1H Nuclear magnetic resonance-based metabolomic study for Cabernet Sauvignon wines in different vintages

An ^{1}H NMR-based metabolomic study was used to characterize 2009, 2010, 2011, and 2012 vintages of Cabernet Sauvignon wines from Ningxia that were vinified in the same fermentation technique. The pattern recognition methods of principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) clearly distinguished vintages of wine, driven by the following metabolites: valine,2,3-butanediol, ethyl acetate, proline, succinic acid, lactic acid, acetic acid, glycerol, gallic acid and choline. The PLS-DA loading plots also differentiated between the metabolites of different vintages. The highest levels of valine, 2,3-butanediol, gallic acid and proline were found in the 2009 vintage wines. The 2011 vintage wines contained the highest levels of lactic acid, and the highest levels of ethyl acetate, succinic acid, glycerol and choline were detected in the 2012 vintage wines. A total of 8 metabolites were selected from the ^{1}H NMR spectra and quantified according to their peak areas, yielding concentrations in a greement with the PLS-DA results.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2332v1 | CC BY 4.0 Open Access | rec: 2 Aug 2016, publ: 2 Aug 2016

^{1}H Nuclear magnetic resonance-based metabolomic study for Cabernet Sauvignon Wines in Different Vintages

Boran Hu[1,*], Qi Zhao[1], Jiangyu Zhu[1], Yaqing Yue[1], Guangnian Lu[1], Hua Li[1] , Fengmin Zhang[2], W. J. Hardie[3]

[1] College of Food Science and Engineering, Yangzhou University, Yangzhou, Jiangsu, China

[2] Testing Center of Yangzhou University, Yangzhou, Jiangsu, China

[3] Department of Earth Sciences, Northern Melbourne Institute of TAFE, Melbourne, Victoria, Australia

[1]Corresponding Author:

Boran Hu[1] DAG

Yangzhou, Jiangsu, China

Email address: 85425674@qq.com

* Supported by the China National Natural Science Foundation Project (No.31271857）

ABSTRACT

An ^{1}H NMR-based metabolomic study was used to characterize 2009, 2010, 2011, and 2012 vintages of Cabernet Sauvignon wines from NingxXia that were vinified in the same fermentation technique. The pattern recognition methods of principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) clearly distinguished vintages of wine, driven by the following metabolites: valine, 2,3-butanediol, ethyl acetate, proline, succinic acid, lactic acid, acetic acid, glycerol, gallic acid and choline. The PLS-DA loading plots also differentiated between the metabolites of different vintages. In the 2009 vintage wines we found gallic acid , valine, proline and 2,3-butanediol provide the highest levels. The 2011 vintage wines contained the highest level of lactic acid, and the highest levels of ethyl acetate, succinic acid, glycerol and choline were detected in the 2012 vintage wines. We picked out 8 metabolites from the ^{1}H NMR spectra, quantified according to the peak areas in total, concentrations in agreement with the PLS-DA results.

Subjects Food Science and Technology, Metabolomic

Keywords Wine, Metabolomics, NMR, Pattern recognition

INTRODUCTION

Wine obtains several metabolites from grape berries during fermentation. Many factors, including the soil, climate, winemaking process, and vintage, contribute to the metabolites composition and content of wines (Son et al., 2009; Rochfort et al., 2010; Forveffle, Vercauteren & Rutledge, 1996; Hu et al., 2015). So far, most studies about wines have focused on the characterization and evaluation of the biological activity of selected extractable components, and the lack of research on the metabolites in wines. The common parameters to evaluate the quality of wine are total soluble solids, alcohol concentration, total acids and total phenols. These basic parameters are significant, while the classical analytical methods can easily detect a great deal of other important compounds. (Son et al., 2008, 2009; Pereira et al., 2005; Amaral & Caro, 2005). These parameters only reflect the health of the wine, cannot fully explain the quality of the wine. Therefore, the analysis of metabolites in wine quality assessment and powerful advanced analysis

methods are needed to determine the metabolites in wines. (Sun et al., 2013; Son et al., 2008)

^{1}H NMR-based metabolomics one pair of potential information extraction and classification of samples provides a great new method to evaluate the metabolic functions. Between proton nuclear magnetic resonance (^{1}H NMR) spectroscopy combined with PCA and PLS-DA has been used to distinguish the wines vinified by same species growing in different geographical regions (Son et al., 2008, 2009; Papotti et al., 2012) and between different cultivars grown in the same geographic region (Rochfort et al., 2010), etc.

In our study, we use PCA and PLS-DA to distinguish different vintages of Cabernet Sauvignon wines.

MATERIALS AND METHODS

We are making it clear that no specific permissions were required for activities and the field studies did not involve protected species.

All wine samples were vinified in Ningxia province (Northwest China). The grapes were grown non-grafted in a single vineyard of uniform soil type (containing gravels main of light sierozem, organic matter content 0.4~1.0 %) in the Helan Mountains of Ningxia Province, located in warm temperate in the northern hemisphere have a dry continental climate with dry summers and severe winters in the last forty years, the average temperature was 15.24 **℃**, rainfall of 264.45 mm and evaporation was 1312.0 mm during the growth vintage (March-October period). Little changes in climate were registered in 2009 to 2012.Its climate information shown in Table 1. The vineyard start planted in 1994.Planted in the north-south line in a single hole, the line spacing is 2.5 meters spacing 1.2m~1.5m, using standardized management.

Table 1. Climate information during growth vintage (March-October period) in 2009-2012 of the vineyard.

Vintages	Average temperature (**℃**)	Rainfall (mm)	Evaporation (mm)
2009	17.65	243.5	1562.1
2010	17.11	233.6	1398.7
2011	15.24	262.2	1423.6
2012	16.52	251.4	1266.7

Sample Origin

Samples were obtained from 2009, 2010, 2011, and 2012 vintages of Cabernet Sauvignon wines, were named S1, S2, S3, S4 respectively,6 samples per year, every sample has 3 parallel. The wines were vinified in the same fermentation technique and same yeast (Lalvin CY 3079), without other chemical adjustment except for potassium metabisulfite (50 mg/L) and not aged in oak barrels. After fermentation, stored the wines in fermenting tank (50 t).

We got 3 parallel samples of each wine from the sampling mouth, every replicate sample was funneled in a brown glass bottle (750 mL), then sealed it with a cork and transported to the laboratory storage(-4°C). The grapes of each vintages were harvested at similar concentrations of reducing sugar and titrable acidity (Table 2). The chemical and physical features of wines meet the China national test standard (GB/T 15038-2006), are shown in Table 3.

Table 2. Grape composition at harvest.

Harvest Date	Cultivar	Reducing Sugar (g/L)	Titrable Acidity (g/L)	pH
2009.8.15	Cabernet Sauvignon	232.7	7.67	3.73
2010.8.16		228.8	6.98	3.88
2011.8.22		220.2	8.32	3.23
2012.8.18		225.61	7.72	3.54

Table 3. Physical and chemical features of the wines.

Index	Vintages			
	2009	2010	2011	2012
Alcohol content % Vol	13.2	12.9	12.4	12.8
Residual sugar g/L	2.20	2.55	3.10	2.50
Total acid g/L	6.4	6.7	6.1	5.8
Volatile acid g/L	0.42	0.45	0.46	0.43
Dry extract g/L	27.9	28.9	27.6	29.1
pH	3.47	3.22	3.56	3.73

Total SO2 mg/L	86	88	82	85
Free SO2 mg/L	31	28	32	33
Methanol mg/L	205	220	214	206
Fe3+ mg/L	2.2	1.9	2.1	2.0
Cu2+ mg/L	0.055	0.053	0.065	0.059
K+ mg/L	946	936	957	955
Ca2+ mg/L	103	97	99	102
Tartaric acid g/L	2.64	2.28	2.32	2.44
Citric acid g/l	0.31	0.28	0.29	0.26
Lactic acid g/L	2.66	2.64	2.73	2.53
Colour tone	12.5	12.8	12.3	12.7
Colour tint	0.83	0.82	0.80	0.81

*Methods of determination of physical and chemical features meet China National Test Standard GB/T15038–2006

NMR Sample Preparation

Ten milliliters of wine was centrifuged at 4000 rpm for 20 min. 3 mL supernatants were frozen at -70°C for 12 h, and then lyophilized for 48 h. The lyophilized wine was dissolved in 400 μL of oxalate buffer (pH =4.0), mixed with 140 μL of D_2O and 60 μL of a 0.75% DSS in D_2O solution, and then in 13000 rpm centrifuged for 20 min. Placed 500 μL supernatants in 5 mm NMR tubes. The chemical shift of DSS provided reference (δ=0) and internal standard quantitative analysis.

