

# Difference of Enzyme Metabolites in Yunnan Arabica Coffee from Different Primary Processing was Analyzed Based on Non-targeted Metabolomics

Li Yang<sup>1</sup>, Yuannan Xu<sup>1</sup>, XueLing Li<sup>2</sup>, Guoyin Chang<sup>1</sup>, Xiaoyan Shang<sup>1,\*</sup>,  
Xurundong Kan<sup>1,\*</sup>

<sup>1</sup> College of Biology and Chemical Science, Pu 'er University, Pu 'er 665000, China

<sup>2</sup> College of Tea and Coffee, Pu 'er University, Pu 'er 665000, China

\* Corresponding Author Email: kanxurundong@163.com

**Abstract.** In order to explore the changes of non-volatile metabolites in Yunnan arabica coffee (*Coffea arabica* L.) by different primary processing methods, the coffee samples were systematically analyzed by non-targeted metabolomics technology in raw and cooked beans of different primary processed coffee beans. The results showed that although the raw materials of coffee beans with different primary processing were the same, there were great differences in metabolites due to different processing methods. Shikic acid and phenyl propionic acid and their derivatives, alkaloids and their metabolites, fatty acids and their metabolites were the main significant differences in coffee samples. Different primary processing methods may have important contributions to the formation of different taste qualities of coffee beans. According to the analysis of metabolic pathway enrichment in Kyoto Encyclopedia of Genes and Genome, different compounds were significantly enriched in metabolic pathways, biosynthesis of secondary metabolites, bio anabolic pathway of amino acids, bio anabolic pathway of cofactors, metabolic pathway of 2-oxo-carboxylic acid and ABC transporter ( $P < 0.05$ ). This study further explored the main non-volatile metabolites that affect the flavor and quality of coffee in different primary processing methods, which is of great significance for the improvement of coffee flavor quality.

**Keywords:** Coffee, Primary processing, non-targeted metabolomics, Non-volatile metabolite.

## 1. Introduction

Coffee (*Coffea* spp.) belongs to the perennial evergreen shrub or small tree of Rubiaceae. It is a tropical cash crop [1-2] with easy cultivation, little investment, fast income and high added value. Among them, the planting area and output of Yunnan coffee account for more than 98% [3-4] of the national coffee industry [3-4]. Benefiting from its unique plateau climate and rich ecological diversity, small coffee is famous for its flavor quality of "thick but not bitter, fragrant but not strong, slightly sour", which promotes the rapid development of Yunnan coffee planting industry [5-6].

As one of the most popular drinks in the world, coffee has a strong market competitiveness in the domestic and foreign markets. Coffee is rich and diverse, influenced by the variety, geographical environment, cultivation management and post-harvest measures, and a series of complex chemical reactions inside coffee beans, thus forming all kinds of coffee flavor substances [7]. The primary processing of coffee includes peeling, peeling, fermentation and drying [8]. Different primary processing methods will affect the enzyme activity, fermentation conditions and microbial activity in coffee fruit, and endow coffee with different flavor characteristics and sensory qualities. The most common primary treatments include sun exposure (dry), water washing (wet), and honey treatment (semi-dry). The sun treatment produced the dried coffee bean [9] without removing the peel. Wash treatment to remove the fresh coffee shell, soak in water to remove pectin, fermented and dried for [10]. Honey treatment retains pectin and dries with the beans, producing the flavor [11] of honey and sugar. However, the primary processing methods of the same type will also be different in the specific operation, which will lead to the change of the internal flavor and substance content of the coffee. Chen et al. [12] found that the content of crude fat, crude protein and caffeine in non-soaking

fermented coffee beans increased compared to the immersion fermentation process. Schock et al. [13] found that different primary processing methods had a significant impact on the total titration acidity (TA), total soluble solid (TSS) and pH of coffee samples. VELASQUEZ et al. [14] found that wet coffee beans had high concentration of volatiles, light lightness, better aroma and higher consumer acceptance than dry coffee beans.

Metabolomics, also known as "systems biological mathematics", is the omics family [15] that studies small molecule metabolites in cells, tissues, organs and organisms. Metabolomics techniques include nuclear magnetic resonance spectroscopy, gas chromatography-ion mobility (GC-IMS) spectroscopy, gas chromatography-mass spectrometer (GC-MS), liquid-mass combination (LC-MS) [16]. Based on its integrity, high throughput and other characteristics, metabolomics technology can detect small molecule compounds with high resolution and high precision, and has been successfully applied in many fields. Mao Honglin et al. [17] explored the compositional differences between De 'ang acid tea and Pu' er tea (cooked tea) based on non-targeted metabolomics methods, and significantly distinguished the metabolites of tea with different fermentation processes. Using transcriptomics and metabolomics, Xiao et al revealed the relationship between flavor formation and microbial gene expression of Sichuan kimchi.

The study with different primary processing methods of coffee beans as the object, using super-efficient pressure liquid chromatography and quadrupole time of flight mass spectrometry combined with untargeted metabolomics technology of non-volatile metabolites in coffee beans comprehensive analysis, by obtaining the metabolites abundance information, to identify different coffee samples, to further reveal the formation mechanism of coffee quality and its regulation to provide theoretical reference.

## **2. Materials and Methods**

### **2.1. Materials and Reagents**

Yunnan small coffee beans: from pu'er city simao district river industrial park coffee farm, picking after tap water cleaning, remove not full and a small amount of immature fruit, used for different primary processing, wash raw beans, sun, honey processing, washed (baked), sun (baked) beans, honey processing (baking) beans, sample number for the WR, SR, HR, WC, SC, HC.

