Journal of Plant Sciences
2021; 9(5): 234-244
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20210905.11
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

^{1}H NMR Metabolic Fingerprints of Cabernet Sauvignon Grapes Produced in the Jinsha River and Lancan River Valleys in the Shangeri-La Region, South China

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To cite this article:

Chunhe Jiang, Kexu Cui, Yue Zhao, Qingyan Sun, Zhiqiang Yu, Yifang Zhu, Ruzhi Mao. ^{1}H NMR Metabolic Fingerprints of Cabernet Sauvignon Grapes Produced in the Jinsha River and Lancan River Valleys in the Shangeri-La Region, South China. *Journal of Plant Sciences.* Vol. 9, No. 5, 2021, pp. 234-244. doi: 10.11648/j.jps.20210905.11

Received: July 5, 2021; **Accepted**: September 3, 2021; **Published**: September 23, 2021

Abstract: Grape berries metabolites determine wine grape and wine quality. The aim of this work was to determine the metabolite profiles of fruit of mature berries of 'Cabernet Sauvignon' of grapes in relation to their growing conditions in high alititude Shangeri-La wine-growing region. Last decade, Proton nuclear magnetic resonance (^{1}H NMR) used to quantify in a single analysis many different compounds in berry extracts. Grape berries were harvested in 2013 on grapevines cultivated in Jinsha River and Lancan River of Shangeri-La, China. After an ethanolic-water extraction, the^{1}H NMR spectra of water-soluble extracts of fruit berriers were run in 15 minutes. Bayesil was development a web system that automatically identifies and quantifies. Principal component analysis (PCA) analysis of berries spectra significantly discriminated mature berries from Jinsha River and Lancan River. ^{1}H NMR spectra of berriers were less discriminanting. OPLS-DA showed significant grapevines cultivated in Jinsha River and Lancan River of Shangeri-La, contributing to the discrimination, were attributed to animo acid, organic acid, alcohol, sugar, Ester. In conclusion, ^{1}H NMR analysis of berry extracts discriminates berries from different origins more efficiently than classical biochemical analyses based on sugar, acidity and Ester. alerting pathway of animo acid, organic acid pathway.

Keywords: Grape Berries, Terroir, Metabolite Profiling, Metabolic Pathway

1. Introduction

Grapevines are economically important for wine production, and most of unique characteristics chemical compounds t are formed during fruit development [1]. However, fruit composition is strongly influenced by the plant's genome, interactions between the natural habitatant, and the vine and fruit/management system, it is hardly to replicate a wine in winery [2]. In recent decades, "terroir" has become an important characteristic to understand how the environment and human behaviors interact in wine development [3]. "Terroir" is a quality characteristic of grape wines in worldily wine-producing regions such as France, Italy, Spain, and Germany, and is defined as the interaction between the plants, the natural environment, and human behavior in vitiscuturepractice [4]. However, the terroir is a development concept, which still extensive in other fruit trees [5]. Nuclear magnetic resonance (NMR), Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-MS (LC-MS) are powerful methods to study the metabolome. Low boiling point substances, small molecules and volatile, GC-MS is a high effectively tool and datasets [6]. LC (HPLC)-MS. HPLC-MS is an attractive method with great potential for rapid and efficient screening of biomarkers [7]. Proton NMRspectroscopy is a powerful and useful tool for metabolite (^{1}H NMR). Qualitative and quantitative analysis. ^{1}H NMR is a nondestructive, noninvasive, and rapid method to determine grape

metabolites [8]. 1In food science, such technology has also been found to be suitable for metabolite qualitative and quantitative analysis in fruit juices and wine [9]. Grape metabolites determine the grape and wine quality and are diverse under variable habitats. Phenolic compounds, major sugars, and organic and amino acids can be representative the metabolic profiles of a complex mixture [8]. In addition, spectra annotation, such as Bayesil, has been developed to automatically identify and quantify metabolites using 1D ^{1}H NMR spectra [10], Metaboanlyst4.0 automatically metabolic pathway analysis [11]. It is challenging to analyst high dimensional from 1D ^{1}H NMR datasets. For that purpose, iprincipal component analysis (PCA) [12], and orthogonal partial least squares-discrimination analysis (OPLS-DA) for the fusion of omics data obtained from multiple sources. OPLS-DA is very useful tool to finding newmodels and pathway in biological studies [6].

The purpose of this work was (1) to use 1D ^{1}H NMR spectra to determine the metabolic profiles of fruit tissues of the Cabernet Sauvignon cultivar of grapes under River Valley wine-growing region, (2) to apply 1D ^{1}H NMR methods to discriminate colleted samples from wine-growing region in Shangeri-La, and (3) to use Bayesil to identify and quantify metabolites in berry samples from growing region in Shangeri-La.

2. Materials and Methods

2.1. Sample Collection Sites

Thirteen Cabernet Sauvignon berry samples were collected from the Lancan River valley of the Shangeri-La wine grape growing region: Cizhong, Rimi, Nitong, Chalitong, Hongpo, Jiulongding, Xidang, Sinong, Bucun, Liutongjian, Adong, Shuori, and Luwa. Fourteen Cabernet Sauvignon berry samples were collected from the Jinshan River valley of the Shangeri-La wine grape growing region: Duotong, Longtong, Benzilan, Waka, Yeri, Dongshui, Dari, Shasitong, Xilongtong, Yingduba, Silu, Sulu, Simuding, and Maoding. From each natural terroir, 200 Cabernet Sauvignon berries were collected.

