Metabolomics Study of Browning Mechanism in Eggplant Fruits

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Research note

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Abstract

Objective Fresh-cut fruits and vegetables is an emerging type of fruits and vegetables processing products for consumers to eat immediately or for the catering industry. Enzymatic browning is one of the crucial problems compromising the flavor and texture of fresh-cut fruit and vegetables. Eggplant is a common vegetable, which is favored by consumers. Accordingly, we used an untargeted metabolomics approach based on liquid chromatography-mass spectrometry (LC-MS) to explore the browning mechanism in peeled eggplant (Solanum melongena L .).

Results: Metabolomics revealed several hundred differential metabolites, including lipids, phenols, sugars and fatty acids. The content of these metabolites changed dynamically as the peeled time increased. The content of polyphenols, especially chlorogenic acid, increased significantly, suggesting that the main substrate for enzymatic browning in eggplant is chlorogenic acid. Furthermore, all the differential metabolite were mapped to KEGG pathway, revealing significant differences in linoleic acid metabolism, tyrosine metabolism glutathione metabolism, pentose phosphate pathway, tropane, piperidine and pyridine alkaloid biosynthesis, phenylpropanol metabolism and glycosylphosphatidylinositol(GPI)-anchor biosynthesis over time. Therefore, we speculate that some metabolic pathways that are closely connected with respiration, glycolysis, ATP synthesis, and phenolic synthesi are disturbed after peeling, under the action of enzymes, eventually leading to browning.

Introduction

Metabonomics was first proposed by Nicholson et al.[1] in 1999 and Fiehn [2] proposed in 2002 that metabolomics is a qualitative and quantitative analysis method for total metabolites in the body of an organism. With the deepening of plant metabolomics research, it has become a forceful tool for probing physiology and biology all aspects of plant [3]. In the study of metabolomics of sorghum root under nitrogen stress demonstrated the biosynthetic capacity of salicylic acid was impaired [4]. In the experiment of different germination capacity of pollen grains of two clones of Chinese fir (Cunninghamia lanceolata (Lamb) Hook) using metabolomics method, it was revealed that in the expression of metabolites across and between clones at all germination stages had significant differences, enriched in 14 metabolic pathways, including flavonoid and flavonol biosynthetic pathways, protein biosynthetic pathways and glycerophospholipid metabolism [5]. In order to explore the mechanism of climate and development regulating the metabolism and antioxidant system of date palm leaves, Du et al. [6] used the non-targeted metabolomics method based on LC-MS to find that young leaves may be more responsive to the environmental change.

Solanum melongena L., commonly known as eggplant, belongs to solanaceae plants, is one of the largest genus of solanaceae plants. It is a common vegetable, rich in polyphenols, dietary fiber, vitamins and other nutrients, and provides a variety of health benefits such as lowering blood lipids, protecting the liver and as an antioxidant, so it is favored by consumers [7]. However, browning of eggplant occurs easily after peeling, which affects its sensory quality and nutritional value, leading to a decline in edibility and commodity. Browning is caused by the destruction of cell structure during the peeling process, resulting in the release of PPO and phenolic substrates. With the occurrence of enzymatic reactions, phenols are catalyzed into quinones and finally polymerizated into brown or black substances. These substances accumulate on the surface of the slices, compromising the flavor of fresh-cut fruits and vegetables [8, 9].

In this study, three groups eggplant samples were selected for metabolic analysis. Eight biological replicates were made for each group. An untargeted metabolomics method based on LC-MS technology combined with qualitative analysis, multivariate statistical analysis and metabolic pathway analysis in differential metabolites was used to explore the browning mechanism in eggplant, so as to offer a feasible theoretical foundation for the processing and production of

fresh-cut fruit and vegetable, and provide a reference for further investigation into the restrain of browning in other fresh-cut products.

Materials And Methods

Plant material

Eggplant cultivar ‘Huqie 5’ is a breeding line produced by our lab at the Shanghai Academy of Agricultural Sciences, Shanghai, China. Pulp of peeled eggplant after 0, 3 and 5 min was taken as experimental materials.