^{1}H NMR Spectroscopy

^{1}H NMR spectra were recorded on a Bruker AVANCE 600 spectrometer, operating at 600.13 MHz 1H frequency and a temperature of 298 K, using a ^{1}H {^{13}C/^{15}N} probe. A NOESYPRESAT pulse sequence was used to suppress the residual water signal. A total of 256 transients were collected into 32,000 complex data points with a spectral width of 7183.9 Hz, an acquisition time of 2.3 s, a mixing time of 100 ms and a relaxation delay of 2 s. The NMR spectra were processed with a line-broadening factor of 0.3 Hz prior to Fourier transformation.

NMR Data Reduction

The NMR spectral data were reduced into 0.005 ppm spectral buckets. The regions corresponding to water (4.6-4.8 ppm), incompletely removed DSS (-0.5–0.5 ppm, 1.74–1.84 ppm and 2.90–2.95 ppm) and ethanol (1.18–1.22 ppm and 3.57–3.72 ppm) were removed by AMIX software. The dataset was then imported into SIMCA-P 12.0 for multivariate statistical analysis.

Pattern Recognition

We used PCA and PLS-DA to check the intrinsic variability of the dataset, and to maximize separate out different vintage wines, respectively. After orthogonal signal correction was applied to eliminate the information that did not contribute to the discrimination, PLS-DA score plots from the ^{1}H NMR spectra of different vintage wines were generated in pairwise comparisons (Nicholson, Lindon & Holmes, 1999; Anastasiadi et al., 2009; Lee, Hong & Lee, 2009a).

Chemicals

All chemical reagents were analytical grade. D_2O (99.9%) and DSS were purchased from SIGMA-ALDRICH.

RESULTS

Metabolite Differences in Wines from Different Vintages

The PCA score plot shows a clear differentiation of the Cabernet Sauvignon wines in different vintages, with good adaptability and high predictive of the model has high statistical values of R^2X (0.867) and Q^2 (0.789) (Fig 1).

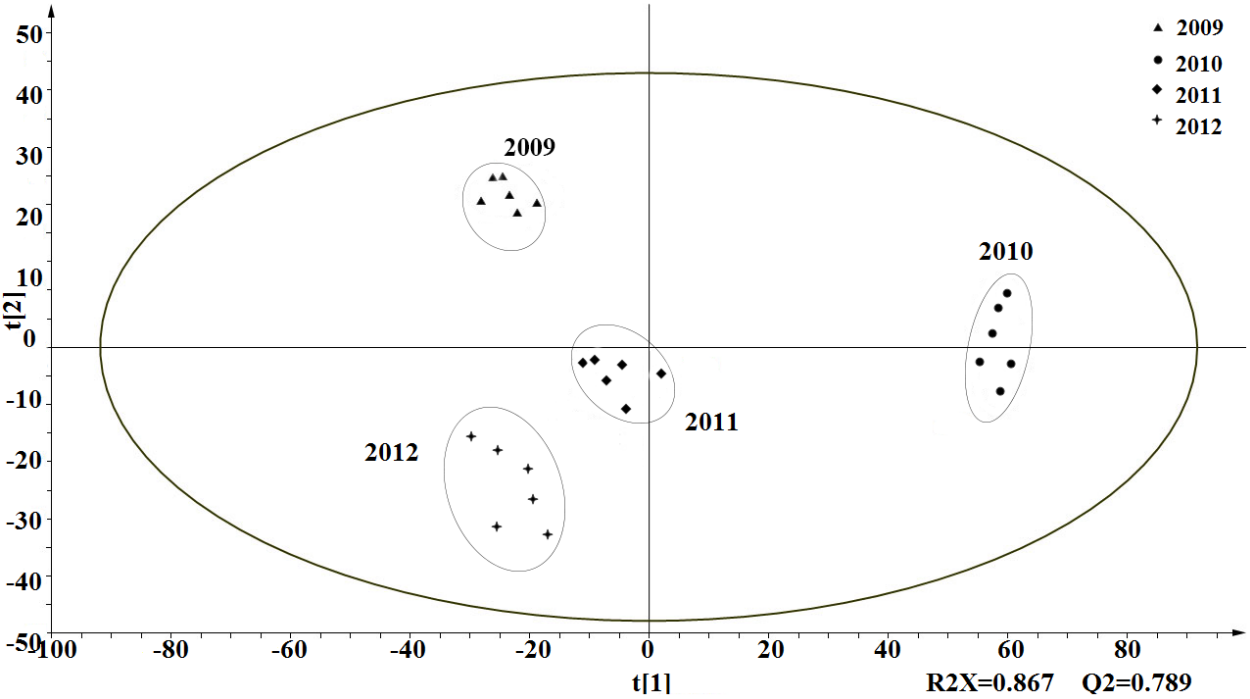


Figure 1 PCA score plot of Cabernet Sauvignon wines of different vintages.

PLS-DA models produces increase comparing the different vintages wines. As shown in Figure 2A the PLS-DA score plots derived from the ^{1}H NMR spectra of the 2009 and 2010 vintages Cabernet Sauvignon wines, has the highest values pairwise comparison of R^2X and Q^2. It has a clear separation between the 2009 and 2010 vintages of wines (Fig 2A). Complementary load plot gives the contribution of metabolites differentiation (Fig 2B). The loading plot represents that metabolites of 2009 vintages is higher than that of 2010. The loading plot revealed a high-level of valine, glycerol, 2,3-butanediol, α-glucose, acetic acid, proline, succinic acid, sucrose, tartaric acid, gallic acid, and tyrosine in the 2009 vintages, while ethyl acetate, lactic acid, choline, β-glucose, and α-D-glucuronic acid were lower level relative in the 2010 vintages.

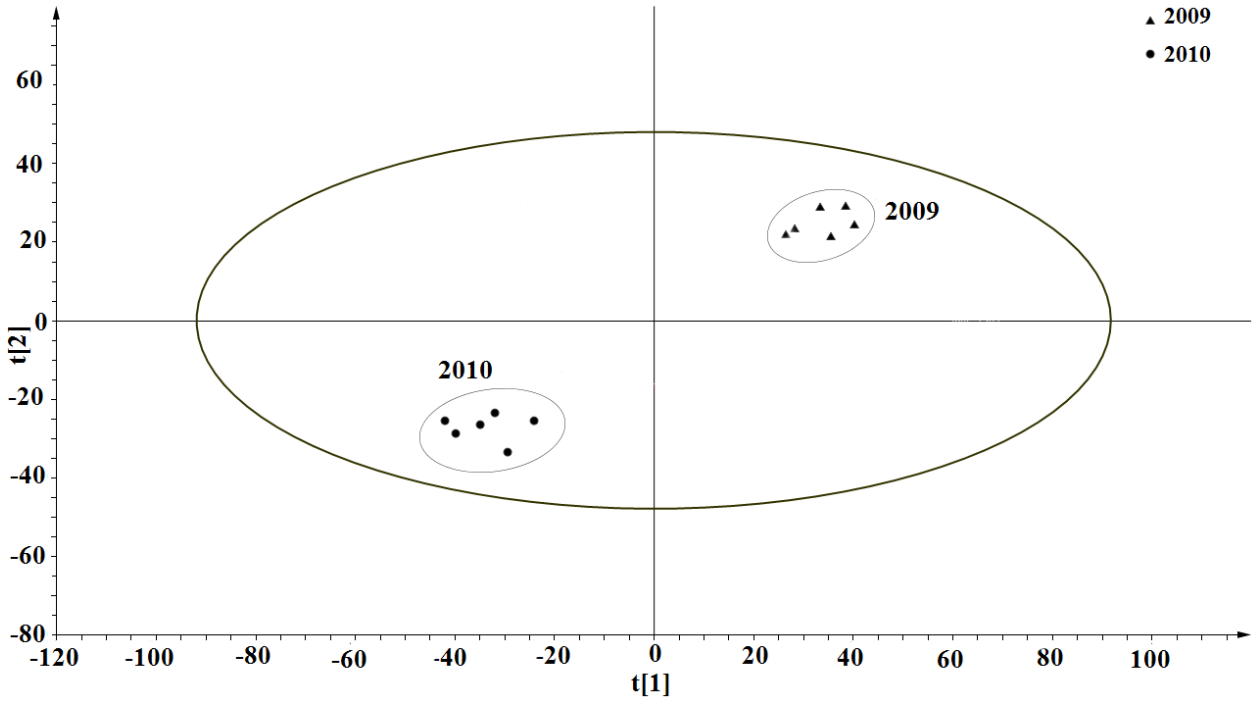


Figure 2A PLS-DA score plot chart from ^{1}H NMR spectra of 2009 and 2010 vintage Cabernet Sauvignon wines.