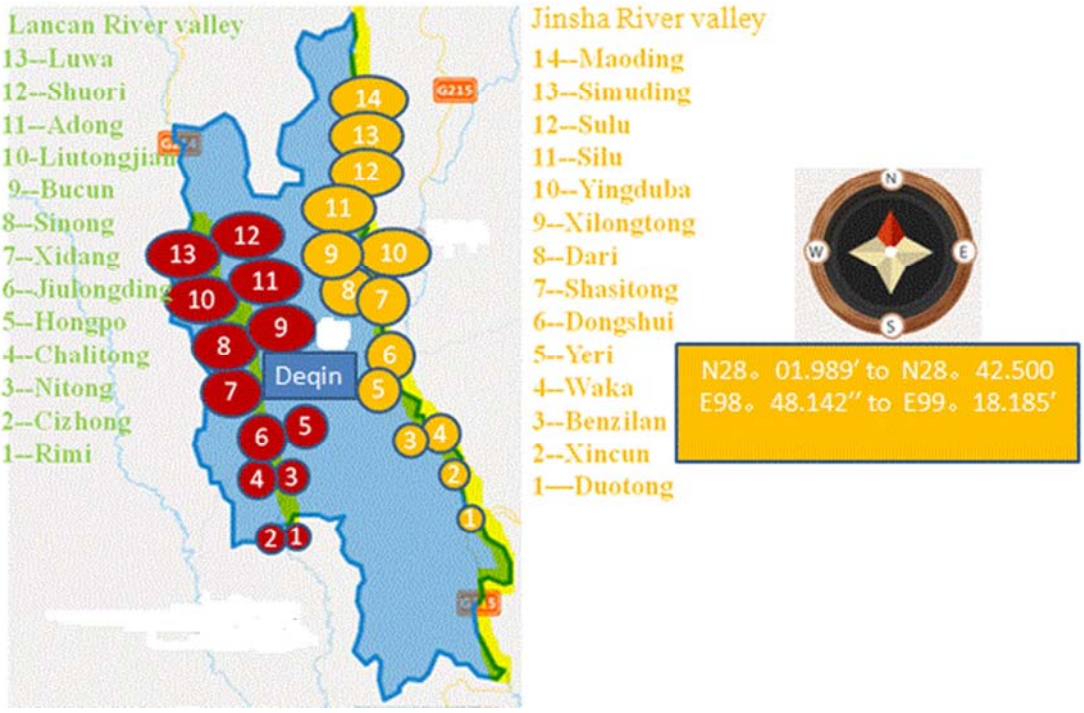


Figure 1. *Sample collection sites in the Lancan River and Jinshan River valleys of the Shangeri-La wine grape growing region.*

2.2. Sample Preparation

The samples were harvested in 2013. For each plot, 200 berries were harvested (after flowering-time, 120 days) from Cabernet Sauvignon cultivated in the Lancan River and Jinshan River valleys of different terroirs of the Shangeri-La wine grape growing region. Each sample was separated into two subsamples, 1 of the 120 berries for routine analyses, and the other of the 80 berries for NMR. Sample grape materials (100 mg) were placed in 2 ml tubes, and 0.35 ml of extraction liquid (Vmethanol: VH2O=8:2) was added. To homogenize the tissue, steels balls were placed in the tubes, and samples were homogenized in a ball mill for 5 min at 70 Hz. They were then centrifuged for 10 min at 12,000 rpm, 4°C. Then, 0.35 ml was transferred from the supernatant into a fresh 1.5 ml EP tube. Samples were dried in a vacuum concentrator without heating. Following this, 0.6 ml of PB buffer was added, and the mixture vortexed for 1 min. It was then centrifuged for 10 min at 12,000 rpm, 4°C, and 0.5 ml of the supernatant was transferred into a fresh NMR tube. NMR analysis was performed when the samples were at room temperature [13].

2.3. 1D ^{1}H-NMR Spectroscopy

Acquisition was performed on extracts corresponding to known fresh weights comprising between 4.9 and 21.2 mg of skins and between 30 and 84 mg of pulp dissolved in 0.5 ml D2O (internal lock). Sodium salt of (trimethyl) propionic-2, 2, 3, 3-d4 acid (TSP) in D2O was added in all samples at a final concentration of 0.01% for chemical shift calibration. 1D ^{1}H-NMR spectra were recorded at 27°C with a Varian 600 spectrometer using a 5 mm inverse probe and fitted with an autosampler. Varian 600 spectrometer Parameter showed (Table 1). Data were phased and baseline corrected using Bayesil online software and to identify and quantify metabolites [14].

2.4. Chemicals

For classical analyses, all chemical reagents were of analytical grade (Mallinckrodt Baker France, Noisy-Le-Sec, France). For NMR, D2O (99.9%) was purchased from EURISO-TOP, and TSP (98%) was from Wilmad-LabGlass, USA.

2.5. Statistical Analyses

Bayesil online used to identification and quantification of metabolites in grapes from Shangeri-La using ^{1}H NMR data

Metaboanlyst6.0 automatically metabolic pathway analysis, Statistical analysis was performed using a combination of two multivariate statistical techniques, principal component analysis (PCA) and orthogonal partial least squares (OPLS-DA), to discriminate the groups. Bayesil online software was used to identify and quantify metabolites in csv format for MetaboAnalayst software and Simca 14.1 (Umetrics, Sweden). ^{1}H NMR data were further processed for PCA (correlation matrix) for 27 samples. OPLS-DA with Simca 14.1 was applied for 27 samples. Twenty-nine test samples randomly selected in each vintage group were used for further validation of the OPLS-DA analysis. Before analyses, NMR data were normalized to the total spectra intensity (without water signal), and the data were mean-centered for each variable. Furthermore, after OPLS-DA data compression, linear discriminant analysis was performed using the two first scores, and the positions of the group centers were calculated. Validation of the groups was estimated from the square Mahalanobis distance after this training phase, group assignation of the 27 test samples was done. Twenty different training sample sets and test samples were used successively to verify the robustness of the OPLS-DA model.

