<tr>
<td>Alanine</td>
<td>δ 1.48 (d, J = 7.3)</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>δ 4.52 (d, J = 2.0)</td>
</tr>
<tr>
<td>Asparagine</td>
<td>δ 2.88 (m)</td>
</tr>
<tr>
<td>Arginine</td>
<td>δ 3.78 (t, J = 6.1), 3.25 (t, J = 6.9)</td>
</tr>
<tr>
<td>Citric acid</td>
<td>δ 2.65 (d, J = 15.1), δ 2.77 (d, J = 15.1)</td>
</tr>
<tr>
<td>Fructose</td>
<td>δ 4.11 (m)</td>
</tr>
<tr>
<td>Glucose</td>
<td>δ 5.24 (d, J = 3.7), δ 4.65 (d, J = 8.0)</td>
</tr>
<tr>
<td>Glutamine</td>
<td>δ 2.14 (m), 2.46 (m)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>δ 3.71 (dd, J = 7.0, 1.9)</td>
</tr>
<tr>
<td>Glycine</td>
<td>δ 3.57 (d, J = 0.9 Hz, 1H)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>δ 1.01 (d, J = 7.1)</td>
</tr>
<tr>
<td>Leucine</td>
<td>δ 0.96 (d, J = 7.5)</td>
</tr>
<tr>
<td>Malic acid</td>
<td>δ 4.33 (dd, J = 9.1, 3.6), δ 2.74 (dd, J = 15.6, 3.6)</td>
</tr>
<tr>
<td>Methionine</td>
<td>δ 2.15 (m), δ 2.65 (t, J = 7.6)</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>δ 4.07 (t, J = 2.9), 3.29 (t, J = 9.4)</td>
</tr>
<tr>
<td>Proline</td>
<td>δ 2.35 (m), δ 3.35 (m)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>δ 7.40 (m)</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>δ 7.82 (dt, J = 7.8, 1.5), 7.47 (m), 6.97 (m)</td>
</tr>
<tr>
<td>Spermidine</td>
<td>δ2.18 (m), 1.80 (m).</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>δ 2.51 (s)</td>
</tr>
<tr>
<td>Sucrose</td>
<td>δ 5.42 (d, J = 2.7)</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>δ 4.36 (s)</td>
</tr>
<tr>
<td>Threonine</td>
<td>δ 1.33 (d, J = 6.6), δ 3.60 (d, J = 4.8)</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>δ 7.74 (d, J = 4.0)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>δ 6.91 (d, J = 8.0)</td>
</tr>
<tr>
<td>Valine</td>
<td>δ 1.02 (d, J = 6.9)</td>
</tr>
</tbody>
</table>
on 1H NMR spectroscopy. Moreover, we could identify the correlation between the metabolites and the harvest time on multivariate analysis. Thus, we identified significant metabolites that are responsible for the quality of grape cv. ‘Cheongsoo’ wine.

Materials and Methods

Plant materials

We measured the fruit characters of ‘Cheongsoo’, such as the length, width, weight and firmness of the fruit at a 7-day harvest interval during a period ranging from July 29 (harvest time I, 85 DAF) to September 29, 2011 (harvest time IV, 120 DAF). Grapes cv. ‘Cheongsoo’ berry collected were measured twenty berries of bunch in the replicated each five grapes at the four different harvest time, using a digital caliper (Mitutoyo, Japan). The firmness was performed using a Texture Analyzer (TA-XT2, Surrey, U.K.) equipped with 2mm probe. Grape qualities characteristic were investigated per a week from 29 July to 29 September from grapes grown by NIHHS in 2011. The samples of grapes collected for Total Soluble Solids (TSS), pH and Titratable acidity (TA) measurements were prepared by removing stems and crushed by hand to prevent breakage of seeds. Total 20 berries of grapes each harvest were collected juice using a presser, and juices were replicate three times at every harvest. Juice samples were analyzed for TSS using a digital refractometer (PAL-1, ATAGO, Japan). The pH was measured using a pH meter (Model 115PD, Istek, Korea). For TA, 20 mL of sample was titrated with 0.1N-NaOH to an end point of pH 8.2. Total acid was converted to tartaric acid equivalents.

Vinification

Grape bunch removed stems from grapes and added potassium metabisulfite (K2S2O5) with the concentration of 100 mg/kg to 10 kg of grape must. Before fermentation, grape must was extracted of the juice and adjusted the sugar content of the grape must to 22°Brix using white sugar. More than 5 hours after application of potassium metabisulfite, active yeasts were inoculated into grapes juice at the ratio of 0.02% of grape juice weight (w/w). While keeping the temperature at 15°C, we fermented grapes juice in a 10L glass fermentation vessel equipped with air lock installations for a month. As the yeast for fermentation, Saccharomyces bayanus (EC-1118, Canada) was selected. After the 1st fermentation, we conducted the 1st isolation of sediment and fermented residual sugar. Sample was analyzed after completely fermented residual sugar and second isolation of sediment.