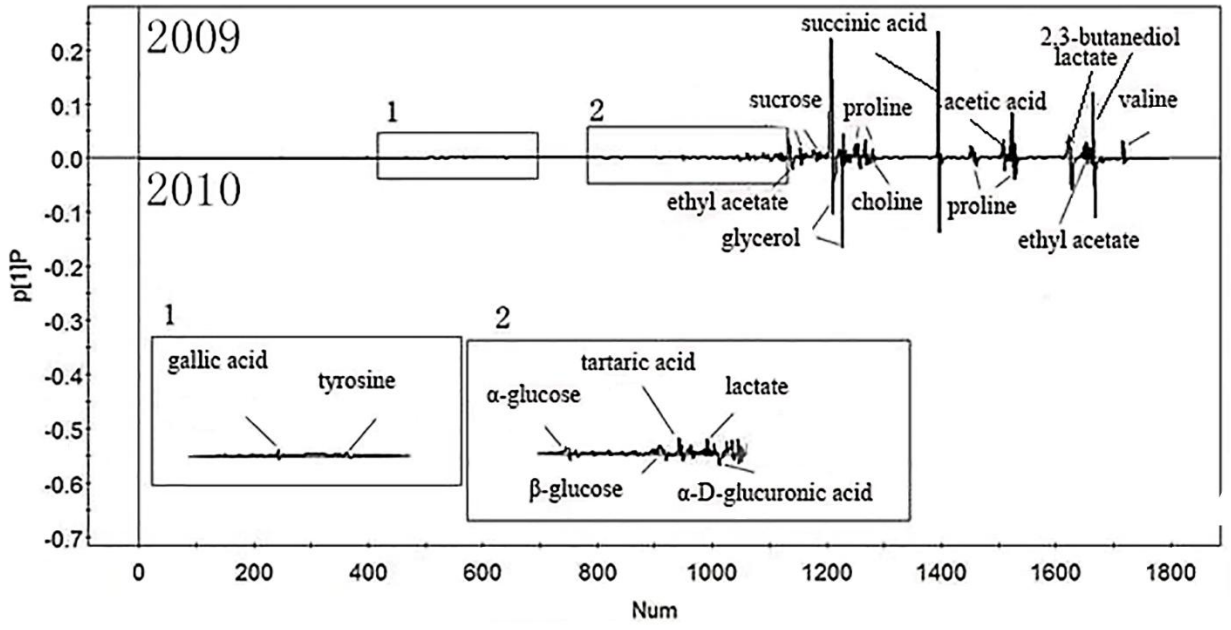


Figure 2B PLS-DA loading plot chart from ^{1}H NMR spectra of 2009 and 2010 vintage Cabernet Sauvignon wines.

The PLS-DA score plot based on the 2009 and 2011 vintages of the Cabernet Sauvignon

wines showed a clear discrimination (Fig 3A), and the loading plot provids the contributed metabolites that to this discrimination (Fig 3B). Higher levels of 2,3-butanediol, ethyl acetate, proline, succinic acid, glycerol, α-glucose, tartaric acid, choline, and sucrose and lower levels of lactate and α-D-glucuronic acid were detected in the 2009 compared to the 2011 vintages.

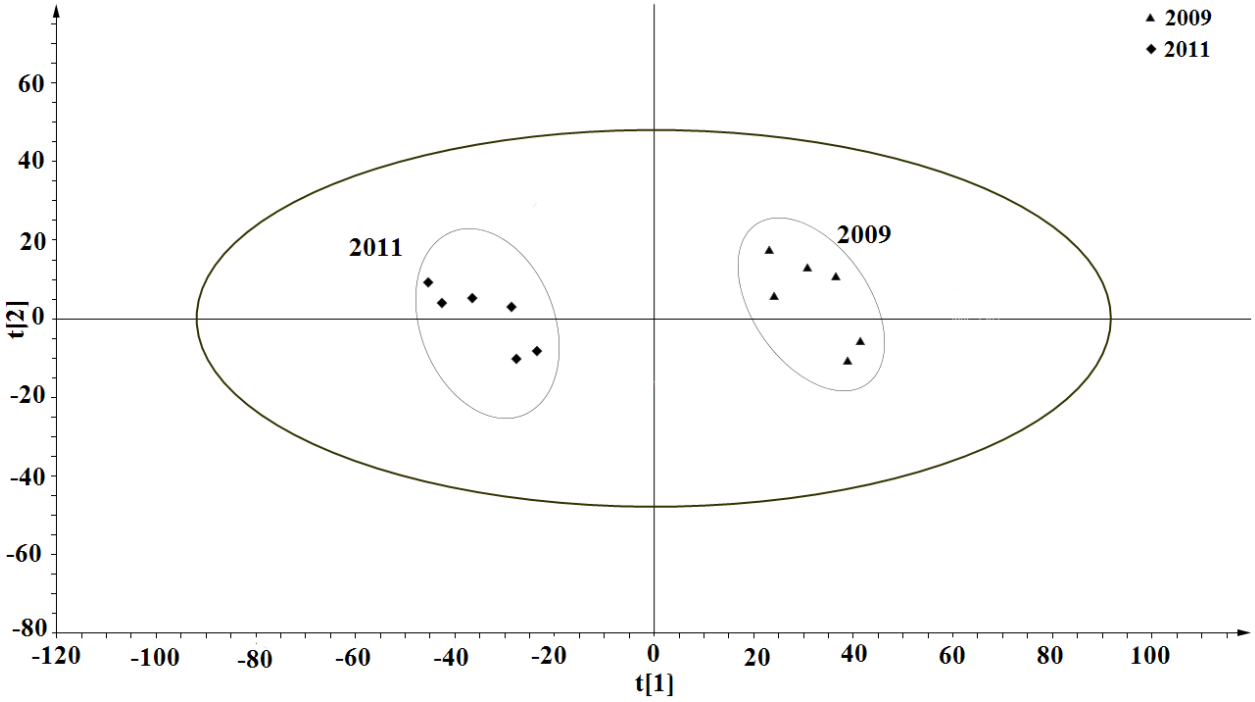


Figure 3A PLS-DA score plot chart from ^{1}H NMR spectra of 2009 and 2011 vintage Cabernet Sauvignon wines.

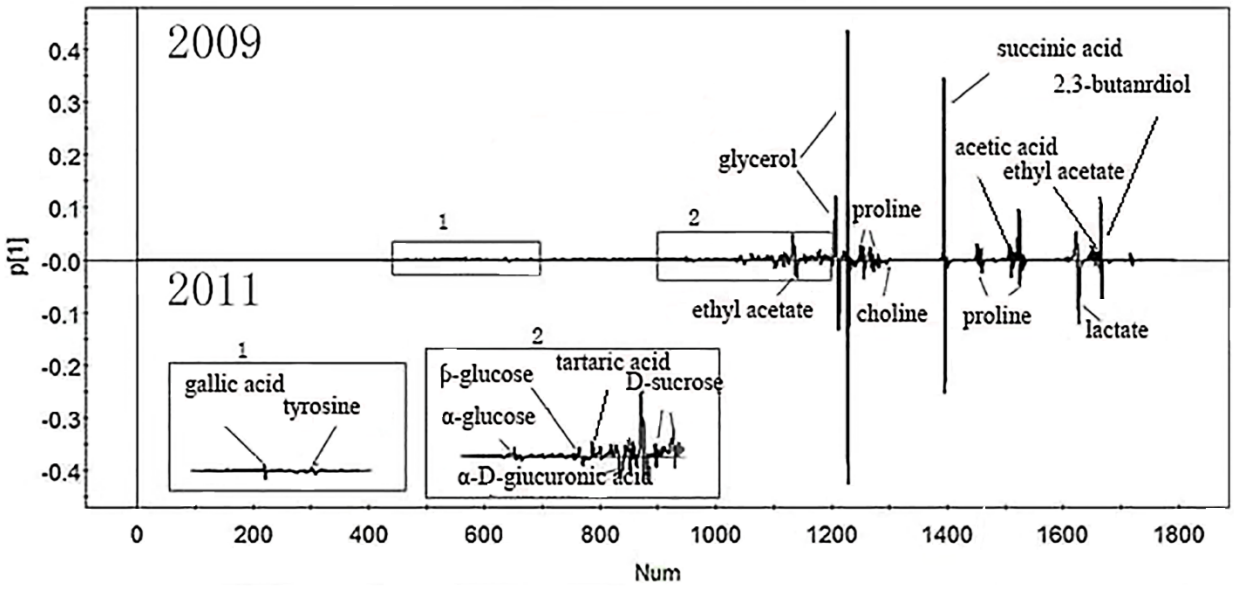


Figure 3B PLS-DA loading plot chart from ^{1}H NMR spectra of 2009 and 2011 vintage Cabernet Sauvignon wines.

The PCA score plot between the 2009 and 2012 vintage Cabernet Sauvignon wines also showed clear separation (Fig 4A) identified by higher levels of valine, 2,3-butanediol, proline, succinic acid, D-sucrose, tartaric acid, gallic acid, α-glucose, and β-glucose and lower levels of lactate, ethyl acetate, acetic acid, glycerol, α-D-glucuronic acid, and choline in the 2009 vintages (Fig 4B).