LC-MS analysis

The analytical instrument of this experiment is The UHPLC system that is composed of liquid-mass coupling system consisted of AB Sciex Triple TOF 5600 high resolution mass spectrometer. It was appiled to analyze metabolic profiles in both TIC + and TIC- modes.

Data preprocessing and statistical analyses

The original data were disposed using progenesis QI made in Waters Corporation from Milford of USA. Then, we constructed a data matrix that was inputed into XCMS software (version 14.0, Umetrics, Umeå, Sweden), in which PCA, PLS-DA and OPLS-DA were executed.

Identification of differential metabolites

The difference metabolites between groups were screened out using the methods of multi-dimensional analysis and one-dimensional analysis. Metabolites were described using progenesis QI (Waters Corporation, Milford, USA) metabolic data software based on public databases (http://www.HMDB.ca/; http://www.lipidmaps.org/) and a self-built database.

KEGG pathway analysis

These differential metabolites were mapped onto the KEGG database, and metabolic pathways were analyzed based on KEGG (http://www.genome.jp/KEGG/pathway.html) pathway analysis.

Results

Multivariate analysis

The data were disposed by multivariate analysis tools (PCA, PLS-DA and OPLS-DA). PCA, PLS-DA and OPLS-DA scoring plots and validation plots of the OPLS-DA models were built for the three contrastive groups: 3 min/CK, 5 min/CK and 3 min/5 min (Fig. 1). All the parameters of these models showed in Additional file 1. R2X represents the cumulative interpretation rate of the multivariate statistical analysis modeling, which generally requires R2X > 0.4, indicating that the model is credible. R2 and Q2 are the parameters of response sequencing test, used to measure whether the model is overfitted. External validation generally requires Q2 < 0 to avoid over-fitting. Internal validation generally requires R2 > 0.5; The closer R2 is to 1, the better the model. In our results, all values for Hotelling’s T2 were 95%, Q2 were < 0 (Q2 = − 0.849 to − 0.741), R2X were > 0.4, and R2 were > 0.5, indicating that the model is reliable.

Differential metabolites

According to the VIP > 1 for the first principal component in the OPLS-DA, and p-value < 0.05 were the criteria for screening differential metabolites, we identified the differential metabolites among the three contrastive groups [see Additional file 2]. The 119 common differential metabolites from the 3 min/CK and 5 min/CK groups were further divided into three sorts represented by A, B and C, as indicated in Additional File 3 based on STC. Furthermore, the metabolites in these three categories were grouped into eight types of compounds (Table 1). Category A included 68 metabolites. These were mainly lipids, fatty acid and carbohydrates. The contents and expression levels of these metabolites are increased gradually. The metabolites of category B comprised 40 compounds, most of which were fatty acids and lipids. The contents and expression levels of these metabolites decreased with increase of the peeled time. There were 11 components in category C, mainly lipids. Interestingly, their contents and expression levels decreased at 3 min, but are increased at 5 min. Furthermore, the contents of chlorogenic acid that associated with browning increased significantly with longer time from peeling. The changes in the contents of these differential metabolites were similar to the expression level changes shown in the heatmap [see Additional file 4].

KEGG pathway analysis

The metabolites in categories A, B and C were mapped, respectively, using the KEGG database onto the KEGG pathways with the following results (Fig. 2).