Methanol, cooked acetonitrile, acetic acid, isopropyl alcohol (pure chromatogram), Shanghai Hengbai Biotechnology Co., Ltd.; Ultra-pure water (ddH 20), Yunnan Baiyao Group Co., Ltd.

### **2.2. Instruments and equipment Prominenece**

UFLC ultra-efficient liquid phase, Shenzhen Yihua New Electronics Co., Ltd.; Orbitrap Exploris 120 High-resolution mass spectrometry, Qingdao Road Bo Jianye Environmental Protection Technology Co., Ltd.; TG 16-WS / H1650 / H1850 centrifuge, Shanghai Yunjin Instrument and Equipment Co., LTD.; The FA-E / EN balance, Changzhou Lucky Electronic Equipment Co., Ltd.; The PS-60AL ultrasound instrument, Shenzhen Redbang Electronics Co., Ltd.; JXFSTPRP-24 Homogenizer, Shanghai Jingxin Technology Co., Ltd.; The LGJ-10C freeze-dryer, Sihuan Freikeyi Technology Development Co., LTD.

### **2.3. Experimental method**

#### **2.3.1. Coffee beans treatment method**

The primary processing of coffee samples refers to the method of [19] et al. Sunset treatment: Clean the fresh coffee fruits and dry them, gradually reduce the water content of the coffee beans to 12%, and then remove the hard peel and shell, and remove the coffee beans. Water washing treatment: clean the fresh fruit and remove the peel and pulp, put the coffee beans containing pectin into a beaker and ferment for 24 h. Rinse the pectin with running water, and then air naturally until the water of the coffee beans is reduced to 12% to get the coffee beans. Honey treatment: wash the fresh coffee fruit

and remove the skin and flesh, directly dry the coffee beans with pectin, and separate the coffee beans.

### 2.3.2. Baking conditions for coffee beans

The roasting method of coffee samples refers to the method of Hong Zishan et al. [20]. As the gas source, the firepower is adjusted to 0.5 kPa, the damper is 4, and the speed is 60 rpm. Bebean temperature 180~190°C, baking time 15~20 min, medium depth baking. Baking date December 23,2023, coffee beans crushed through 80 mesh sieves.

### 2.3.3. and the metabolite extraction

Ground the coffee samples, weigh 25 mg of coffee samples into the EP tube, add homogenate beads, add 1000  $\mu$  L of extract (methanol: acetonitrile: water: 2:2:1 (V / V), containing isotopic labeled internal standard mixture), mix well for 30s, homogenate at 35 Hz for 4min, ice bath for 5 min, repeat 3 times; -40°C for 4°C, 12000 rpm for 15 min, 12000 rpm for 15 min, and the supernatant was taken for [21-22].

### 2.3.4. Computer detection

Phenomenex Kinetex C18 (2. 1 mm 50 mm, 2. 6 mm  $\mu$  m) liquid chromatography column, mobile phase A is aqueous phase (containing 0.01% acetic acid), phase B is isopropanol: acetonitrile (1:1, V / V), sample temperature: 4°C, sample volume: 2  $\mu$  L.

Orbitrap Exploris 120 The mass spectrometer enables the primary and secondary mass spectrometry data acquisition under the control of the control software (Xcalibur4.4, Thermo). Sheath gas flow rate: 50 Arb, auxiliary pressure: 15 Arb, capillary temperature: 320°C, resolution (Full MS), resolution (MS 2) are 60000,15000, collision energy: SNCE 20 / 30 / 40, spray voltage: 3.8 kV (positive ion mode) or-3.4 kV (negative ion mode).

## 2.4. Data processing

After the data were converted to mzXML format by ProteoWi zard software, the retention time correction peak identification, peak extraction, peak integration, peak alignment, and the peaks were identified using the co-written R program package and self-built secondary mass spectrum database. Differential analysis of the metabolome data was performed by PCA, OPLS-DA, Venn plot, and volcano plot using the R package. Significant different metabolites were selected by Fold Change (FC) 2 and P-value <0.05 criteria, and pathway analysis was conducted through the human metabolome database (human metabolome database, HMDB), Kyoto Encyclopedia of Genes and Genome Pathways (Kyoto encyclopedia of genes and genomes, KEGG) and other databases.

## 3. Results and analysis

### 3.1. Metabolite profile analysis

QC samples were tested, and the peak retention time and peak area of total ion flow (Total Ion Current, TIC) of QC samples were analyzed to determine the repeatability of metabolite extraction and detection, so as to guarantee the quality of data [23]. The total ion flow map of the QC sample is shown in Figure 1, where the TIC retention time and peak area overlap well, indicating good instrument stability, normal signal, less variation caused by instrument deviation throughout the experiment, and reliable data.