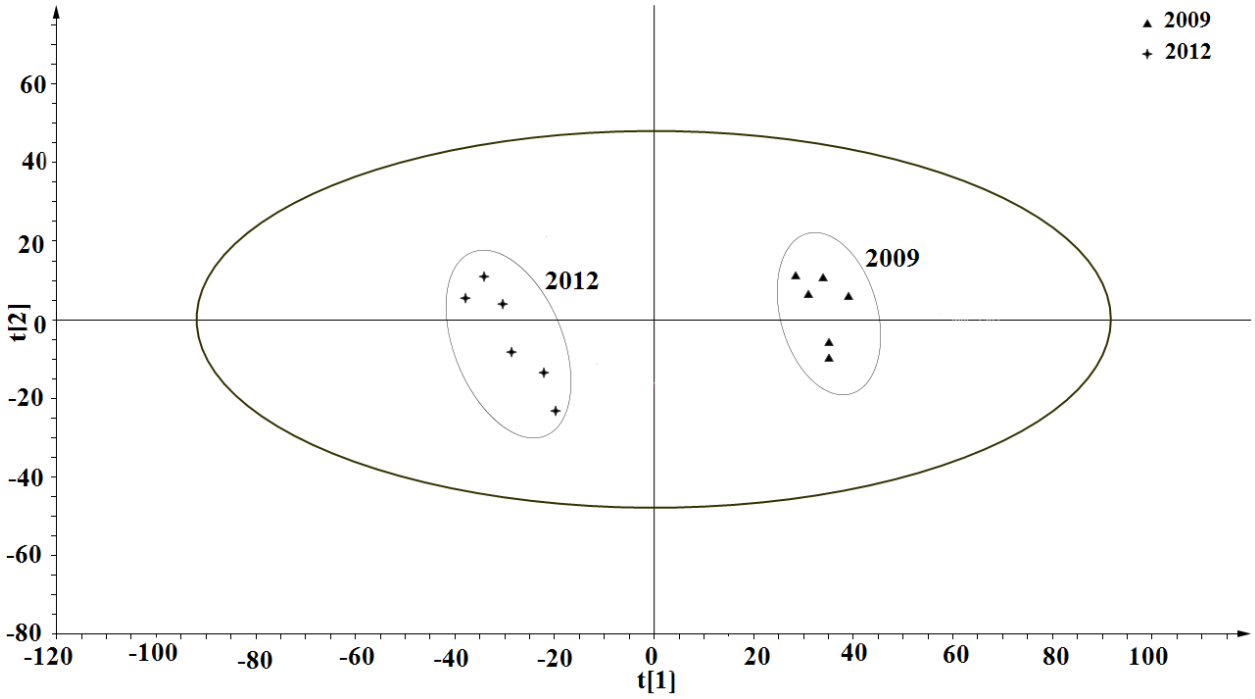


Figure 4A PLS-DA score plot chart from ^{1}H NMR spectra of 2009 and 2012 vintage Cabernet Sauvignon wines.

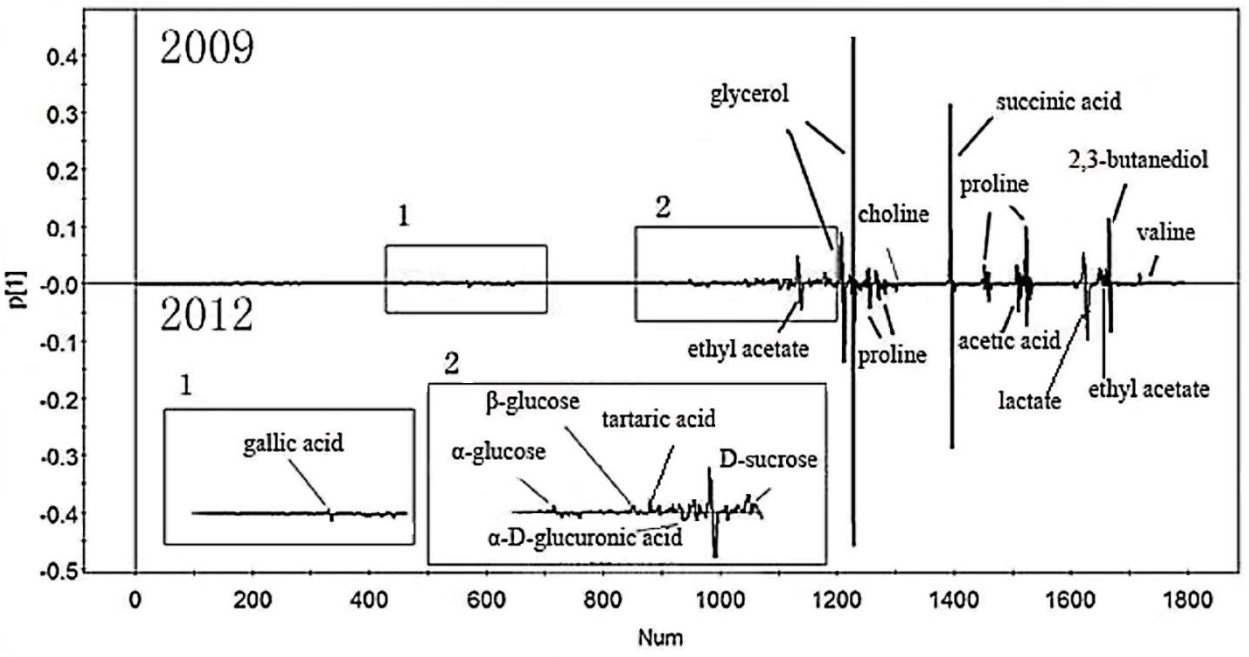


Figure 4B PLS-DA loading plot chart from ^{1}H NMR spectra of 2009 and 2012 vintage Cabernet Sauvignon wines.

The PCA score plot between 2010 and 2011 vintage Cabernet Sauvignon wines also showed significant separation (Fig 5A). The loading plot illustrates that choline, proline, and 2,3-butanediol in higher levels and valine, lactic acid, succinic acid, and glycerol in lower levels compared to those in the 2010 vintages and 2011 vintages (Fig 5B).

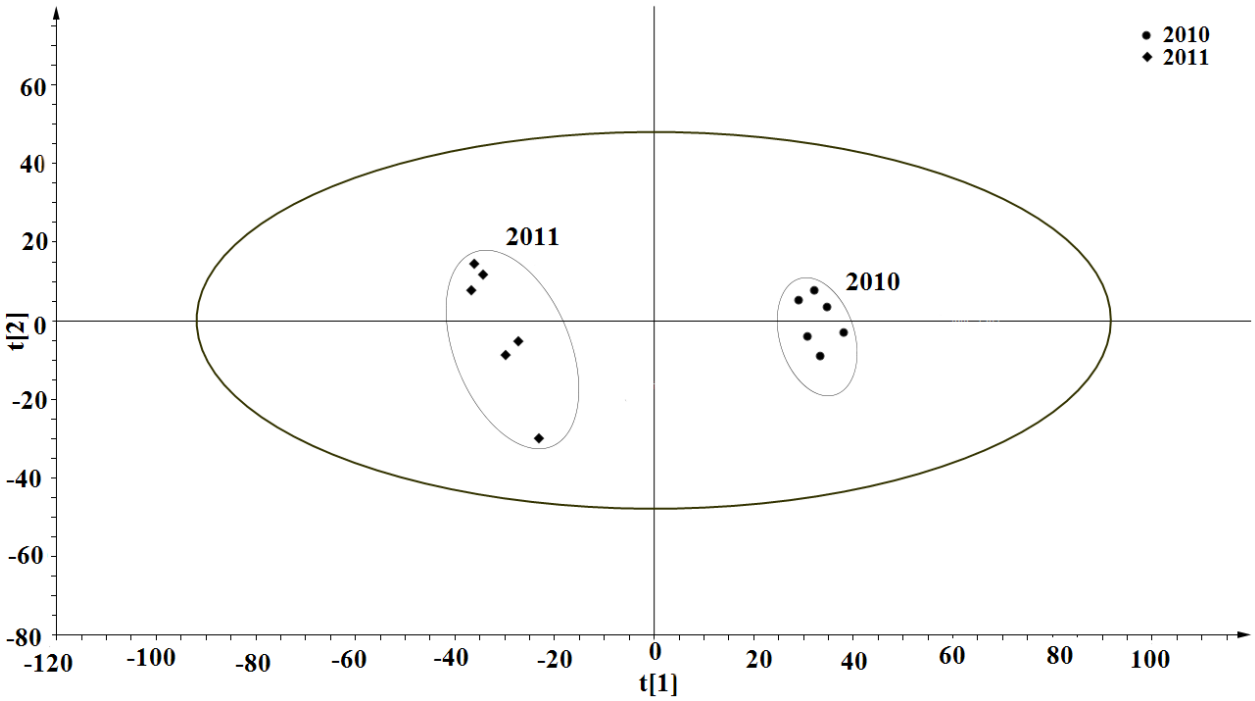


Figure 5A PLS-DA score plot chart from ^{1}H NMR spectra of 2010 and 2011 vintage Cabernet Sauvignon wines.