The metabolites of category A were mainly enriched in 18 metabolic pathways: Linoleic acid metabolism, Pentose phosphate pathway, Nitrogen metabolism, Glycosylphosphatidylinositol (GPI)-anchor biosynthesis, Autophagy-other, Taurine and hypotaurine metabolism, Galactose metabolism, Aminoacyl-tRNA biosynthesis, Arginine biosynthesis, Arginine and proline metabolism, Alanine, aspartate and glutamate metabolism, C5-Branched dibasic acid metabolism, Glutathione metabolism, Butanoate metabolism, Histidine metabolism, ABC transporters, Glyoxylate and dicarboxylate metabolism, and Glycerophospholipid metabolism. Of these, Glycosylphosphatidylinositol (GPI)-anchor biosynthesis, Linoleic acid metabolism, Autophagy-other and Nitrogen metabolism showed extremely significant differences in the 3 min/CK and 5 min/CK two comparative groups at the $p < 0.01$ level of significance, while the others revealed a significant difference ($p < 0.05$). There was a remarkable difference ($p < 0.05$) in the metabolites of category B that were only enriched in one metabolic pathway, Tropane, piperidine and pyridine alkaloid biosynthesis. Finally, the metabolites of category C were enriched in Tyrosine metabolism and had a remarkable difference ($p < 0.05$) similarly.

Discussion

Enzymatic browning of fruits is an extremely complex process. Phenols, as important substrates of enzymes, play a key role in the browning that occurred during the fruits and vegetables deeply processing, process of fruits and vegetables [10]. Fruit tissue structure and regionalization of the cell space are damaged by fresh-cut operations, destroying partitions between PPO and substrate polyphenols and allowing contact between enzymes and substrates for catalysis by PPO of the transformation of contact the transformation of, phenolic compounds into quinone compounds [11, 12]. Melanins are accumulated through secondary enzyme reactions, inducing the enzymatic browning reaction [13–16]. Liu et al. [17] studied the reaction mechanism of enzymatic browning during potato processing, and analyzed the correlation between substrates and browning. Their results showed that phenolic compounds are closely related to browning, and chlorogenic acid is an important substrate leading to potato enzymatic browning. The phenolic compounds identified in banana peels in recent years were mainly dopamine, with dopaminerase oxidation being the main factor for banana browning [18]. Chlorogenic acid and epicatechin are the phenolic compounds with the highest contents in apple fruits [19], while the substrates in mango are mainly coffeic acid and ferulic acid [20]. Mishra et al. [21] also showed that browning of eggplant was related to the content increased of polyphenols. In this study, the content of chlorogenic acid accumulated at an extremely significant level ($p = 0.0025$), but the content of flavonoids

such as soybean vanillin did not change significantly, suggesting that the main substrate of enzymatic browning in eggplant is chlorogenic acid.

Phenylalanine ammonialyase (PAL) plays a momentous role in regulating the production of phenolic substances in the phenylalanine pathway and is an important enzyme regulating browning [22]. In this study, we detected the phenylpropanol metabolism pathway in the 3 min/CK comparison, with the accumulation of L-tryptophan and chlorogenic acid up-regulated. The biosynthesis pathway for flavonoids was found in the 3 min/CK and 5 min/CK comparisons, where the participation of quercetin was down-regulated. Mechanical injury by peeling can significantly increase PAL activity, and phenols are changed by the phenylpropanol metabolism pathway and flavonoids biosynthesis pathway. At the same time, the membrane system is destructed, and the regional distribution of enzymes and substrates is disrupted, allowing phenols to be catalyzed by PPO under action of reactive oxygen species (ROS), eventually generating brown substances. In addation, mechanical damage when fruits and vegetables are cut, destroys the plant ROS metabolism balance, a large increase of ROS may be lead to the cell membrane lipid peroxidation and destroy the integrity of the cell, which caused the cell aging and death [23–25]. In this study, glutathione metabolism was found in all three groups, and the content of L-glutamate involved in this pathway increased, while the content of threonine decreased. Therefore, we hypothesized that glutathione in fruits and vegetables is oxidized through glutathione metabolism after eggplant is peeled, and the content of L-glutamic acid is increased while the content of threonine is decreased, which accelerating aging and affecting the flavor and nutrition of fruits and vegetables.