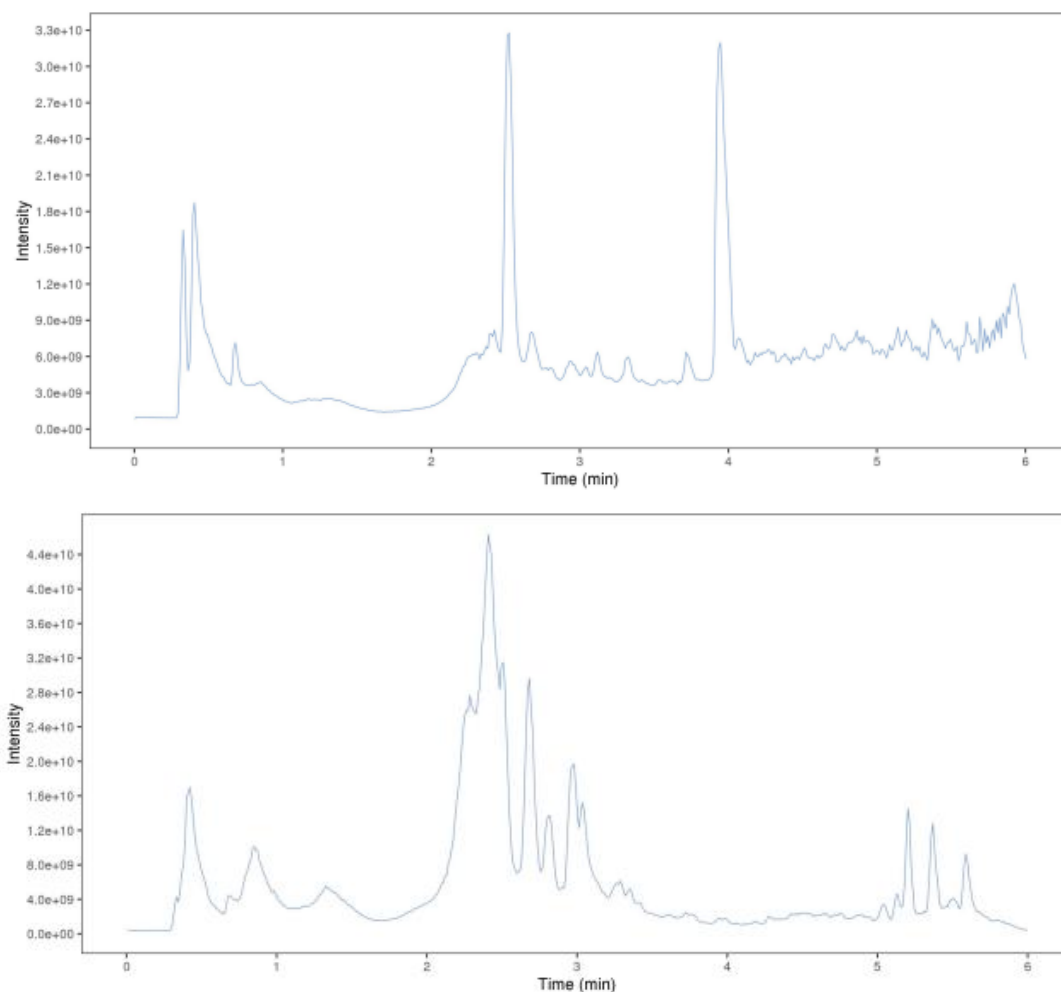


**Figure 1.** UHPLC-0E-MS positive and negative ion mode TIC of QC sample

### 3.2. Metabolomics difference analysis

According to Figure 2, the contribution rate of PC1 was 70.7% and that of PC2 was 10.8%, indicating that the fit of PCA model was stable and reliable, and all the samples were within 95% confidence interval, indicating that the overall distribution trend of the samples is good and effectively distinguished, indicating that the metabolites differ significantly in Yunnan, which can be used for subsequent analysis.

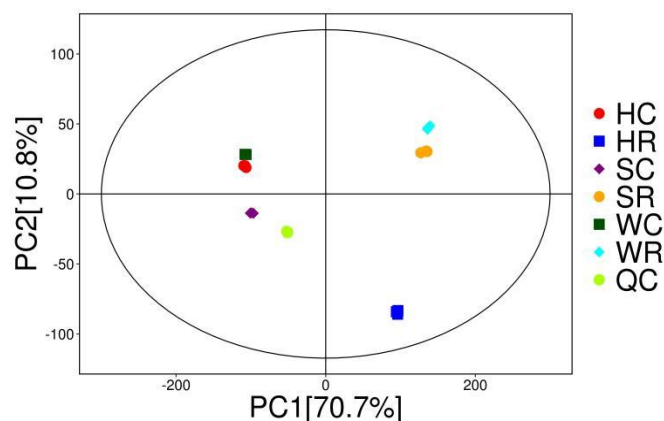


**Figure 2.** PCA analysis of different substances in Yunnan small-grain coffee with different primary processing methods

Note: WR, washed raw beans; SR, sun exposed raw beans; HR, honey treated raw beans; WC, washed (baked) beans; SC, sun cooked (baked) beans; HC, honey treated cooked (baked) beans, the

same below.

To obtain a higher level of separation, a discriminant analysis was performed using the classical supervised discriminant model OPLS-DA, drawing the score plot and cross-verifying the model 200 times as shown in Figure 3. The  $Q^2$  of the OPLS-DA model represents the predictive power of the model, The closer this value is to 1 indicates that the more reliable the model is, In different primary bean comparison models,  $Q^2$  was greater than 0.99, And verify that the intersection between the regression line and the vertical axis is all below zero, All  $Q^2$  values were below the original values, Suggesting that none of the OPLS-DA models constructed from the different data matrices showed an overfitting phenomenon, Showed that the constructed model is more reliable, Most of the data were simulated within, Coffee samples with different primary processing methods are distributed on the left and right sides of the confidence interval, The differentiation effect is more obvious, Showed that the sample composition differences were very significant, Differential metabolite search for [17] can be performed.