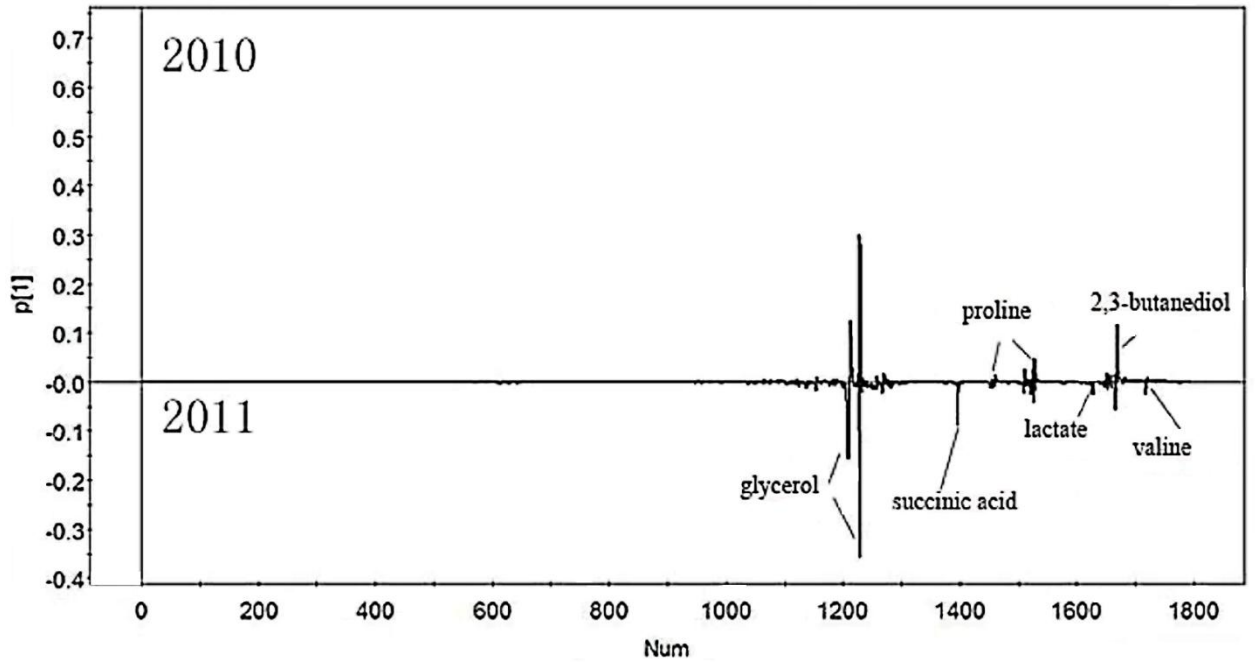
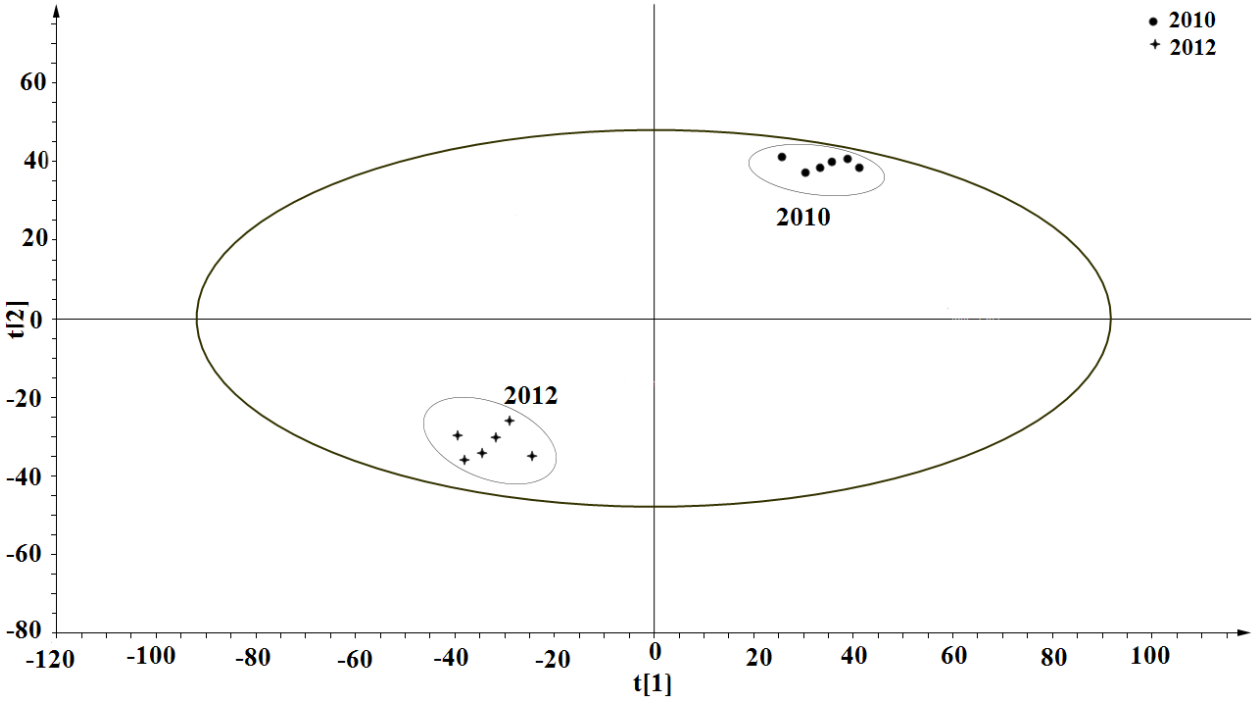


Figure 5B PLS-DA loading plot chart from ^{1}H NMR spectra of 2010 and 2011 vintage Cabernet Sauvignon wines.

The PCA score plot also showed significant differentiation between the Cabernet Sauvignon

159 wines vinified in 2010 and 2012 vintages (Fig 6A). Relatively higher levels of valine and 2,3-
160 butanediol and lower levels of lactic acid, proline, acetic acid, succinic acid, choline, glycerol,
161 and ethyl acetate in Cabernet Sauvignon wines vinified in 2010 vintages, compared to those
162 vinified in 2012 vintages, can be observed in the PLS-DA loading plot (Fig 6B).



163

164 **Figure 6A** PLS-DA score plot chart from ^{1}H NMR spectra of 2010 and 2012 vintage Cabernet
165 Sauvignon wines.

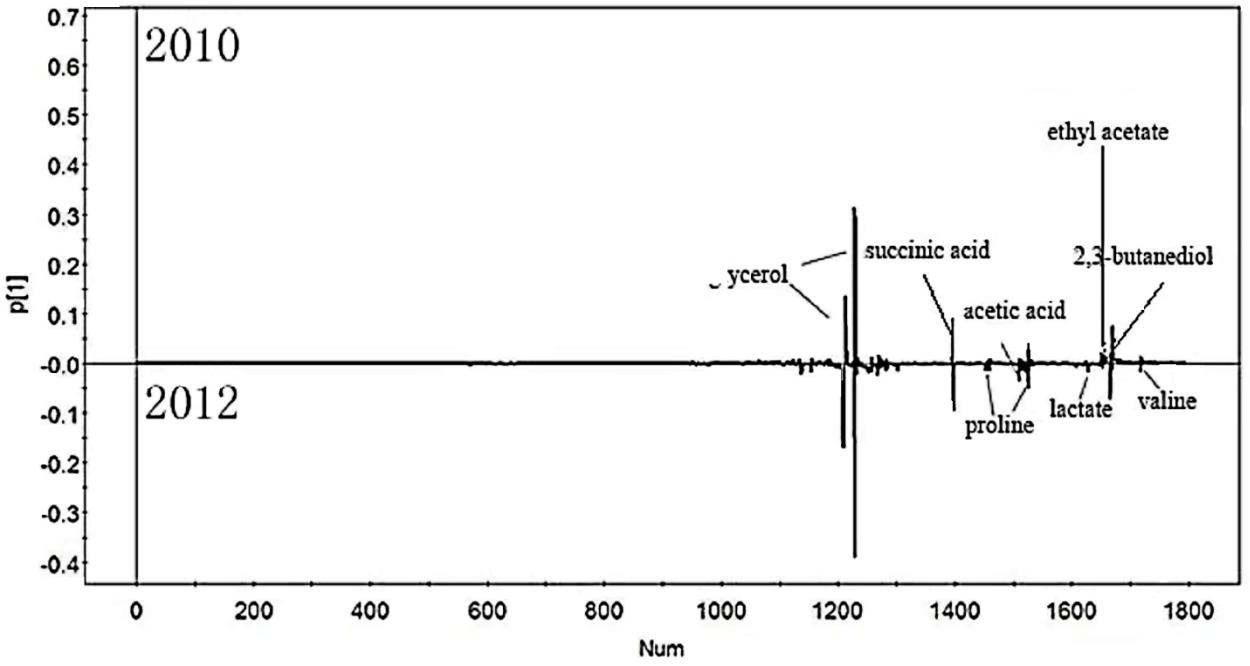


Figure 6B PLS-DA loading plot chart from ^{1}H NMR spectra of 2010 and 2012 vintage Cabernet Sauvignon wines.