Furthermore, the analysis of metabolites gave a comprehensive overview of the active pathways after eggplant is peeled. Glycosylphosphatidyl-inositol anchored protein(GPI), is an integral protein involved in the endoplasmic reticulum and plasma membranes on GPI [26, 27]. Like all fatty acids, Linoleic acid can be perceived as a material source of cellular energy [28, 29]. Moreover, when it is released from membrane phospholipids, can be enzymatically oxidized into all kinds of derivatives participate in cell metabolism and signal transduction [30]. The pentose phosphate pathway provides substrates for essential biosynthetic pathways [31]. In this study, linoleic acid metabolism, the glycosylphosphati-dylinositol (GPI)-anchor, and pentose phosphate pathways at the three different treatment stages after peeling were significantly different. Therefore, we hypothesize that these metabolic pathways may play an important role in the browning of fruits and vegetables.

Conclusion

The present investigation clearly demonstrated that metabolomics analysis is a good tool for explaining the browning mechanism of eggplant by detecting changes in the expression of metabolites and differences in metabolic pathways after peeling. Several hundred differential metabolites were identified in eggplant for at 3 min and 5 min after peeling, including lipids, phenols, sugars and fatty acids etc. The metabolite contents changed dynamically as the peeled time increased. Furthermore, by analyzing the KEGG pathway of these differential metabolites, we found that some metabolic pathways were perturbed after peeling, such as glutathione metabolism, pentose phosphate pathway, phenylpropanol metabolism and tyrosine metabolism.The identified metabolites and metabolic pathways may have specific effects on various aspects of fruit and vegetable browning. We established an untargeted metabolomics method based on LC-MS technology to explain the mechanism of eggplant browning, which provides new ideas and perspectives for understanding fruit browning in the future.

Limitations

The study established an untargeted metabolomics method based on LC-MS technology to explain the mechanism of eggplant browning, which may lay the foundation for better understanding the mechanism of browning during the fruits

and vegetables deeply processing. However, the specific molecular mechanism of Eggplant browning remains to be further studied.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The data charts supporting the results and conclusions are included in the article and additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors Contributions

DZ and XW put forward and designed the research, ZZ and AZ carried out the preparation of the experiment. SZ and JS performed the treatment of test materials. XL analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Abbreviations

LC-MS: Liquid Chromatography-Mass Spectrometry

TIC+: The positive Ion Current

TIC-: The negative Ion Current

PCA: Principal Component Analysis

PLS-DA: Partial Least Squares Discriminant Analysis

OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis

VIP: Variable Importance in the Projection

PPO: Polyphenol Oxidase

PAL: Phenylalnine Ammonialyase

RPT: Response Permutation Tests

KEGG: Kyoto Encyclopedia of Genes and Genomes

HMDB: Human Metabolome Database

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Table

	Benzenoids	Fatty acids	lipids	Organic acids	Organoheterocyclic compounds	Carbohydrates	Amines	Hydrocarbons
A	1	13	22	6	9	17	0	0
B	3	14	13	3	4	1	1	1
C	1	1	7	2	0	0	0	0