Total polyphenols and anthocyanins

Total anthocyanins and total polyphenols analyses were done after removing stems. Total 10 g of grapes were randomly selected three replications. Skins were separated from the pulp. Skins were blotted on paper towels to remove any residual pulp. Skin sample of 1 g and 10 mL of 10% formic acid in methanol were extracted for 24 hours at dark place. The mixture was centrifuged at 4,000 rpm for 10 min at 4°C. The supernatant was filtered through a 0.45 μm membrane filter (HAWP Millipore Co., Bedford) and then analyzed. Total anthocyanin and total polyphenol contents were analyzed by spectrophotometer method according to Mazza et al. (1999). Tannin content of wine was determined by Folin–Ciocalteau procedure, using tannic acid as standard (Folin and Ciocalteu 1927). The volatile acid was measured by titration methods. These procedures were recommended by the D.O.C.E. Volatile acidity is determined in the solution obtained from distillation of wine, pretreatment of the sample is not necessary. The result was expressed as mg of acetic acid per 1 L of wine.

Sensory test

Sensory test of wine, prepared by harvest time, was carried out to find out the possibility of brewing and the optimum harvesting time. Sensory analysis of wines was evaluated with ten expert tasters affiliated to the Korea Sommelier Association (KSA). Explanation of the descriptive terms applied to this wine was sufficiently held before the sensory analysis, to give information about the characteristics of score cards. Eight different descriptive terms were used: clarity (degree of clearness), color (definition of color), flavor, aroma intensity, acidity intensity, sweet intensity, body (sense of feeling in the mouth) and balance/harmony of eight different terms. Samples were presented in wine taster glasses with random order. These descriptors were scored from 0 to 5 points. Very poor or very low intensity was corresponded to 0 and very good or very strong intensity to 5. Before the tasting sessions, wines were stored at 8 ± 1°C for 24 h.

Sample extraction

Proton NMR analysis of wine extracts was modified to Deborde et al. (2009). Total 2ml of wines were collected at the difference harvest time. Collected wines were N2 gas evaporation with evaporator (Eyela, Japan). The dried extracts were titrated with KOD to pH 6 in 400mM potassium phosphate buffer in D2O, and lyophilized again. The dried samples were solved in 1ml of D2O with sodium salt (trimethylsilyl propionic-2,3,3-d4 acid; TSP) at a final concentration of 0.75% for chemical shift standard. Total 0.5ml supernatant of each sample was transferred into 5mm NMR tubes.

NMR analysis

Extraction samples were analyzed Bruker 600MHz NMR using a 5mm TXI probe and fitted with an autosampler. Each spectrumspectrum was acquired with 128 scans of 64K data points with a spectral width of 12,376 Hz, a pulse angle of 30°, an acquisition time of 2.65 s and recycle delay of 1s per scan in order to allow complete relaxation and absolute quantification. Spectra were acquired under an automation procedure (automatic shimming and automatic sample loading) requiring about 8 min per sample. Acquired NMR FID files were Fourier transformed, phased and baseline corrected using MNOVA 8.0 software (Mestrelab Research, Santiago, Spain) following procedure of manufacture. The assignment of each metabolite used ppm and 1H–1H J-resolved plot. Assigned results were compared own DB and other literatures (Deborde et al. 2006; Fan TW 1996)

Statistical analysis

Random three replicate was used for four sampling dates for bunches. Fruit characters and wine characters were compared to means significant at P≤0.05 with Tukey’s HSD test of one-way ANOVA using R program (Ver 2.13.0). Statistical analysis of metabolic profiling with Principal component analysis (PCA), and bidirectional orthogonal projections to latent
structures (OPLS) were analyzed with the SIMCA-P+ software (v12.0, Umetrics, Umea, Sweden).

**Conclusion**

Significant metabolites, affecting fruit and wine quality at the different harvest times, were identified using metabolite profiling and multivariate statistical techniques. Proline, tartaric acid, and arginine were identified major metabolites affecting wine quality. The content of proline in wine was increased late harvest time. Previously reported proline was affected sensory test such as wine body. Our result shown increasing proline content and wine body score at the harvest time III, IV. Also tartaric acid content related wine acidity. In other metabolites were significantly different at the harvest time. In these results were possible to adjust GC/M proton NMR method, metabolic profiling and multivariate statistical technique.

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