The PLS-DA score plot showed clear separation between the 2011 and 2012 vintage Cabernet Sauvignon wines based on the first component (Fig 7A). The corresponding loading plot showed relatively high load levels of valine, lactic acid, and succinic acid, with low levels of 2,3-butanediol, proline, acetic acid, choline, glycerol, D-sucrose, acetate, α-glucose, gallic acid, and tyrosine in the 2011 vintages, compared with the 2012 vintages (Fig 7B).

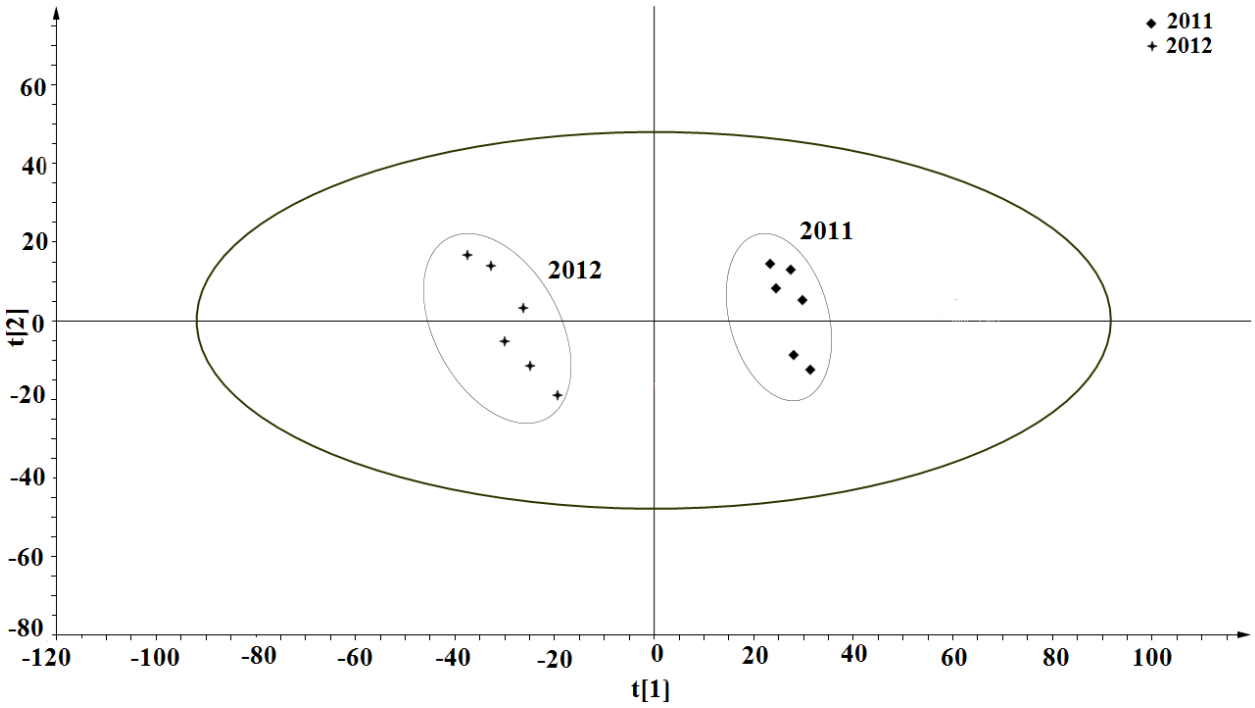


Figure 7A PLS-DA score plot chart from ^{1}H NMR spectra of 2011 and 2012 vintage Cabernet Sauvignon wines.

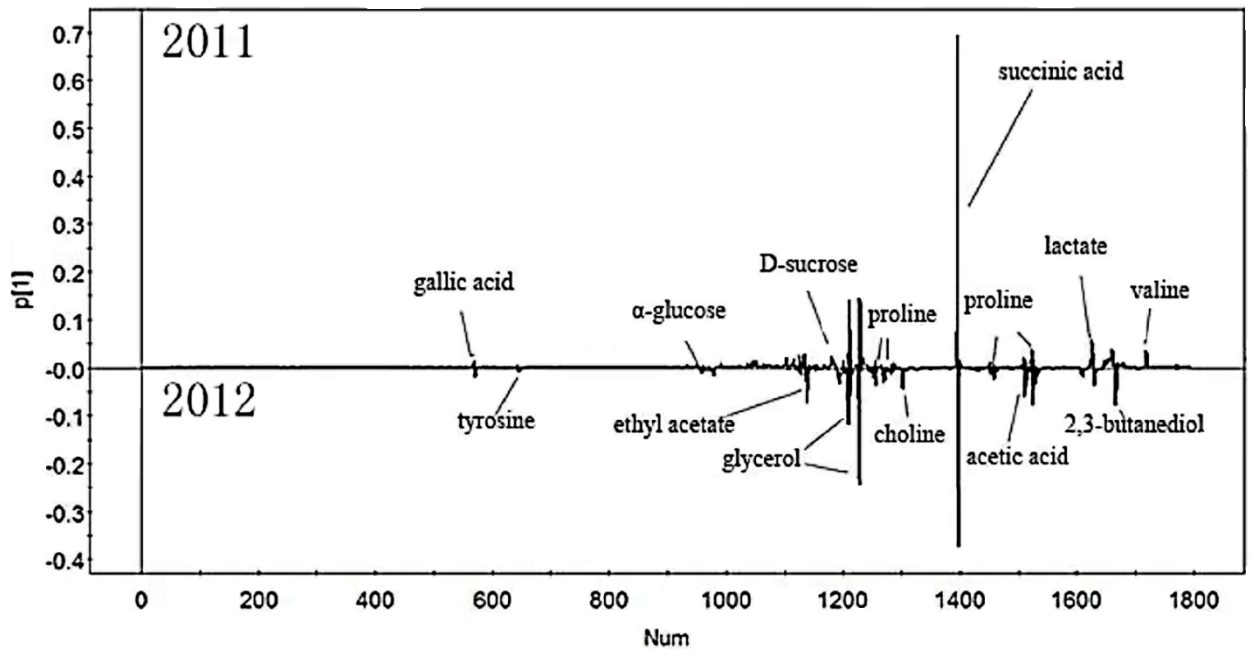
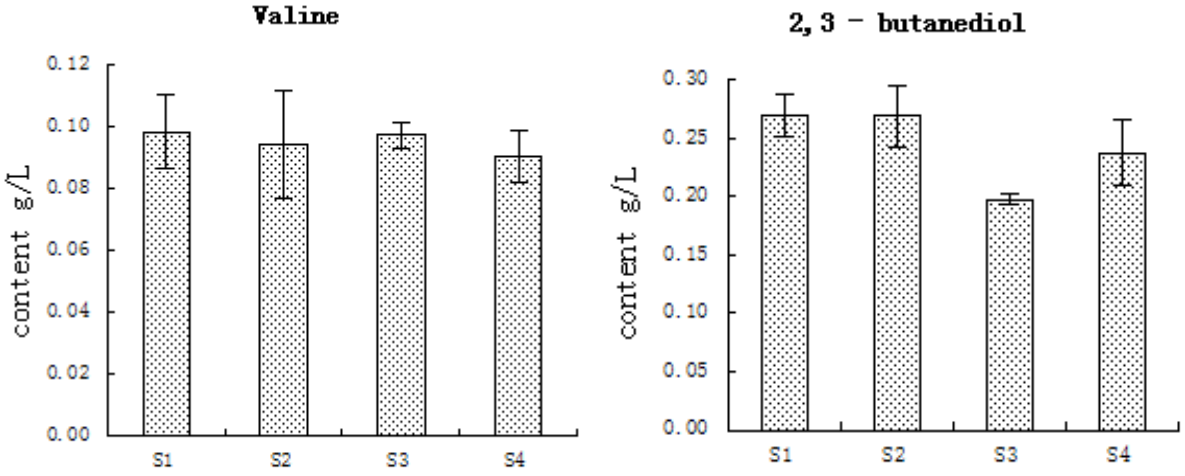


Figure 7B PLS-DA loading plot chart from ^{1}H NMR spectra of 2011 and 2012 vintage Cabernet Sauvignon wines.