Table 1 Classification of metabolites

Figures

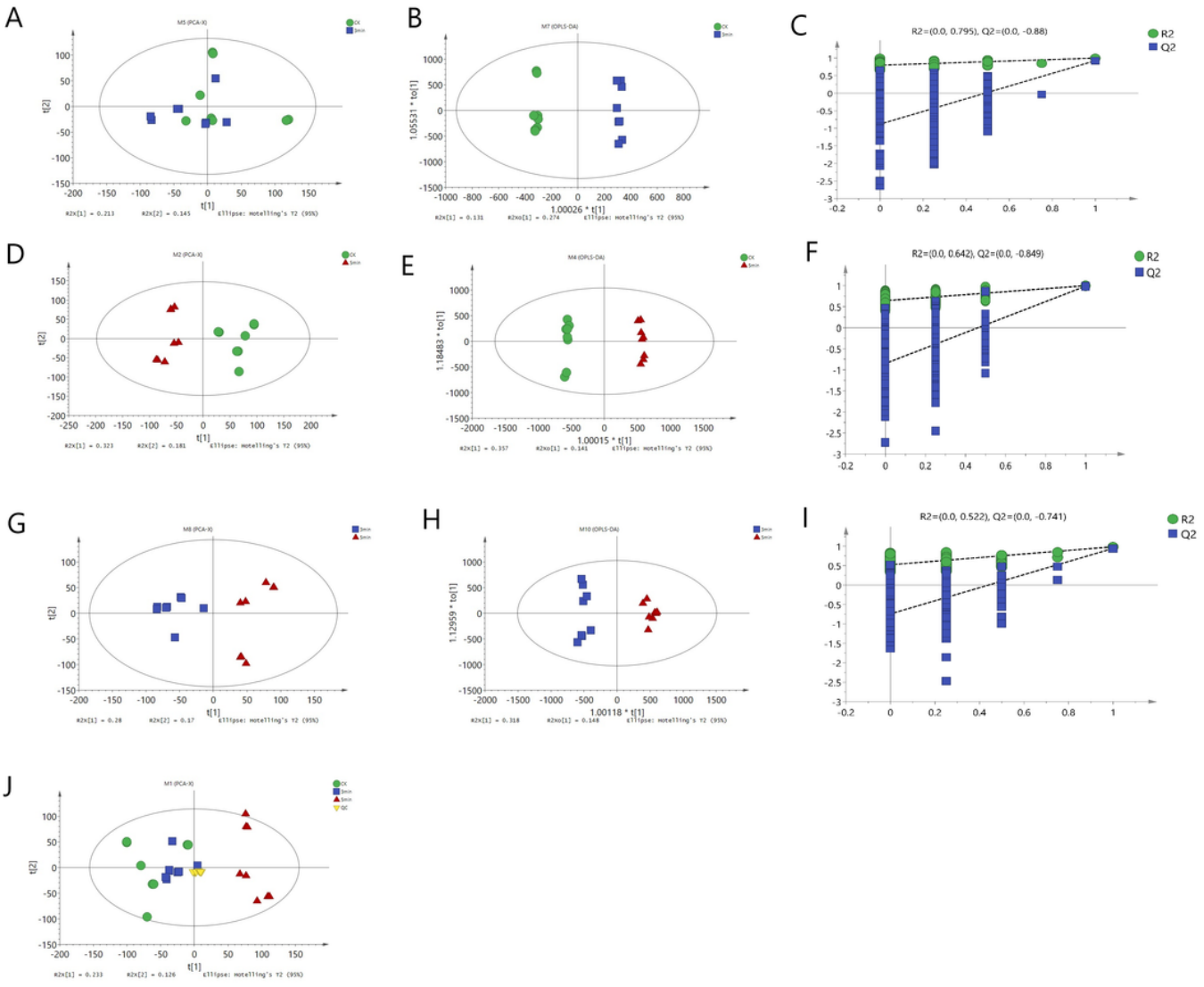

Figure 1

Scores of PCA, PLS-DA, OPLS-DA and validation plots of OPLS-DA for 3min/CK、5min/CK and 3min/5min. (a) PLS-DA for 3min/CK. (b) OPLS-DA for 3min/CK. (c) validation plots of OPLS-DA for 3min/CK. (d) PLS-DA for 5min/CK. (e) OPLS-DA for 5min/CK. (f) Validation plots of OPLS-DA for 5min/CK. (g) PLS-DA for 3min/5min. (h) OPLS-DA for 3min/5min. (i) Validation plots of OPLS-DA for 3min/5min. (j) Score scatter plots of PCA for all comparative groups

