Metabolites comparison in post-fermentation stage of manual (mechanized) Chinese Huangjiu (yellow rice wine) based on GC–MS metabolomics

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ABSTRACT
In order to understand the differences of metabolites and their key metabolic pathways between traditional manual and mechanized Huangjiu, gas chromatography-mass spectrometry (GC–MS) combined with non targeted metabolomics was used to track and monitor Huangjiu in the whole post-fermentation stage. The results showed that 25 metabolites and 14 metabolites were identified as differential metabolites in manual and mechanized Huangjiu, respectively (VIP > 1, P < 0.05); three metabolic pathways had significant effects on differential metabolites (−log (P) > 1, impact > 0.01). Compared with the two kinds of Huangjiu, 21 kinds of metabolites were identified as differential metabolites (VIP > 1, P < 0.05); four metabolic pathways had significant effects on differential metabolites (−log (P) > 1, impact > 0.01). This study is helpful to gain insight into the underlying mechanism of flavor formation during the post-fermentation process of Huangjiu and provide a theoretical basis for the industrial development.

1. Introduction

Chinese Huangjiu (yellow rice wine), a traditional Chinese alcohol beverage, is popular among Chinese customers. Huangjiu is honored as national banquet alcohol beverage due to a large number of flavor substances. The brewing technology of Huangjiu is divided into manual Huangjiu and mechanized Huangjiu (Fig. S1). The mechanized Huangjiu is fermented in a large stainless-steel tank with yeast and wheat starter as saccharifying and fermenting agents, and the fermentation cycle is generally 24 days. The distiller’s yeast is cultivated by pure breed, and the wheat Qu is mixed by natural culture and pure breed. Traditional manual Huangjiu uses naturally cultivated wheat Qu and yeast as saccharifying and fermenting agents, and the fermentation cycle is generally 72 days. In the first fermentation stage, pottery jar is used as fermentation container, and in the second fermentation stage (post-fermentation), pottery jar is used as fermentation container (Xie et al., 2021). The fermentation of Huangjiu requires a post-fermentation process with a long time at low temperature. In the post-fermentation stage, besides the further action of yeast to produce ethanol, the main purpose is to produce the special flavor substances of Huangjiu and make the flavor of Huangjiu more harmonious. Therefore, it is of great significance to deeply understand differences in metabolites and metabolic pathways in post-fermentation stage between traditional manual and mechanized Huangjiu for improving the brewing technology. As an important part of systems biology, metabolomics, which is able to detect dozens or even hundreds of endogenous metabolites, has been widely applied in food research (Aihua et al., 2011; Pang et al., 2019; Saigusa et al., 2020; Wishart et al., 2011). Analytical techniques used in metabolomics mainly include nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (HPLC-MS) (Pang et al., 2019). Compared with other methods, GC–MS has the advantages of low cost, good repeatability, high resolution and small matrix effect (Aihua et al., 2011; Saigusa et al., 2020; Wishart et al., 2011), and it is still the main analysis method of metabolomics. Such as Jang et al.(2014), the metabolites of seven industrial vinegar products and two traditional vinegar products were analyzed by gas chromatography time of flight mass spectrometry (GC-TOF-MS). Chuangye et al. (2018) analyzed the

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metabolites, and used stoichiometry combined with the analysis technology of volatile components of liquor products; and used stoichiometry combined with the electronic nose technology to identify the geographical origin of camellia oil (Peng et al., 2020).

However, the current reports on Chinese Huangjiu mainly focus on the changes of flavor substances during fermentation. There are few studies on the differences, dynamic changes and key metabolic pathways of metabolites between traditional manual and mechanized Chinese Huangjiu. To analyze the differences that existed between traditional manual Huangjiu and mechanized Huangjiu, the key differential volatile substances and key differential metabolic pathways were identified. How to determine key differential volatile compounds and key differential metabolic pathways to analyze the differences between traditional manual and mechanized Huangjiu?

In this study, GC–MS combined with chemometric analysis was used to explore the key metabolic pathways of the differential metabolites between the two kinds of Huangjiu in the post-fermentation process, which would help to gain insight into the underlying mechanism of flavor formation and provide a theoretical basis for the improvement of quality during the industrial development of Huangjiu.