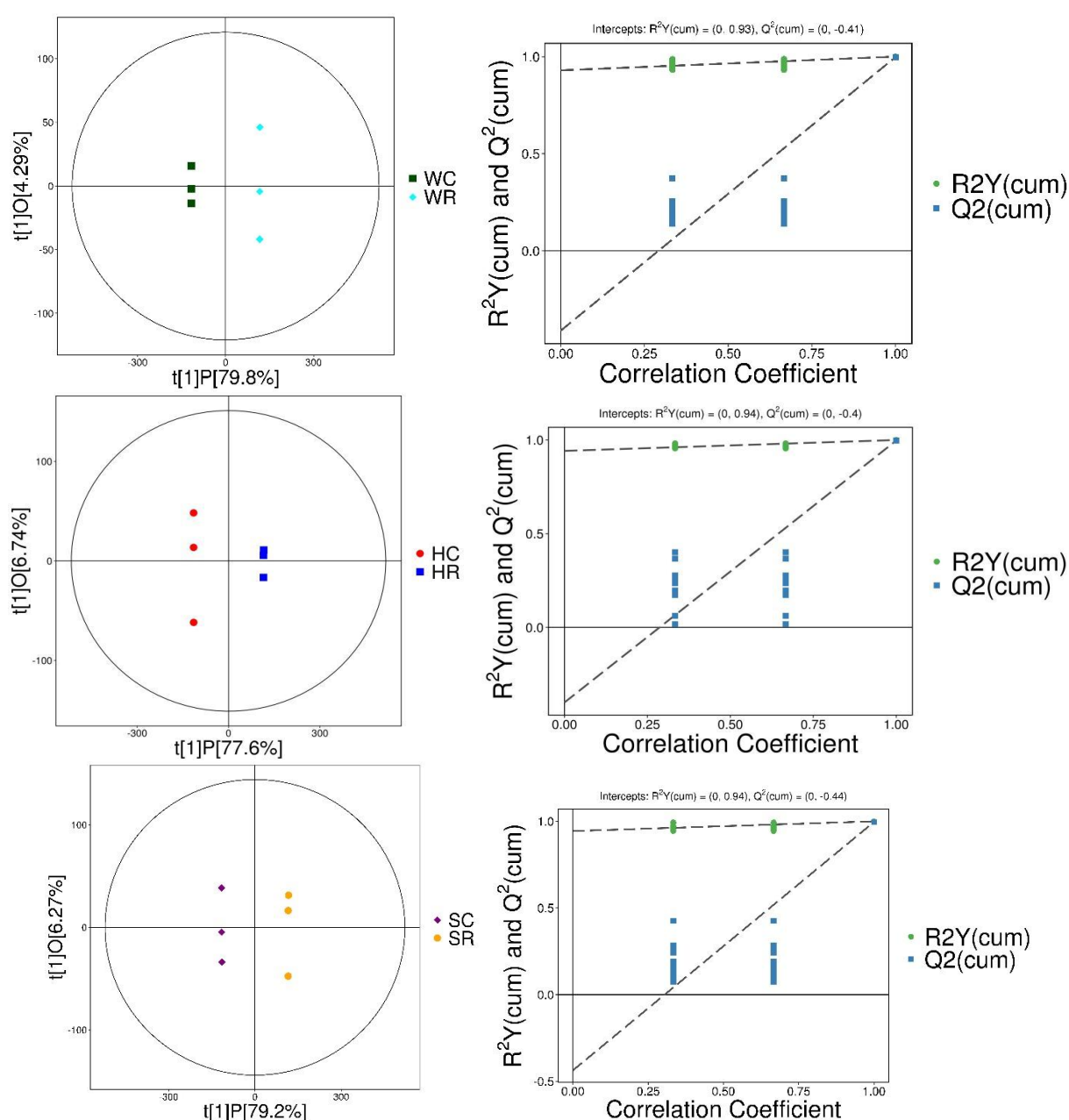


**Figure 3.** OPLS-DA analysis scores and verification charts for different primary processing methods of Yunnan small coffee  
(A. HR vs HC, B. WR vs WC, C. SR vs SC)

### 3.3. Analysis of the differential metabolites

To further screen the differential metabolites of Yunnan grain coffee in different primary processing methods, For the differential metabolite analysis between HR and HC, WR and WC, and SR and SC groups, With  $P < 0.05$  and  $FOLD\ CHANGE > 2$  as indicators, In the positive and negative ion mode, Initial screening of differential metabolites detected from secondary mass spectrometry analysis, According to the volcano map, Figure 4, The results showed that there were of Yunnan different in in methods, A total of 2066 substances were detected in HR and HC group, Of these, 334 significantly differential metabolites were selected, Including 24 species of amino acids, 12 species of polyketides, 90 species of shikimate and phenylpropanic acid, 88 species of alkaloids, 14 species of carbohydrates, 24 terpenes, 56 fatty acids and 26 other classes. It was found that the higher substances in the HR group included myristic acid, malic acid, p-hydroxyphenyl pyruvic acid, epicatechin, lauric acid, and the relatively low substances were resveratrol, Chinese icin I, and ginkgolide C. The higher substances in the HC group included epicatechin, myristic acid, malic acid, catechol, and hydroquinone, and the relatively small substances were pseudopoline, potato ash, and epigallocatechin gallate. Myristic acid, malic acid and epicatechin were high-content substances shared by HR and HC groups.

In WR and WC group, 2082 species were detected, of which 396 significantly different metabolites were selected, including 35 amino acids, 10 polyketides, 96 herbal acid and phenylpropanic acid, 103 alkaloids, 20 carbohydrates, 33 terpenes, 62 fatty acids and 37 other species. It was found that the higher substances in the WR group included malic acid, epicatechin, 9-oxadine acid, 9,10-epoxy stearic acid, N-acetyl-L-phenylalanine, etc., and the relatively small substances were resveratrol and citrulline. The higher substances in the WC group included epicatechin, malic acid, catechol, hydroquinone, catechol, and the relatively small substances were sanzhu-isopanhexanine and canlitone.