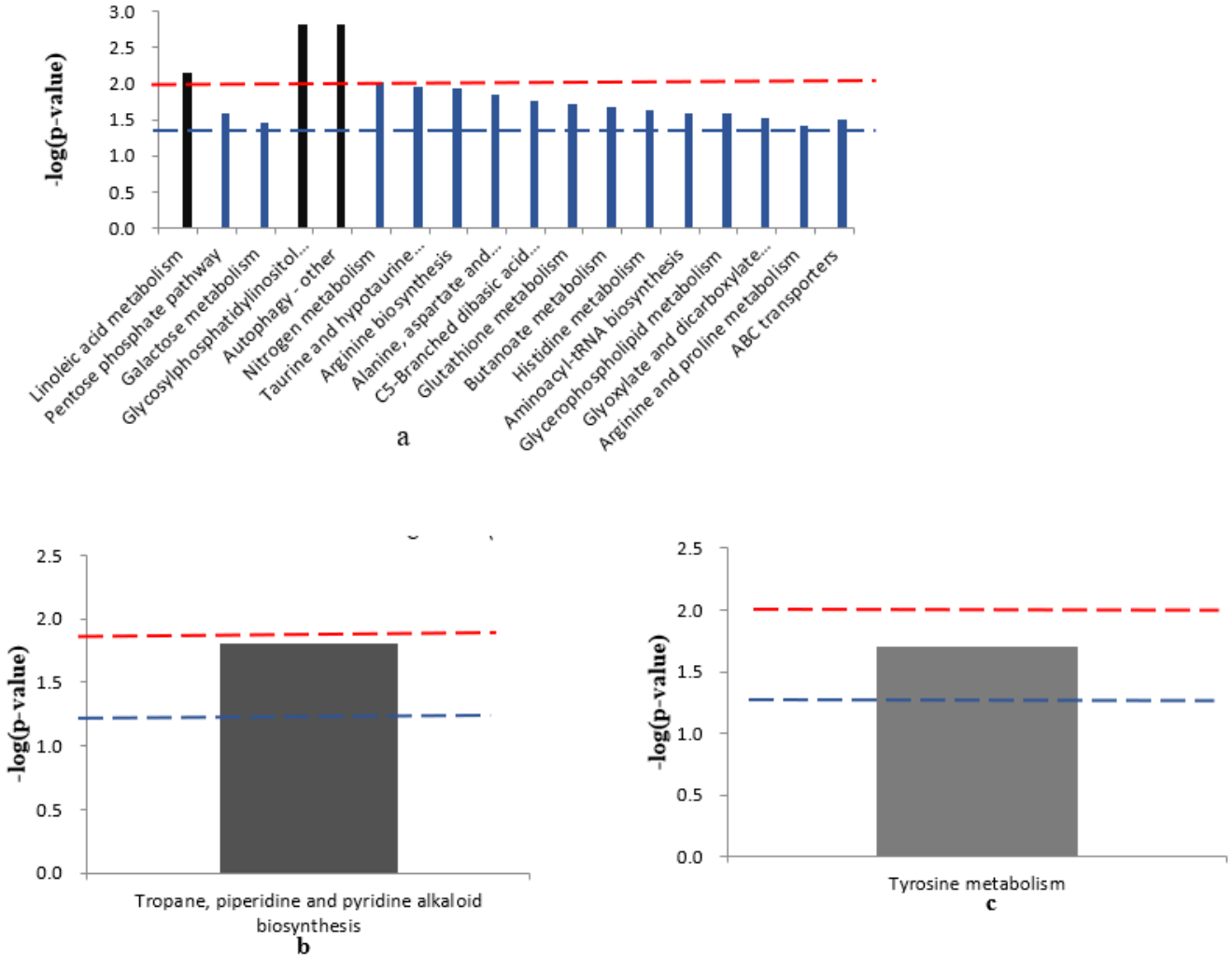

Figure 2

The differential metabolic pathways (a) The differential metabolic pathways that be enriched by the metabolites of category (A). (b) The differential metabolic pathway that be enriched by the metabolites of category (B). (c) The differential metabolic pathway that be enriched by the metabolites of category (C). The red line indicates that the p-value=0.01, and the blue line indicates that the p-value=0.05. When the top of a column is higher than the blue or red line, the metabolic pathway shows significant differences.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile4Heatmap.docx
- Additionalfile3Differentialmetabolitesclassification.xlsx
- Additionalfile2Thedifferentialmetabolites.xlsx

- Additionalfile1Parametersfortheassessmentofmodels.docx