Quantitative Analysis

The PLS-DA analysis revealed small differences in the metabolite compositions and large differences in the metabolite concentrations in the Cabernet Sauvignon wines vinified from 2009 to 2012 vintages. The abbreviations S1-S4 represent the 2009-2012 vintages, respectively. The concentration of valine in the four vintages in order from high to low was S1>S3>S2>S4; the concentration of 2,3-butanediol from high to low in the order of four vintages was S1>S2>S4>S3; the concentration of glycerol in the four vintages in order from high to low was S4>S1>S3>S2; the concentration of ethyl acetate in the four vintages in order from high to low was S4>S2>S1>S3; the concentration of succinic acid in the four vintages in order from high to low was S4>S3>S1>S2; the concentration of lactate in the four vintages in order from high to low was S3>S4>S1>S2; the concentration of choline in the four vintages in order from high to low was S4>S2>S1>S3; the concentration of gallic acid in the four vintages in order from high to low was S1>S4>S3>S2.

From the ^{1}H NMR spectra totally selected 8 metabolites, and calculated concentrations according to their peak areas. The results of this quantitative analysis (Fig 8) agree with the PLS-DA results.



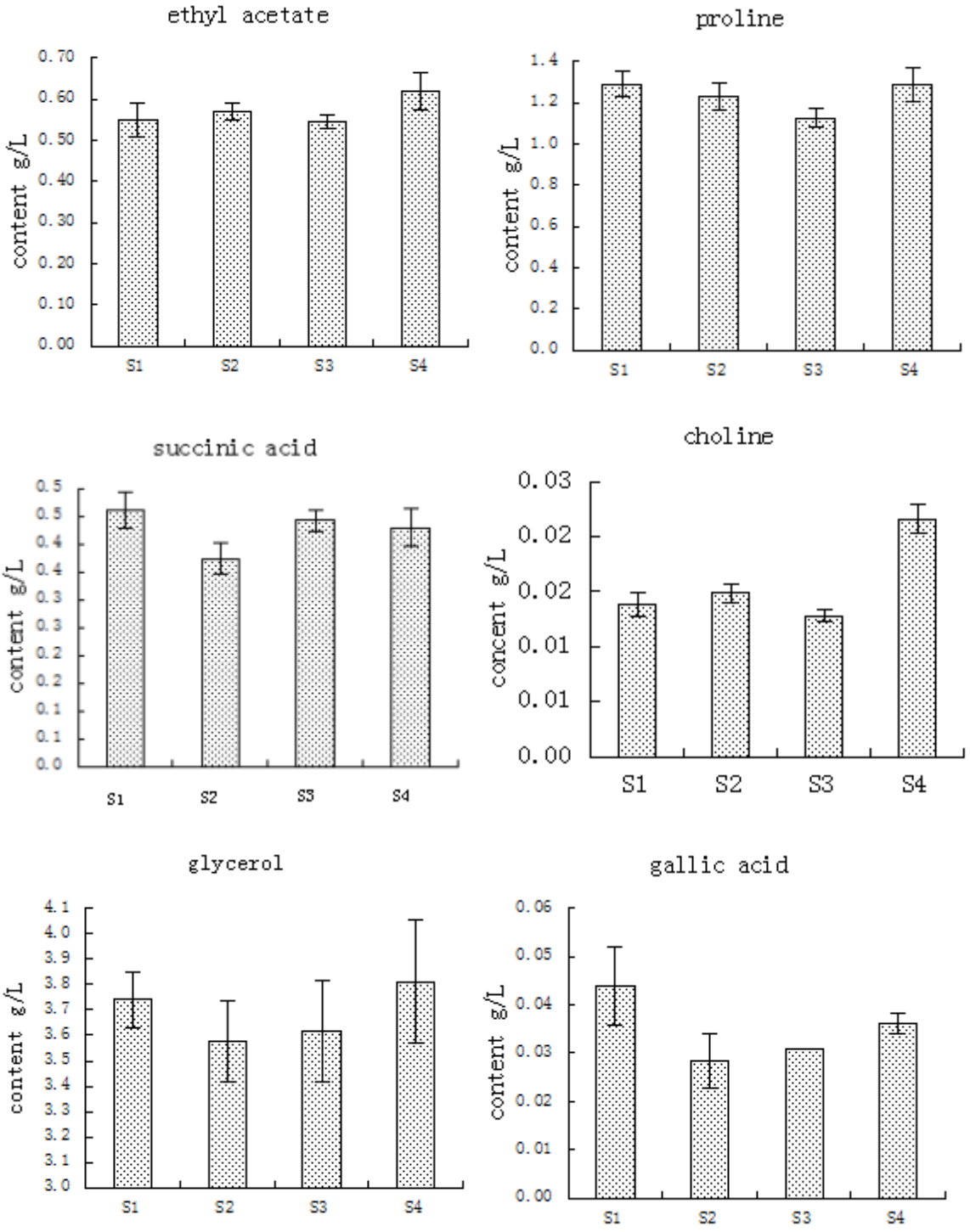


Figure 8 Comparison of the main metabolite concentrations in the 2009-2012 vintage wines.

* Error bars means standard deviations.

DISCUSSION

204 **Polyols and Ethyl Acetate**

205 In the present study, ethanol composes a large proportion of the wines, and the difference in the
206 ethanol content of each wine is quite small. Ethanol signals of the samples were so intense in the
207 spectra that they cover the other components signals less abundant. Therefore, ethanol was not a
208 major discriminating compound.

209 2,3-Butanediol is a by-product of fermentation in wine, probably from the reduction of
210 acetoin or pyruvic acid (Romano et al., 2003; Son et al., 2009). Because the taste threshold of
211 2,3-butanediol is 150 mg/L, it does not usually affect the flavor. However, the average content of
212 2,3-butanediol in each wine was approximately 0.243 g/L, which will make the wine slightly
213 bitter and sticky feeling. In our study, the 2011 vintage wines contained the highest levels of 2,3-
214 butanediol.

215 Glycerol is formed as a by-product of alcohol fermentation. The pH, sulfite concentration,
216 grape variety, fermentation temperature, yeast, and nitrogen composition of the wine influence
217 the level of glycerol (Radler & Schutz, 1982; Vineyardner, Rodrigue & Champagne, 1993; Son et
218 al., 2009). In our study, the winemaking conditions, such as the sulfite concentration,
219 fermentation temperature, and yeast, were nearly the same. Therefore, the glycerol contents may
220 have resulted from the sugar contents in the grape berries.

221 **Organic Acids**

222 Tartaric acid, malic acid and citric acid in wine mostly derive from the grape berries. The
223 concentration of tartaric acid in grape berries usually remains stable despite increases in berry
224 volume during maturation. The concentration of tartaric acid in grape berries usually remains
225 stable. Precipitation is related to the brewing conditions, including fermentation temperature, pH
226 and concentration of potassium and calcium (Viggiani & Morelli, 2008; Son et al., 2009).
227 Therefore, tartaric acid in wines cannot be revineyarded as a biomarker for describing the
228 characteristics of wines.

229 The lactate contents in wines higher show that malolactic fermentation has occurred, in
230 which bacteria completely transformed into lactic acid, citric acid and malic acid (Avenoza et al.,

2006; Larsen, Van Den Berg & Engelsen, 2006). So we cannot detect malic acid or citric acid in dry red wine.

Succinic acid is the main nonvolatile organic acid present during alcoholic fermentation and MLF (Son et al., 2009). Succinic acid is very stable and does not change with age, as one of the major metabolic products.

Amino Acids

The wine amino acids have different origins. Released from dead yeast or at the end of fermentation some are indigenous to the grape can be partially or fully metabolized by yeast; others are vinified by proteins enzymatic degradation of (Košir & Kidrič, 2002). Classically, alanine used in the growth of yeast in wine, so little is detected in the finished wine product. Proline is not a nutrient used by yeast and can therefore be used as a biological marker of wine. Lee et al. (2009b) states that the proline content in wine depends on environmental factors and grape varieties. Among the 4 different vintages of Cabernet Sauvignon wine tested, the 2009 vintage had the highest proline content, and the 2011 vintage had the lowest level of proline. This pattern may have resulted from the longer sunshine and less rainfall in 2009.

Another amino acid biomarker, valine, was also revealed by the PLS-DA analysis. Valine is used by yeast during fermentation and appears with yeast autolysis.

Choline

Choline is precursor of glycine betaine, and betaine is related to homocysteine. The average level of choline in wines is 5.6 mg/100 g (Zeisel et al., 1991; Mickelbart, Chapman & Collier-Christian, 2006). In our study, the 2012 vintage Cabernet Sauvignon wines had the highest levels of choline, whereas the 2011 vintage had the lowest levels.