3. Results

3.1. Bayesil Identification and Quantification of Metabolites in Grapes from Shangeri-La Using ^{1}H NMR Data

Cabernet Sauvignon cultivars were harvested from two wine-growing areas in Shangeri-La during 2013. A typical 1D ^{1}H-NMR spectrum is presented Figure 2. The H-NMR peaks were between 2.0 and 5.5. Bayesil online software identified and quantified 82 metabolites, including 27 amino acids, 21 organic acids, 8 alcohols, 5 sugars, 3 esters, and 2 ketones (Table 6).

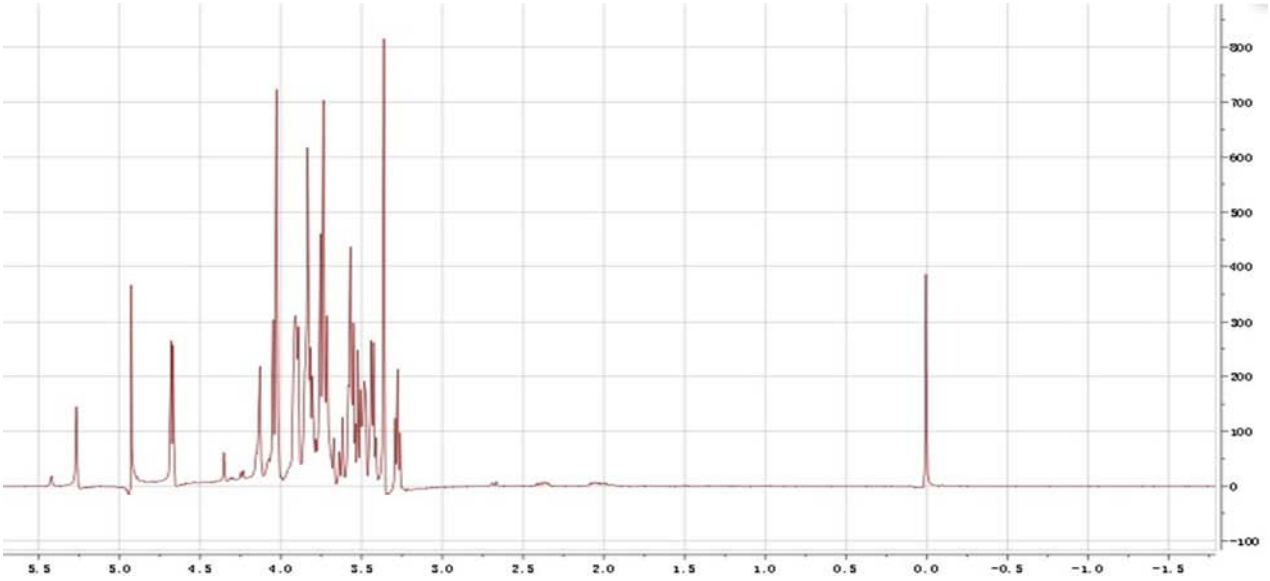
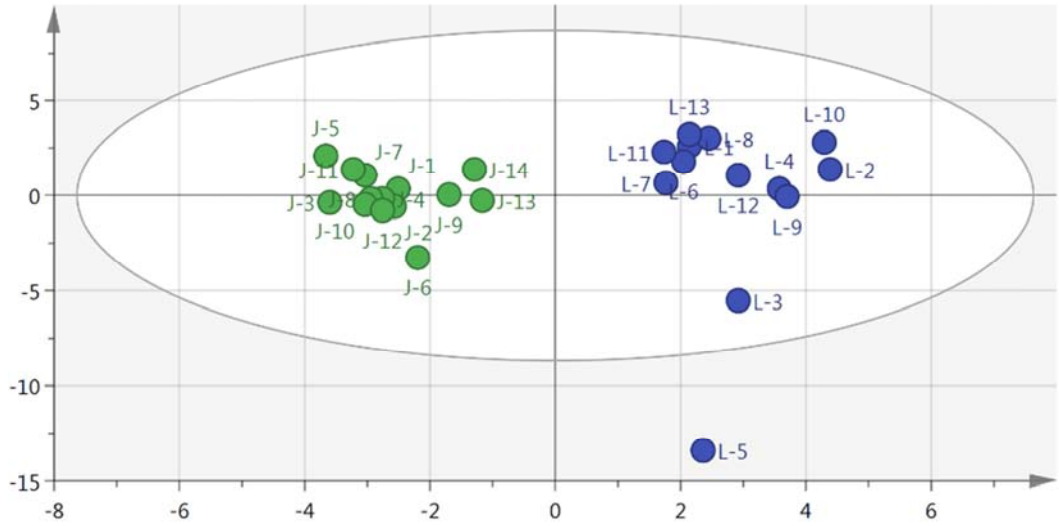


Figure 2. *Representative ^{1}H NMR spectrum for berries harvested in Shangeri-La.*