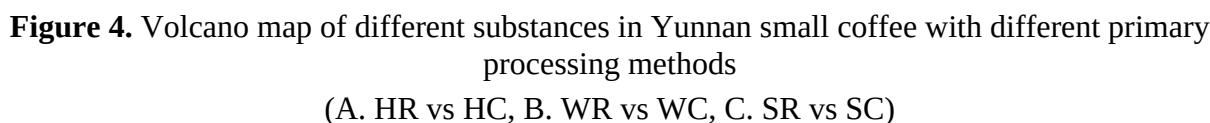
In SR and SC groups, 2099 substances were detected, of which 409 significantly different metabolites were selected, including 30 amino acids, 12 polyketides, 96 herbal and phenylpropanic acids, 108 alkaloids, 19 carbohydrates, 40 terpenes, 37 fatty acids and 37 other species. The substances found to be higher in the SR group included p-hydroxyphenylpyruvate, 9-oxadine acid, 9,10-epoxy stearic acid, (9Z, 11E, 13S) -13-hydroxocadecyl-9,11-dienic acid, 1- (9Z, 12Z-octadecadienoyl) -sn-glycerol-3-phosphocholine, with relatively small levels of resveratrol and citrulline. The higher substances in the SC group included ramipril, catechol, hydroquinone, p-hydroxyphenylpyruvate, ethyl caffeate, rotenone and 1-methyl-1,2,5,6-tetrahydronicotinate.

In conclusion, shikimate and phenylpropionate and its derivatives, alkaloids and their metabolites, fatty acids and their metabolites were the main significant differences between HR and HC, WR and WC, and SR and SC groups. Organic acids are the most abundant class of compounds in coffee, giving coffee beans different acidity. Malic acid is a kind of aliphatic acid, which is the metabolite of coffee beans itself. The most important carboxylic acid that affects the perceived acidity, increases the vitality and hierarchy of coffee. Its high concentration will make the coffee drinks produce sour [24]. Citric acid is a tricarboxylic acid compound that not only gives acidity to food, but also provides fruit and berry flavor [25]. Batista et al. found that wet fermentation of coffee beans could significantly improve the quality, flavor, aroma and sensory [26] of coffee drinks by directly increasing the level of key precursor organic acids. Caffeine is a xanthine derivative, the most abundant alkaloid in coffee beans, and is one cause of the bitter taste of coffee [27]. Calababine is a pyridine derivative, second only to caffeine in content, is an aroma precursor, degradation products include pyrazine, furan, alkyl pyridine and pyrrole, help coffee to produce niacin in the process of roasting to form an ideal flavor characteristic [28-29]. Grease is one of the most important ingredients in roasted coffee beans, and it is considered to be an important carrier of roasted aroma and flavor, helping to increase the texture and taste of coffee by [30]. The composition of saturated fatty and unsaturated fatty acids, mainly stearic acid, palmitic acid, oleic acid, linoleic acid, linolenic acid and arachidonic acid, endows coffee with more fruity fragrance, floral and creamy [31-32]. Different primary processing methods of coffee beans will affect the change of metabolite content, and then

Figure 2 is a volcano plot showing the differential abundance of metabolites in the plasma of patients with COVID-19. The y-axis represents  $-\log_{10} P\text{-value}$  (ranging from 3 to 9), and the x-axis represents VIP (Variable Importance in Projection) (ranging from 0.0 to 1.1). A vertical dashed line at  $\text{VIP} = 0.5$  separates down-regulated metabolites (blue dots on the left) from up-regulated metabolites (red dots on the right). Several metabolites are labeled with their names and corresponding VIP values. A legend on the right indicates the status of each metabolite: blue for down-regulated, grey for not significant, and red for up-regulated. A legend at the bottom indicates the size of the dots: small for 0.0 and large for 1.1.

**Metabolites labeled in Figure 2:**

- Resolvin, D1
- (L)-cyclo-octaparglyphenylalanine
- 14-oxo-6,9-dihydro-2,5-bisoxan-8-ylpropen-2-yl acetate
- MD1279
- Neotame
- Octadecanoic acid
- Propionic acid
- 14-Ethylphenyl-1,3-benzoxazol-2-amine
- 4-Guainolamine, N (4-ethoxyphenyl)-4-(2-methylimidazol-2-yl)pyridin-3-yl
- 13-dimethyl-5,5,7a,9,14b-pentamethyl-2,3,7a,13,14,14a,14b-octahydro-4H-3-ba-apofuran[3,4-d]isopropyl-4,5-quinolin-12(1H,5H)-one
- 2-Thiazolamine, N (4-ethoxyphenyl)-4-(2-methylimidazol-2-yl)pyridin-3-yl
- MD1279
- Cryptochlorogenic acid
- Novobiose
- 9,11,18-Trihydroxy-4,16-epoxystigmar-5,8(14),15-one-7-one (Ecgonine)
- 7,8,8'-Trihydroxydihydrochalcone, 4-glucoside
- Triolein
- 14-Ethylphenyl-1,3-benzoxazol-2-amine
- Octadecanoic acid



Differential metabolite enrichment analysis, and the results are shown in Figure 5. The figure shows that the pathway enrichment analysis of all significantly different metabolites between HR and HC groups by the KEGG database, 81 differential metabolic pathways, The metabolic pathway ranked in the top 15 is shown in Figure 5A, The top 5 pathways with the most enriched differential metabolites were the metabolic pathways mainly including lauric acid, 6-hydroxyniacin, hesperetin, caproic acid and other 95 significant differential metabolites; The biosynthesis of secondary metabolites mainly includes 49 different metabolites, 2-ketovallutaric acid, stearic trienoic acid and indoppyruvate; Biosynthetic metabolic pathways of cofactors include hepinic acid, uroblack acid, isocitrate, 5, 6-dimethyl benzimidazole-and p-hydroxyphenylpyruvate were 17 significant metabolites; The biosynthetic and metabolic pathways of amino acids include phenylpyruvate, methionine, N-acetyl-L-glutamate, aspartate, citrulline and other 15 significantly different metabolites; The ABC transporters mainly include (2R, 3R, 4R)-2, 3,4, 5,6-pentahydroxyhexanaldehyde, phytate, 6-hydroxymelatonin, deoxy uridine and other 15 differentially significant metabolites.