Carbohydrates

Glucose and fructose are the main sugars in the grape. When grape maturity begins, the glucose content in the grape is higher than the fructose content, and as the grape matures, both contents become nearly equal by harvest time. Dry wine refers to wine with a sugar level less than or

equal to 4.0 g/L and we detected sucrose, α-glucose and β-glucose, and the differentiation of the concentrations of these three sugars was small. Therefore, we cannot revineyard the carbohydrate in these wines as characteristic metabolites.

CONCLUSIONS

^{1}H NMR-based metabolomics was used to study the metabolite differences in different vintages of Cabernet Sauvignon wines. Pattern recognition showed clear differentiation between the wines vinified in 2009, 2010, 2011, and 2012 vintages.

Pattern recognition methods clearly differentiated between the wines vinified in different vintages. Responsible for the differentiation of the metabolites were identified as 2,3-butanediol, ethyl acetate, valine, proline, succinic acid, lactate, acetic acid, glycerol, gallic acid and choline. Wines were vinified in the same fermentation technique, yeast and grape varieties, therefore, climatic factors such as average temperature, rainfall, evaporation and so on are the main reason for the difference of the metabolites in different vintages wines. Probably the higher average temperature and evaporation, less rainfall in 2009 increase the sugar content of the grapes and enable the grapes to reach optimum ripeness. This has contributed to the 2009 vintage wines have the highest level of valine, 2,3-butanediol, gallic acid and proline. Grapes from a long, slow ripening season due to the lower average temperature, higher rainfall and evaporation in 2011 and 2012. The 2011 vintage wines contained the highest level of lactic acid, and the highest levels of ethyl acetate, succinic acid, glycerol and choline were detected in the 2012 vintage wines.

Selected metabolites were selected from the ^{1}H NMR spectra and quantified according to their peak areas. The results of the quantitative analysis agree with the PLS-DA results.

It seemed that this NMR based metabonomics approach can effectively classify wine. For wine, certification of a vintage's geographical indications, as well as adulteration and quality monitoring, provide the theoretical basis and technical support.

ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (No. 31271857).

REFERENCES

Amaral FM, Caro MSB. 2005. Investigation of different pre-concentration methods for NMR analyses of Brazilian white wine. *Food Chemistry* 93:507–510. DOI: 10.1016/j.foodchem.2004.09.039.

Anastasiadi M, Zira A, Magiatis P, Haroutounian SA, Skaltsounis AL, Mikros E. 2009. ^{1}H NMR-based metabonomics for the classification of Greek wines according to variety, region, and vintage. Comparison with HPLC Data. *Journal of Agricultural and Food Chemistry* 57:11067–11074. DOI: 10.1021/jf902137e.

Avenoza A, Busto JH, Canal N, Peregrina JM. 2006. Time course of the evolution of malic and lactic acids in the alcoholic and malolactic fermentation of grape must by quantitative ^{1}H NMR (qHNMR) spectroscopy. *Journal of Agricultural and Food Chemistry* 54:4715–4720. DOI: 10.1021/jf060778p.

Forveffle L, Vercauteren J, Rutledge DN. 1996. Multivariate statistical analysis of two-dimensional NMR data to differentiate grapevine cultivars and clones. *Food Chemistry* 57:441–450. DOI: 10.1016/0308-8146(95)00220-0.

Vineyardner N, Rodrigue N, Champagne CP. 1993. Combined effects of sulfites, temperature, and agitation time on production of glycerol in grape juice by *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology* 59:2022–2028.

Hu B, Yue Y, Zhu Y, Wen W, Zhang F, Hardie JW. 2015. Proton nuclear magnetic resonance-Spectroscopic discrimination of wines reflects genetic homology of several different grape (V. vinifera L.) cultivars. *PLoS One* 10:e0142840. DOI: 10.1371/journal.pone.0142840.

Košir IJ, Kidrič J. 2002. Use of modern nuclear magnetic resonance spectroscopy in wine analysis: determination of minor compounds. *Analytica Chimica Acta* 458:77–84. DOI: 10.1016/S0003-2670(01)01549-5.

Larsen FH, van den Berg F, Engelsen SB. 2006. An exploratory chemometric study of ^{1}H NMR spectra of table wines. *Journal of Chemometrics* 20:198–208. DOI: 10.1002/cem.991.

311 Lee JE, Hong YS, Lee CH. 2009a. Characterization of fermentative behaviors of lactic acid
312 bacteria in grape wines through ^{1}H NMR- and GC-based metabolic profiling. *Journal of*
313 *Agricultural and Food Chemistry* 57:4810–4817. DOI: 10.1021/jf900502a.
314 Lee JE, Hwang GS, Van Den Berg F, Lee CH, Hong YS. 2009b. Evidence of vintage effects on
315 grape wines using ^{1}H NMR-based metabolomic study. *Analytica Chimica Acta* 648:7–6.
316 DOI: 10.1016/j.aca.2009.06.039.
317 Mickelbart MV, Chapman P, Collier-Christian L. 2006. Endogenous levels and exogenous
318 application of glycinebetaine to grapevines. *Scientia Horticulturae* 111:7–16. DOI:
319 10.1016/j.scienta.2006.07.031.
320 Nicholson JK, Lindon JC, Holmes E. 1999. 'Metabonomics': understanding the metabolic
321 responses of living systems to pathophysiological stimuli via multivariate statistical
322 analysis of biological NMR spectroscopic data. *Xenobiotica* 29:1181–1189. DOI:
323 10.1080/004982599238047.
324 Papotti G, Bertelli D, Graziosi R, Silvestri M, Bertacchini L, Durante C, Plessi M. 2013.
325 Application of one- and two-dimensional NMR spectroscopy for the characterization of
326 protected designation of origin lambrusco wines of modena. *Journal of Agricultural and*
327 *Food Chemistry* 61:1741–1746. DOI: 10.1021/jf302728b.
328 Pereira GE, Gaudillere J-P, Van Leeuwen C, Hilbert G, Lavialle O, Maucourt M, Deborde C,
329 Moing A, Rolin D. 2005. ^{1}H NMR and chemometrics to characterize mature grape berries
330 in four wine-growing areas in Bordeaux, France. *Journal of Agricultural and Food*
331 *Chemistry* 53:6382–6389. DOI: 10.1021/jf058058q.
332 Radler F, Schütz H. 1982. Glycerol production of various strains of *Saccharomyces*. *American*
333 *Journal of Enology and Viticulture* 33:36–40.
334 Rochfort S, Ezernieks V, Bastian SEP, Downey MO. 2010. Sensory attributes of wine influenced
335 by variety and berry shading discriminated by NMR metabolomics. *Food Chemistry*
336 121:1296–1304. DOI: 10.1016/j.foodchem.2010.01.067.

337 Romano P, Fiore C, Paraggio M, Caruso M, Capece A. 2003. Function of yeast species and
338 strains in wine flavour. *International Journal of Food Microbiology* 86:169–180. DOI:
339 10.1016/S0168-1605(03)00290-3.
340 Son HS, Kim KM, van den Berg F, Hwang GS, Park WM, Lee CH, Hong YS. 2008. ^{1}H nuclear
341 magnetic resonance-based metabolomic characterization of wines by grape varieties and
342 production areas. *Journal of Agricultural and Food Chemistry* 56:8007–8016. DOI:
343 10.1021/jf801424u.
344 Son H-S, Hwang G-S, Ahn H-J, Park W-M, Lee C-H, Hong Y-S. 2009. Characterization of wines
345 from grape varieties through multivariate statistical analysis of ^{1}H NMR spectroscopic
346 data. *Food Research International* 42:1483–1491. DOI: 10.1016/j.foodres.2009.08.006.
347 Sun SY, Che CY, Sun TF, Lv ZZ, He SX, Gu HN, Shen WJ, Chi DC, Gao Y. 2013. Evaluation
348 of sequential inoculation of *Saccharomyces cerevisiae* and Oenococcus oeni strains on the
349 chemical and aromatic profiles of cherry wines. *Food Chemistry* 138:2233–2241. DOI:
350 10.1016/j.foodchem.2012.12.032.
351 Viggiani L, Morelli MA. 2008. Characterization of wines by nuclear magnetic resonance: a work
352 study on wines from the Basilicata region in Italy. *Journal of Agricultural and Food*
353 *Chemistry* 56:8273–8279. DOI: 10.1021/jf801513u.
354 Zeisel SH, Da Costa KA, Franklin PD, Alexander EA, Lamont JT, Sheard NF, Beiser A. 1991.
355 Choline, an essential nutrient for humans. *Journal of the Federation of American*
356 *Societies for Experimental Biology* 5:2093–2098.