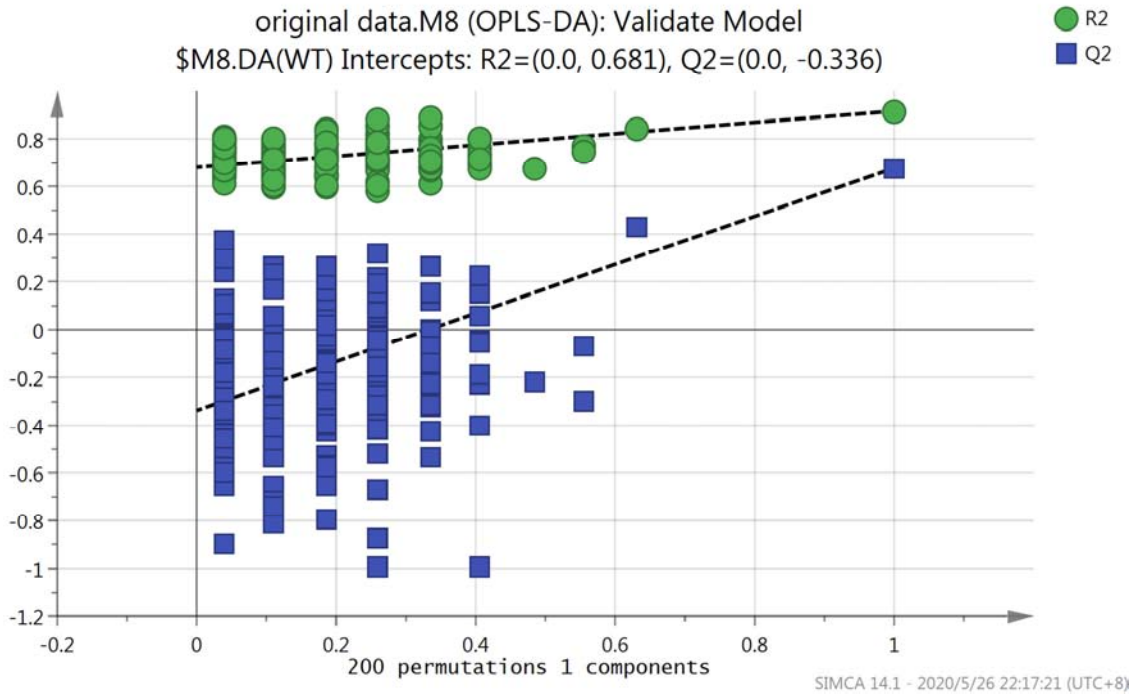
3.2. Multivariate Statistical Analysis of ^{1}H NMR Datas from Cabernet Sauvignon Cultivars from Shangeri-La

Twenty-seven samples of Cabernet Sauvignon grapes were collected from the wine-growing areas in Shangeri-La. A typical ^{1}H NMR spectrum of berry is presented in PCA on 27 domains of each ^{1}H NMR spectrum was used to explore the variability of the terroirs; Com1 represented 13.5% of variables, Com2 represented 10.6% variables, and the

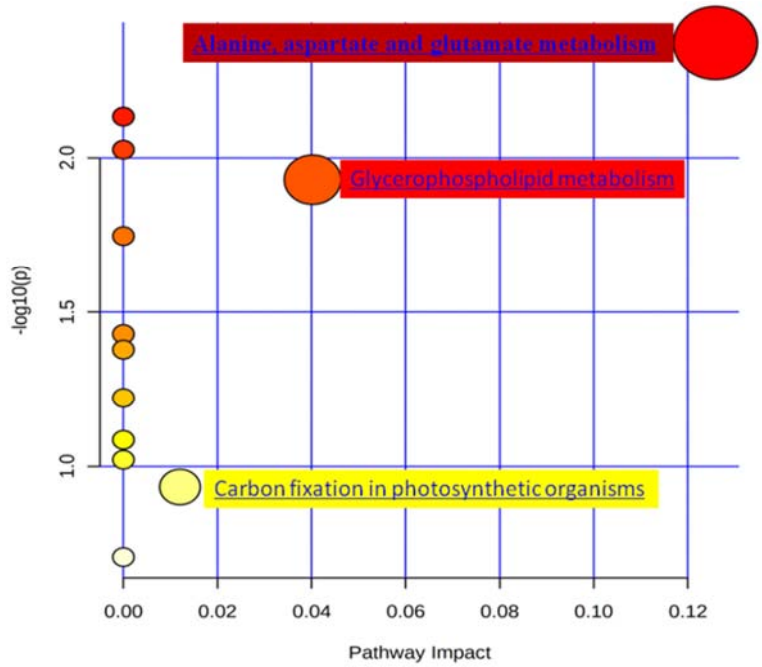
metabolites were unseparated and nondiscriminant (Figure 4). However, OPLS-DA model analysis of 27 samples of grapes indicated the Jinsha River and Lancan River valleys to be well separated (R2=0.916, Q2=0.681) (Figure3a and Figure 3b). Bayesil online software identified and quantified organic acids such as Pyroglutamic acid, Choline, Malonic acid, Trimethylamine, L-Aspartic acid, L-Asparagine and Ethanolamine whose contents were significantly increased (VIP >1, p value <0.001) in the Jinsha River valley vs. the Lancan River valley, while Acetone, Mannose, L-Proline, Caffeine, Methylamine and Methylmalonic acid contents were significantly decreased (VIP >1, p value <0.001). We could use those significant metabolites to predict whether the fruit came from the Jinsha River or Lancan River valleys (Table 4, Table 5 and Figure 3a, Figure 3b). Metabolic pathway showed that in the Jinsha River valley vs. the Lancan River valley, grapes had enhanced Alanine, aspartate and glutamate metabolism, Carbon fixation in photosynthetic organisms and Glycerophospholipid metabolism;. However, Jinsha River valley compared to the Lancan River valley had decreased Fructose and mannose metabolism, Valine, leucine and isoleucine degradation and Arginine and proline metabolism. (Figure 3c , Figure 3d).