148

include guanosine, phosphate, uridine, 6-O-  $\alpha$ -D-galactor-pyran-yl-  $\alpha$ -D-pyran glucose and other 17 different significant metabolites; The 2-oxycarboxylic acid metabolism pathway mainly included 14 different metabolites including linotonic acid, glutamate, p-hydroxyphenylpyruvate, and 3-methyl-2-oxybutyrate.

Pathway enrichment analysis was performed on the SR against all significantly different metabolites in the SC group, 83 differential metabolic pathways, Among the top 15 metabolic pathway (Figure 5C), The top 5 pathways with the most enriched differential metabolites were metabolic pathways mainly including methyl malonic acid, 6-hydroxynicotinic acid,  $\gamma$ -linolenic acid, dopamine, and pregnenolone sulfate and other 101 significant differential metabolites; The biosynthesis of secondary metabolites mainly includes linolenic acid, coutoic acid, 3-dehydroquinic acid, novobiocin and other 52 different metabolites; The biosynthetic and metabolic pathways of amino acids include tryptophan, citrulline, phenylpyruvate, 2-amino-6-oxyadipic acid, 3-methyl-2-oxybutyrate and other 16 different metabolites; Biosynthetic and metabolic pathways of cofactors include 5, 6-dimethylbenzimidazole, pantothenic acid, 4-hydroxybenzoic acid, p-hydroxyphenylpyruvate and other 16 different significant metabolites; The 2-oxycarboxylate metabolic pathway mainly includes 15 different metabolites including isocitrate, tyrosine, 3-methyl-2-oxybutyrate, 2-oxogadipic acid, and glucothioside.

Studies show that the enrichment analysis of KEGG metabolic pathways can provide a powerful reference for deeply exploring the intricate biological processes in plant metabolism. [33]. In conclusion, differential compounds are significantly enriched in metabolic pathways, secondary metabolites biosynthesis, biosynthesis of amino acids, biosynthesis of cofactors, ABC transporters, 2-oxycarboxylic acid metabolic pathway, which may be related to the continuous accumulation of metabolites in fruit ripening and growth stage and the compound changes under high temperature [34]. Research found that the largest number of differential metabolites enrichment is the metabolic pathway, followed by secondary metabolites biosynthesis pathway, possibly due to the accumulation of primary metabolites, secondary metabolites synthesis provides rich raw materials and energy, which leads to the intermediate and final products in the secondary metabolic pathway aggregation in cells, help cells play their physiological function, such as against pathogens, attract pollinators or adapt to environmental changes such as [35]. Amino acids in coffee play an important role in Maillard reaction, which is an important precursor of many aromatic compounds; Miao Yue et al. [36] By analyzing the differential metabolites of Yunnan small coffee, using seven important amino acid metabolism routes, they are the precursors of many natural products and can be used to distinguish the metabolites of different varieties of coffee.