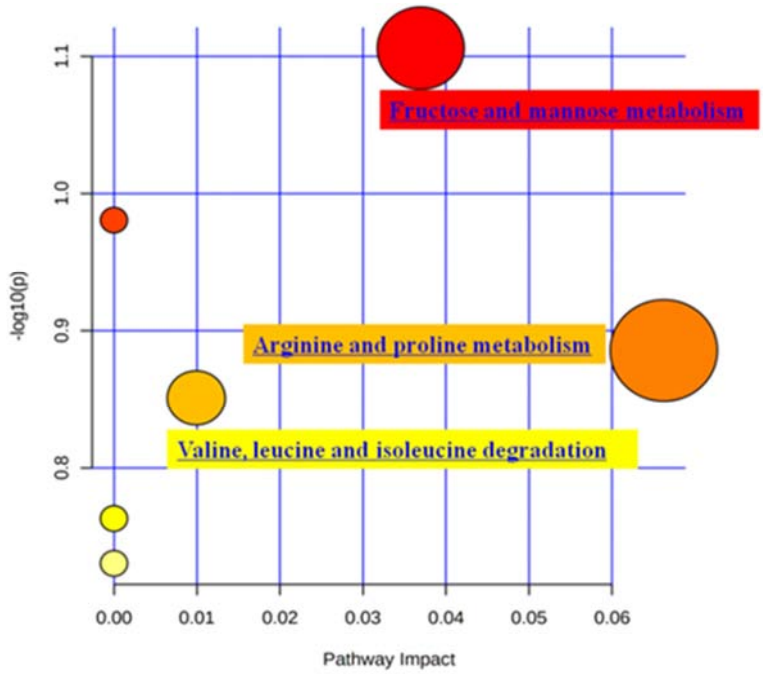
a



b



c



d

Figure 3. *a. OPLS-DA model ^{1}H NMR spectrum for an extract of berries harvested in Shangeri-La; b. OPLS-DA model ^{1}H NMR spectrum for an extract of berries harvested in Shangeri-La, The variance displayed in the plot above is the explained variance for R2=0.916. J and L are well separated: c. Jinsha River vs. Lancan River valley terroir's relation to the Cabernet Sauvignon grape metabolic pathway: d. Jinsha River vs. Lancan River valley terroir's relation to the Cabernet Sauvignon grape metabolic pathway.*

4. Discussion

The OPL-DA model showed ten organic acids that were biomarkers of the Jinsha River valley vs. the Lancan River valley for Cabernet Sauvignon grapes, indicating an enhanced pyroglutamic acid pathway for improved functions such as activity against Zymoseptoria tritici; which enhancing berries fresh weight and resistance to stressed plants[15]. The Jinsha River valley compared to the Lancan River valley also showed an enhanced succinic acid pathway, which can function by adapting to CO_2 partial pressure, improving the CO_2 fixation rate, and resisting Citrobacter amalonaticus disease [16]. The Jinsha River valley compared to the Lancan River valley also showed an enhanced fumaric acid pathway, which is known to inactivate foodborne pathogens in apple juice and is a useful additive to

improve wine microbiological stability and freshness, by reducing of SO_2 levels [17]. In addition, the Jinsha River valley compared to the Lancan River valley showed an enhanced malonic acid pathway. The Jinsha River valley also had an enhanced hippuric acid pathway, which can improve leaching or sorption of N_2O emissions and resistance to toluene exposure, and is a biomarker of high altitude [18]. The Jinsha River valley compared to the Lancan River valley had decreased methylmalonic acid and guanidoacetic acid pathways, which can downregulate the pentose phosphate pathway, and a decreased ascorbic acid pathway, which can reduce vitamin digestion and absorption from grapes [19, 20]. The model also showed an enhanced 1,5-anhydrosorbitol pathway, in addition to 2-hydroxybutyrate and acetone, which have functions that are unknown.

The OPL-DA model showed that in the Jinsha River valley vs. the Lancan River valley, grapes had enhanced aminoacyl-tRNA biosynthesis; glycine, serine, methionine, cysteine, and threonine metabolism; L-carnitine, creatinine, and creatine. However, Jinsha River valley compared to the Lancan River valley had decreased arginine and proline metabolism. The OPL-DA model showed an enhanced caffeine pathway in the Jinsha River valley via xanthine alkaloid compounds, which have multiple functions in biologic processes, including antioxidant activity, and can alter the cellular redox and inflammatory status in a dose-dependent manner [21].

5. Conclusion

Cabernet Sauvignon grapes produced in the Jinsha River and Lancan River valleys were analyzed by 1D ^{1}H NMR for metabolite profiling to clear the wine-growing terroirs in Shangeri-La. The 1D ^{1}H NMR spectra of mature berries cultivated in different river valleys indicated clear differences in composition, i.e., sugars, alcohols, esters ,organic acids and amino acids, phenolic compounds, sugars, and ketones. These variations could be due to differences in the Jinsha River and Lancan River valley soil and climate. Results vital to the terroir soil and climate in vineyards for the composition of grapes and strengthen the concept of terroir used to characterize the environmental conditions in which the grapevines are produced. The different river valleys had altered metabolic pathways to adapt to each valley's soil and climate. The Jinsha River valley showed enhancement of most organic， phenolic compounds and amino and organic acids . In the future, this technique can be used to understand environmental effects on the quality of grapes, and 1D ^{1}H NMR spectra can be used to determine which grapes come from which wine-growing regions.

Acknowledgements

Many thanks to all viticulturists and farmers located in Shangeri-La and the Shangeri-La Wine Co. Ltd. for providing the plant material (grapes) for the analyses.

Appendix

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

Table 1. *NMR Parameter.*

Parameter	Value
Comment	Water Suppression Spectrum
Origin	Varian
Owner	
Site	
Spectrometer	vnmrs
Author	
Solvent	D2O
Temperature	25
Pulse Sequence	noesypr1d
Experiment	1D
Number of Scans	64
Receiver Gain	30
Relaxation Delay	0
Pulse Width	18.4
Acquisition Time	0.9984
Acquisition Date	2013-11-06T20:53:03
Modification Date	2013-11-06T21:15:16
Spectrometer Frequency	599.91
Spectral Width	8012.8
Lowest Frequency	-1075.5
Nucleus	^{1}H
Acquired Size	8000
Spectral Size	16384

Table 2. *OPLS-DA Analyst and VIP.*

Var ID (Primary)	M1.VIP[1+1+0]	2.44693 * M1.VIP[1]cvSE
Pyroglutamic acid	1.91792	1.11016
Succinic acid	1.8103	1.22807
Methanol	1.71546	1.30709
Trimethylamine	1.63476	0.938466
Ascorbic acid	1.62655	0.53103
L-Asparagine	1.62583	1.00869
Ethanolamine	1.48414	0.897396
Fumaric acid	1.44891	1.65961
Choline	1.43334	1.39759
Dimethylglycine	1.35758	1.08098
L-Alanine	1.33769	1.79089
Malonic acid	1.32152	1.19884
Caffeine	1.31332	0.974932
Oxalacetic acid	1.31198	1.55167
L-Carnitine	1.29146	2.23089
Methylmalonic acid	1.28162	1.41357
Acetone	1.27304	0.858142
1,5-Anhydrosorbitol	1.22362	1.55553
L-Cysteine	1.21893	1.77824
Hippuric acid	1.20837	2.69032
Creatinine	1.20276	2.56836
L-Proline	1.19751	1.7423
Creatine	1.18296	1.18397
Xanthine	1.17331	2.34942
Gluconic acid	1.15971	0.615109
2-Hydroxybutyrate	1.07305	1.60922
Guanidoacetic acid	1.05351	1.66458
L-Serine	1.02802	1.4338

Table 3. *Rank Freq.*

	Rank Freq.	Importance	D	N	
L-Proline	1	0.009410999	High	Low	1
Methylmalonic acid	1	0.006127278	High	Low	2
Pyroglutamic acid	1	0.005674744	High	Low	3
Isobutyric acid	1	0.00393668	High	Low	4
Acetone	1	0.003883864	High	Low	5
Methylamine	1	0.003742189	High	Low	6
Glycine	1	0.002976002	High	Low	7
L-Valine	1	0.002476788	High	Low	8
Ascorbic acid	1	0.001938579	High	Low	9
Propylene glycol	0.98	0.002299847	High	Low	10
Isopropanol	0.98	0.001814926	High	Low	11
Dimethylglycine	0.94	0.004738728	High	Low	12
L-Aspartic acid	0.94	0.003617911	High	Low	13
Creatine	0.94	0.003401096	High	Low	14
Acetoacetate	0.94	0.002398812	High	Low	15
L-Isoleucine	0.9	0.001776976	High	Low	16
3-Hydroxyisobutyrate	0.84	0.002491495	High	Low	17
Taurine	0.78	0.002754264	High	Low	18
1-Methylhistidine	0.76	0.001742504	High	Low	19
L-Carnitine	0.74	0.001896167	High	Low	20
Xanthine	0.72	0.001536996	High	Low	21
Caffeine	0.7	0.001665526	High	Low	22
Ethanolamine	0.66	0.001113826	High	Low	23
L-Cysteine	0.64	0.00161287	High	Low	24
L-Methionine	0.6	0.001250286	High	Low	25
Alpha-Lactose	0.58	0.00112128	High	Low	26

	Rank Freq.	Importance	D	N	
Hippuric acid	0.58	0.000978114	High	Low	27
p-Hydroxyphenylacetic acid	0.58	0.000884373	High	Low	28
L-Fucose	0.54	0.001197595	High	Low	29
L-Lactic acid	0.54	0.000916649	High	Low	30
Phosphorylcholine	0.52	0.001393003	High	Low	31
Isovalerate	0.52	0.001122008	High	Low	32
Sarcosine	0.5	0.001218498	High	Low	33
Creatinine	0.48	0.001148504	High	Low	34
Betaine	0.46	0.001085574	High	Low	35
5-Aminopentanoic acid	0.42	0.000895584	High	Low	36
Guanidoacetic acid	0.4	0.001117602	High	Low	37
3-Methylhistidine	0.4	0.001044249	High	Low	38
3-Hydroxybutyric acid	0.4	0.000940507	High	Low	39
L-Glutamic acid	0.38	0.001039835	High	Low	40
EDTA	0.38	0.000951908	High	Low	41
Myoinositol	0.38	0.000840038	High	Low	42
Urea	0.38	0.000700116	High	Low	43
Trimethylamine	0.36	0.000901441	High	Low	44
Ethanol	0.34	0.000741788	High	Low	45
2-Hydroxybutyrate	0.34	0.00062663	High	Low	46
1,5-Anhydrosorbitol	0.32	0.001214268	High	Low	47
D-Galactose	0.32	0.000699793	High	Low	48
Mannose	0.3	0.000627261	High	Low	49
L-Cystine	0.28	0.00072196	High	Low	50
Fumaric acid	0.28	0.000564467	High	Low	51
Butyric acid	0.26	0.000694745	High	Low	52
2-Oxoisovalerate	0.26	0.000484992	High	Low	53
Hypoxanthine	0.24	0.000503547	High	Low	54
Glycerol	0.24	0.000422207	High	Low	55
Malonic acid	0.22	0.00048087	High	Low	56
3-Hydroxyisovaleric acid	0.2	0.000403159	High	Low	57
Ornithine	0.2	0.000390291	High	Low	58
Choline	0.2	0.00038213	High	Low	59
Dimethylsulfone	0.18	0.00045064	High	Low	60
Sorbitol	0.18	0.000408649	High	Low	61
D-Glucose	0.16	0.000385665	High	Low	62
Succinic acid	0.12	0.00039065	High	Low	63
L-Serine	0.12	0.000354945	High	Low	64
2-Oxoglutaric acid	0.12	0.000335306	High	Low	65
Dimethylamine	0.12	0.000286766	High	Low	66
Formic acid	0.12	0.000273776	High	Low	67
L-Alanine	0.08	0.000320253	High	Low	68
L-Histidine	0.08	0.00030547	High	Low	69
Gluconic acid	0.06	0.000446887	High	Low	70
Oxalacetic acid	0.06	0.000337989	High	Low	71
Acetic acid	0.04	0.0002113	High	Low	72
Pyruvic acid	0.02	0.000132002	High	Low	73
L-Asparagine	0.98	0.003703431	High	Low	74
Uracil	0.16	0.000391291	Low	High	75
L-Arginine	0.64	0.00132991	Low	High	76
Citric acid	0.12	0.000312099	Low	High	77
p-Cresol sulfate	0.94	0.001631714	Low	High	78
L-Threonine	0.06	0.000300007	Low	High	79
Lysine	0.82	0.002322154	Low	High	80
L-Phenylalanine	0.98	0.00289456	Low	High	81