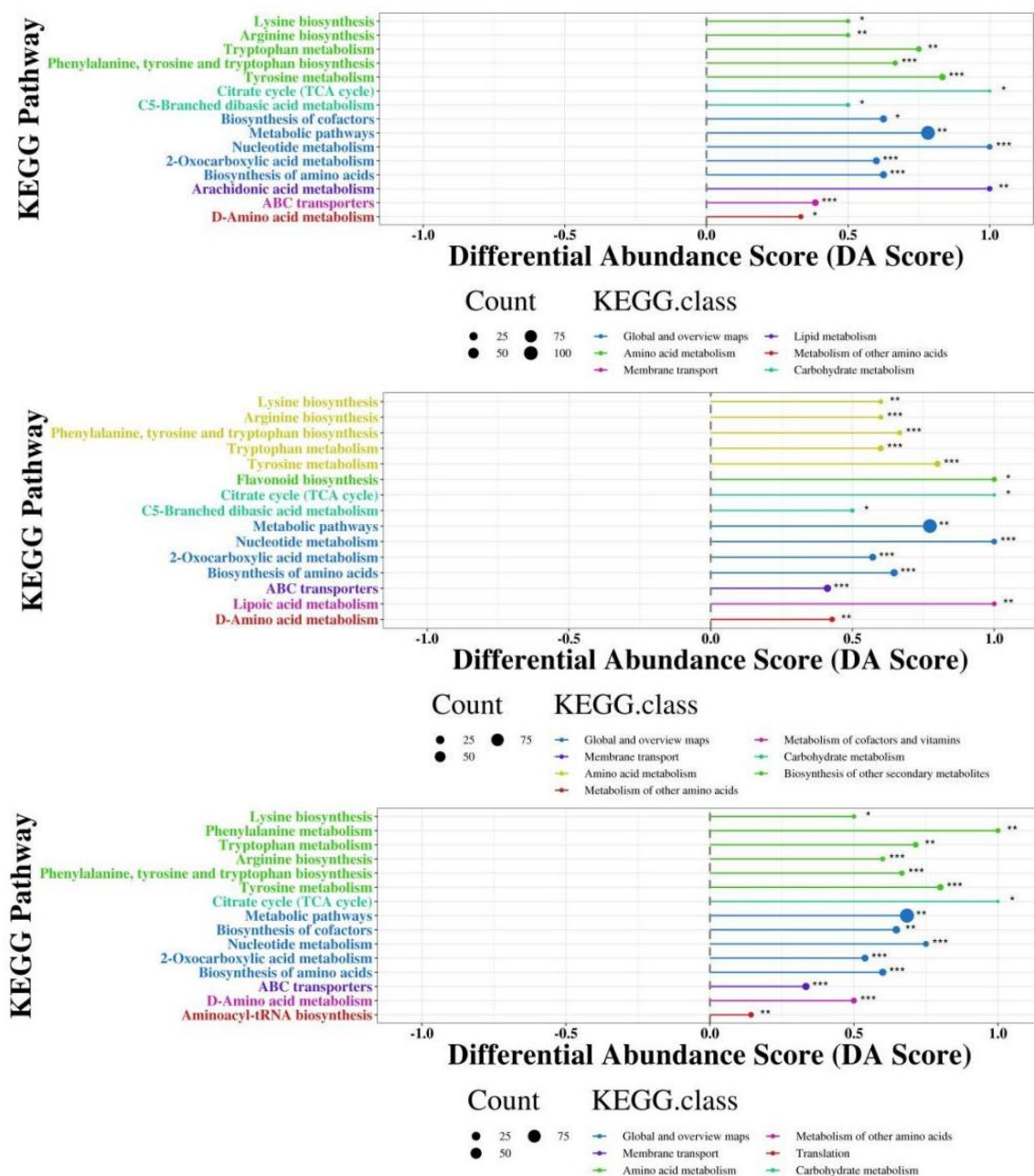


**Figure 5.** KEGG enrichment of differential metabolites

## 4. Conclusion

Through ultra-efficient liquid chromatography-quadrupole flight-time mass spectrometry technology to detect different primary processing method of coffee bean metabolites, found that the raw materials the same different primary processing way of coffee, and there are large metabolites, shikimic acid and phenylpropanic acid and its derivatives, alkaloids and their metabolites, fatty acids and their metabolites is the main significant differences in coffee samples. Kyoto encyclopedia of genes and genomes metabolic pathway enrichment analysis found that different primary processing coffee bean metabolism pathway is similar, different compounds in the metabolic pathway, secondary metabolites biosynthesis, amino acids, biosynthesis of cofactors, 2-oxygen carboxylic acid metabolic pathway, ABC transporter pathways significantly enriched ( $P < 0.05$ ). This study further excavates the main non-volatile metabolites that affect the flavor and quality of coffee with different primary processing methods, which is of great significance for the improvement of coffee flavor quality.

## Acknowledgements

Funding source of this project: Fund Project: Joint Project of Basic Research of Local Undergraduate Universities (Part) in Yunnan Province (202301BA070001-124).

## References

- [1] Aaron, P., Davis, A. P., et al. (2006). An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae). *Botanical Journal of the Linnean Society*, 152 (4), 465 - 512.
- [2] Dong, W., Hu, R., Zhao, J., et al. (2020). Dynamics of volatile compounds in raw coffee beans during accelerated storage revealed by HS-SPME-GC/MS and electronic nose analysis. *Journal of Food Science and Technology*, 57 (7), 250 - 262.
- [3] Dong, W., Hu, R., Long, Y., et al. (2019). Comparative evaluation of the volatile profiles and taste properties of roasted coffee beans as affected by drying method and detected by electronic nose, electronic tongue, and HS-SPME-GC-MS. *Food Chemistry*, 272, 723 - 731.
- [4] Wang, Y., Wang, X., Hu, G., et al. (2023). Comparative studies of fermented coffee fruits post-treatments on chemical and sensory properties of roasted beans in Yunnan, China. *Food Chemistry*, 423, 136332.
- [5] Dong, W., Tan, L., Zhao, J., et al. (2015). Characterization of fatty acid, amino acid and volatile compound compositions and bioactive components of seven coffee (*Coffea robusta*) cultivars grown in Hainan Province, China. *Molecules*, 20 (9), 16687 - 16708.
- [6] Wang, Y., Xiao, B., Tan, C., et al. (2020). Optimization of extraction technology and antioxidant activity analysis of total flavonoids from Arabica coffee pericarp. *Food Science & Nutrition*, 8 (2), 385 - 393.
- [7] Barrios-Rodriguez, Y. F., Salgado-Cervantes, M. A., et al. (2020). Effect of the postharvest processing method on the biochemical composition and sensory analysis of Arabica coffee. *Engenharia Agrícola*, 40 (2), 177 - 183.
- [8] Wu, H., Gu, J., Bekhit, A. E. D., et al. (2022). Effect of processing on bio accessibility and bioavailability of bioactive compounds in coffee beans. *Food Bioscience*, 46, 101373.
- [9] Firdaus, N. K., Aunillah, A., Pranowo, D., et al. (2023). The effect of various harvesting techniques and drying methods on the yield and quality of Liberoid coffee. *IOP Conference Series: Earth and Environmental Science*, 162 (1), 012034.
- [10] Febrianto, N. A., Sanny, O., et al. (2021). From plantation to cup: Changes in bioactive compounds during coffee processing. *Foods*, 10 (11), 2827.
- [11] Drabińska, N., Azeem, M., et al. (2023). Application of sorbent-based extraction techniques in food analysis. *Molecules*, 28 (24), 1863.
- [12] Chen, Y., Jiang, K., Hu, G., et al. (2019). Impact of primary processing methods on quality attributes of Yunnan Arabica coffee. *Journal of Food Processing and Preservation*, 43 (12), e14256.
- [13] Xiao, K., Zhai, H., Hu, R., et al. (2023). Effects of different primary processing methods on flavor compounds and sensory characteristics of coffee beans. *Food Science & Technology*, 44 (3), 1 - 16.
- [14] Velasquez, S., Banchon, C. (2023). Influence of pre-and post-harvest factors on the organoleptic and physicochemical quality of coffee: A short review. *Journal of Food Science and Technology*, 60 (10), 2526 - 2538.
- [15] Wang, N., Chen, S., Zhou, Z. (2020). Age-dependent characterization of volatile organic compounds and age discrimination in Chinese rice wine using GC/MS-based metabolomics. *Food Chemistry*, 323, 126900.
- [16] Song, X., Zhao, M., Chen, F., et al. (2020). Geographical authentication of strong aroma-type baijiu using GC×GC-TOFMS metabolomics. *Food Chemistry*, 314, 126098.
- [17] Mao, H., Yang, L., Xiao, R., et al. (2022). Metabolomic differentiation of De'ang sour tea and Pu-erh ripened tea using UPLC-QTOF-MS. *Food Research International*, 157, 111432.
- [18] Xiao, M., Peng, Z., Hardie, W. J., et al. (2022). Microbial succession and flavor formation during Sichuan Paocai fermentation revealed by multi-omics analysis. *LWT-Food Science and Technology*, 153, 112474.
- [19] Bi, X., Zhang, X., Fu, X., et al. (2023). Effects of different primary processing methods on quality characteristics of coffee cherries. *Postharvest Biology and Technology*, 195, 112158.