Table 4. *Organic acid composition.*

Type of Metabolites	number	Metabolites composition	N	Peak Mean	Std. dev.
	1	Butyric acid	27	0.89	1.20
	2	Acetic acid	27	0.89	1.00
	3	Ascorbic acid	27	4788.02	1731.64
	4	Citric acid	27	26.51	21.77
	5	Guanidoacetic acid	27	249.73	273.73
	6	Fumaric acid	27	0.88	2.15
	7	Formic acid	27	0.90	0.85
	8	L-Glutamic acid	27	33.66	28.06
	9	L-Aspartic acid	27	166.71	214.01
	10	Methylmalonic acid	27	1.76	3.61
Organic acid	11	2-Oxoglutaric acid	27	8.38	15.34
	12	Oxalacetic acid	27	30.40	17.60
	13	Pyruvic acid	27	25.47	16.86
	14	Succinic acid	27	16.79	12.38
	15	Pyroglutamic acid	27	49.56	43.46
	16	3-Hydroxybutyric acid	27	3.36	7.79
	17	Gluconic acid	27	864.70	554.38
	18	Isobutyric acid	27	0.81	3.86
	19	Malonic acid	27	114.53	239.22
	20	5-Aminopentanoic acid	27	0.77	1.11
	21	3-Hydroxyisovaleric acid	27	1.46	3.64

Table 5. *Animo acid composition.*

Type of Metabolites	number	Metabolites composition	N	Mean	Std. dev.
	1	1-Methylhistidine	27	20.41	47.08
	2	Betaine	27	87.49	84.17
	3	L-Carnitine	27	10.33	38.55
	4	Creatine	27	90.33	94.27
	5	Dimethylamine	27	2.24	7.53
	6	Dimethylglycine	27	41.66	64.33
	7	Choline	27	25.11	53.38
	8	Glycine	27	1641.70	488.45
	9	Ethanolamine	27	992.69	803.46
	10	Hypoxanthine	27	1.10	1.02
	11	L-Alanine	27	0.84	1.95
	12	L-Proline	27	107.98	133.77
	13	Methylamine	27	2.61	5.77
Animo acid	14	L-Threonine	27	1.25	3.18
	15	L-Asparagine	27	376.64	514.90
	16	L-Isoleucine	27	0.29	0.86
	17	L-Serine	27	1260.90	1326.68
	18	L-Cystine	27	72.21	119.28
	19	Ornithine	27	6.19	11.60
	20	Taurine	27	653.40	678.86
	21	Sarcosine	27	5.77	14.40
	22	Xanthine	27	3.21	9.76
	23	3-Methylhistidine	27	1.00	2.45
	24	Creatinine	27	108.72	171.40
	25	L-Cysteine	27	158.38	257.45
	26	Trimethylamine	27	13.47	22.08
	27	Phosphorylcholine	27	0.91	2.04

Table 6. *Alcohol, Sugar, Ester, Ketone and Others composition.*

Type of Metabolites	number	Metabolites composition	N	Mean	Std. dev.
Alcohol	1	Ethanol	27	3.56	8.64
	2	Glycerol	27	156.16	208.57
	3	Methanol	27	7318.20	11675.80
	4	Myoinositol	27	178.23	182.05
	5	Sorbitol	27	29.83	29.95
	6	1,5-Anhydrosorbitol	27	217.51	346.65
	7	Caffeine	27	25.24	41.12
	8	p-Cresol sulfate	27	0.01	0.03
Sugar	1	D-Glucose	27	2711.31	1031.48
	2	D-Galactose	27	85.52	86.64
	3	Mannose	27	123.86	182.33
	4	L-Fucose	27	0.54	1.79
	5	Fructose	27	1272.59	1005.78
Ester	1	2-Oxoisovalerate	27	0.86	2.02
	2	3-Hydroxyisobutyrate	27	0.98	1.39
	3	Isovalerate	27	1.04	2.19
Ketone	1	Acetone	27	0.65	0.54
	2	Dimethylsulfone	27	16.03	35.28
Others	1	Urea	27	3039.54	2380.43
	2	Uracil	27	1.49	2.18
	3	EDTA	27	6.54	11.82

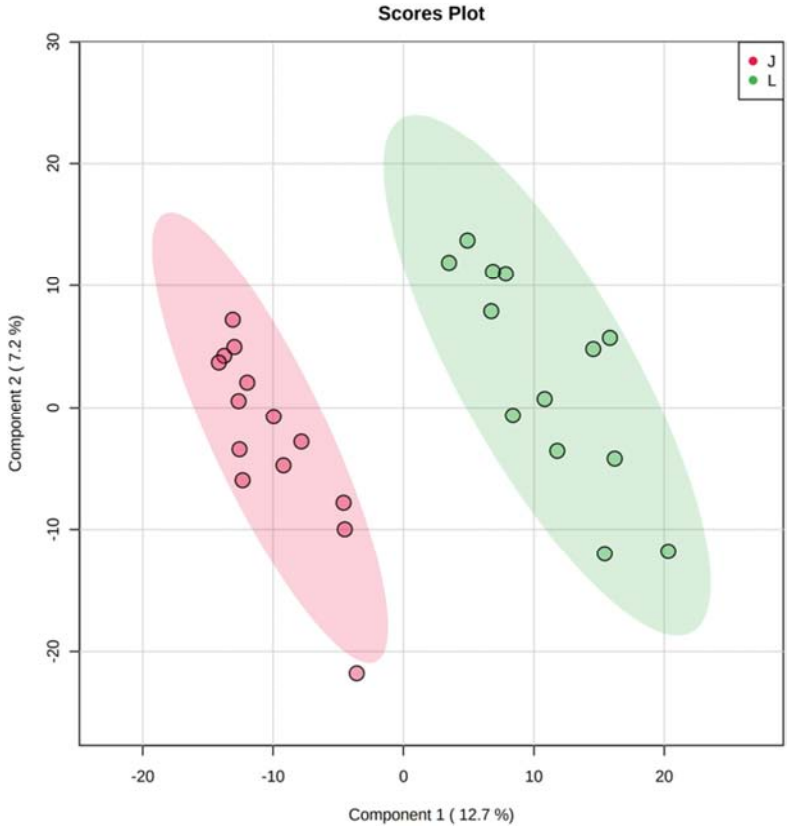


Figure 4. *PCA on 27 domains of each [1]H NMR spectrum was used to explore the variability of the terroirs; Com1 represented 13.5% of variables, Com2 represented 10.6% variables.*

References

[1] Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Gerós H. (2006). Biochemical changes throughout grape berry development and fruit and wine quality. Structure. 1.

[2] Torgrip R, Åberg K, Alm E, Schuppe-Koistinen I, Lindberg J. (2008). A note on normalization of biofluid 1D 1 H-NMR data. Metabolomics. 4: 114-21.

[3] Smart R. (2012). Evaluating the data and discussion in Wine, Terroirand Climate Change. Wine & Viticulture Journal. 27: 15-6.

[4] Leeuwen CV. (2010). Terroir: the effect of the physical environment on vine growth, grape ripening and wine sensory attributes. Managing Wine Quality. 83: 273-315.

[5] Anesi A, Stocchero M, Santo SD, Commisso M, Zenoni S, Ceoldo S, Tornielli GB, Siebert TE, Herderich M, Pezzotti M. (2015). Towards a scientific interpretation of the terroir concept: plasticity of the grape berry metabolome. BMC Plant Biology.

[6] Boccard J, Rutledge DN. (2013). A consensus orthogonal partial least squares discriminant analysis (OPLS-DA) strategy for multiblock Omics data fusion. Analytica Chimica Acta. 769: 30-9.

[7] Duan L, Ma A, Meng X, Shen GA, Qi X. (2020). QPMASS: A parallel peak alignment and quantification software for the analysis of large-scale gas chromatography-mass spectrometry (GC-MS)-based metabolomics datasets. Journal of Chromatography A. 1620: 460999.

[8] Picone G, Trimigno A, Tessarin P, Donnini S, Rombola AD, Capozzi F. (2016). ^{1}H NMR foodomics reveals that the biodynamic and the organic cultivation managements produce different grape berries (Vitis vinifera L. cv. Sangiovese). Food Chemistry. 213: 187-95.

[9] Pereira GE, Gaudillere JP, Leeuwen CV, Hilbert G, Maucourt ML, Deborde C, Moing A, Rolin D. (2006). ^{1}H NMR metabolite fingerprints of grape berry: Comparison of vintage and soil effects in Bordeaux grapevine growing areas. Analytica Chimica Acta. 563: 346-52.

[10] Maulidiani, Rudiyanto, Mediani A, Khatib A, Ismail A, Hamid M, Lajis NH, Shaari K, Abas F. (2017). Application of BATMAN and BAYESIL for quantitative ^{1}H-NMR based metabolomics of urine: discriminant analysis of lean, obese, and obese-diabetic rats. Metabolomics. 13: 131.

[11] Ciampa A, Dell'Abate MT, Florio A, Tarricone L, Gennaro DD, Picone G, Trimigno A, Capozzi F, Benedetti A. (2019). Combined magnetic resonance imaging and high resolution spectroscopy approaches to study the fertilization effects on metabolome, morphology and yeast community of wine grape berries, cultivar Nero di Troia. Food Chemistry. 274: 831-9.

[12] Abdi H, Williams LJ, Valentin D. (2013). Multiple factor analysis: principal component analysis for multitable and multiblock data sets. Wiley Interdisciplinary reviews: computational statistics. 5: 149-79.

[13] Sun X, Lyu G, Luan Y, Yang H, Zhao Z. (2019). Metabolomic Study of the Soybean Pastes Fermented by the Single species Penicillium glabrum GQ1-3 and Aspergillus oryzae HGPA20. Food Chemistry. 295: 622-9.

[14] Ravanbakhsh S, Liu P, Bjordahl TC, Mandal R, Grant JR, Wilson M, Eisner R, Sinelnikov I, Hu X, Luchinat C. (2015). Accurate, fully-automated NMR spectral profiling for metabolomics. PloS one. 10: e0124219.

[15] Mejri S, Siah A, Abuhaie CM, Halama P, Magnin-Robert M, Randoux B, Reignault P, Rigo B, Ghinet A. (2019). New salicylic acid and pyroglutamic acid conjugated derivatives confer protection to bread wheat against Zymoseptoria tritici. Journal of the science of food and agriculture. 99: págs. 1780-6.

[16] Amulya K, Kopperi H, Mohan SV. (2020). Tunable production of succinic acid at elevated pressures of CO2 in a high pressure gas fermentation reactor. Bioresource Technology. 309: 123327.

[17] Morata A, Bauelos MA, López C, Chenli S, Lepe J. (2019). The oenological interest of fumaric acid: Stop malolactic fermentation and preserve the freshness of wines. BIO Web of Conferences. 15: 02034.

[18] Bertram JE, Clough TJ, Sherlock RR, Condron LM, O'CALLAGHAN M, Wells NS, Ray JL. (2009). Hippuric acid and benzoic acid inhibition of urine derived N2O emissions from soil. Global Change Biology. 15: 2067-77.

[19] Brand E, Brand FC (2003). Guanidoacetic Acid. Organic Syntheses. Place. Published

[20] Tanaka A, Isshiki G. (1995). [Methylmalonic acid]. Pediatrics. 51: 1012-5.

[21] Yuan JF, Hu JW, Chen ZY, Wang DH. (2020). Screening of cardioprotective diseases bioactive components from Danshen extracts and LC–MS analysis. Biomedical Chromatography. 34: e4823.