- [20] Hong, Z., Tan, C., Miao, Y., et al. (2020). Geographical discrimination of coffee beans using HS-GC-IMS fingerprinting. *Food Chemistry*, 342, 128348.
- [21] Ma, X., Li, X., Zou, J., et al. (2021). Metabolomic analysis of root exudates in *Salix viminalis* inoculated with *Crucibulum laeve*. *Tree Physiology*, 41 (3), 46 - 55.
- [22] Kong, Y., Chen, L., Cheng, E., et al. (2024). Non-targeted metabolomics reveals dynamic changes during Xinyang Maojian tea processing. *Food Chemistry: X*, 21, 101128.
- [23] Chen, Y., Yan, R., Lu, X., et al. (2023). Chemical variation in aged *Citrus reticulata* 'Chachi' peel revealed by widely targeted metabolomics. *Journal of Agricultural and Food Chemistry*, 71 (21), 6543 - 6552.
- [24] Wu, S., Wang, Z., Zhang, H., et al. (2018). Simultaneous determination of six organic acids in roasted coffee beans using HPLC. *Journal of Food Composition and Analysis*, 74, 112 - 115.
- [25] Li, X., Li, H., Yan, J., et al. (2024). Effects of ultra-low temperature treatment on flavor characteristics and antioxidant activity of specialty coffee. *Food Chemistry Advances*, 3, 100506.
- [26] da Mota, M. C. B., Batista, N. N., Dias, D. R., et al. (2022). Impact of microbial self-induced anaerobiosis fermentation (SIAF) on coffee quality. *Food Bioscience*, 47, 101640.
- [27] Jiang, L., Ding, Y., Jiang, F., et al. (2014). Nitrogen-doped graphene/carbon nanotube nanocomposite for enhanced sensing of caffeine and vanillin. *Analytica Chimica Acta*, 833, 22 - 28.
- [28] Chen, Q., Dong, W., Wei, C., et al. (2020). Integrated ultrasonic-microwave assisted extraction of green coffee oil. *Industrial Crops and Products*, 151, 112405.
- [29] Cordoba, N., Fernandez-Alduenda, M., Moreno, F. L., et al. (2020). Coffee extraction: A review of parameters influencing physicochemical characteristics and flavor. *Trends in Food Science & Technology*, 96, 45 - 60.
- [30] Dong, C., Dong, W., Cheng, J., et al. (2022). Evolution of fatty acids, volatile compounds and active components during coffee roasting. *Food Chemistry*, 397, 133755.
- [31] Lyu, W., Liu, Y., Yang, K., et al. (2015). Formation mechanisms of key flavor compounds during coffee roasting. *Food Chemistry*, 187, 394 - 400.
- [32] Ren, H., Zhou, B. (2018). Changes in volatile compounds and antioxidant activity during coffee roasting. *Journal of Food Biochemistry*, 42 (6), e12690.
- [33] Li, X., Pei, G., Liu, L., et al. (2017). Metabolomic analysis of lipid accumulation in glucose-tolerant *Cryptocodium cohnii*. *Bioresource Technology*, 235, 87 - 95.
- [34] Wu, Y., Zhang, C., Yang, H., et al. (2022). Metabolomic differentiation between *Rubus* spp. and *Morus* spp. fruits. *Journal of Agricultural and Food Chemistry*, 70 (30), 790 - 797.
- [35] Fu, A., Wang, Q., Mu, J., et al. (2021). Multi-omics analysis reveals chayote evolution and fruit development. *Horticulture Research*, 8 (1), 35.
- [36] Miao, Y., Tan, C., Peng, C., et al. (2022). Characterization of Arabica coffee beans using UHPLC-QE-MS metabolomics. *Journal of Food Science*, 87 (11), 355 - 367.