2. Materials and methods

2.1. Samples

The manual Huangjiu was obtained from Gu Yue Long Shan, Zhejiang Province, and the sampling time points were days 2, 4, 6, 16, 26, 36, 54, 72. One batch was taken for each process, and 250 mL was taken after mixing the bottles and recorded once.

The mechanized Huangjiu was obtained from Gu Yue Long Shan in Zhejiang Province, the sampling time points were days 1, 2, 3, 4, 13, 21. One batch was taken for each process, and 250 mL was collected after mixing in bottles and recorded once.

All samples were aseptically sealed and put into –80°C ultra low temperature freezer for further use.

2.2. Samples preparation

The 100 μL manual Huangjiu sample was taken in a 1.5 mL EP tube, and then added 300 μL of extraction solution (methanol) followed by addition of 5 μL ribitol, vortexed for 30 s; sonication for 10 min (ice water bath); samples were centrifuged at 12,000 r/min for 15 min at 4°C; the supernatant 30 μL was pipetted into a 1.5 mL EP tube, and 10 μL of each sample was mixed to form a QC sample; the extract was dried in a vacuum concentrator; to the dried metabolites, 100 μL methoxyamine salt reagent (methoxyamine hydrochloride dissolved in pyridine 20 mg/mL), mixed gently, and placed in the oven for 30 min at 80°C; methoxamine salt reagent 100 μL (methoxamine hydrochloride was dissolved in pyridine 20 mg/mL) was added to the dried metabolites, mixed gently, and incubated at 80°C for 30 min in the oven. To each sample, 100 μL bis (trimethylsilyl) trifluoroacetamide, incubating the mixture at 70°C for 1.5 h; the 100 μL bis (trimethylsilyl) trifluoroacetamide was added to each sample, and incubated the mixture at 70°C for 1.5 h. The samples were cooled to room temperature, and 5 μL of saturated fatty acid methyl ester (dissolved in chloroform) was added to the mixed samples. Samples were randomly and sequentially tested on the machine. This method was improved on the basis of Kanani et al. (2008) and Zeki et al. (2020).

2.3. GC–MS analysis

The extraction head was removed from the sample bottle and quickly inserted into the gas chromatograph inlet (240°C, 5 min) to start GC/MS detection and analysis (Li et al., 2008). Gas Chromatography (Azcuingilpica et al., 2008) conditions: The carrier gas is high-purity helium (flow rate 1 mL/min); the temperature of the injection port was set at 40°C for 5 min, then increased to 120°C at 5°C/min and 240°C at 10°C/min (Xian et al., 2019), and then its operating temperature is 240°C; connecting rod 150°C; sample injection in splitless mode. MS conditions: ion source (EI) temperature 230°C; EI ionization source 70 eV, quadrupole temperature 150°C, mass scanning range m/z 35–450; transmission line temperature 250°C (Chen et al., 2019).

2.4. Bioinformatic analysis of the untargeted metabolomics dataset

Mass spectral data of both liquor samples were analyzed using chromato software (V 4.3|xleco) for peak extraction, baseline correction, deconvolution, peak integration, peak alignment, and so on. For the qualitative work on substances, the LECO fhnx database was used, including mass spectral matches and retention time index matches. Peaks with a call rate below 50% or a relative standard deviation greater than 30% in QC samples were finally removed. Peaks below 50% call rate or greater than 30% relative standard deviation in QC samples were removed after filling.

2.5. Statistical analysis

SPSS 25 was used to analyze variance of fermentation process data of manual and mechanized Huangjiu samples (n = 20), and only metabolites with P value less than 0.05 were defined as significant differences. SIMCA 14.1 software was used for data conversion and centralized formatting, and then modeling and analyzing the data of two kinds of Huangjiu samples (Fig. 3).

2.6. Stoichiometry analysis

The measured data (peak values, sample names and relative substance contents are included) were subjected to principal components analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). The quality of the constructed PCA was assessed by R²X and Q² and the quality of the constructed OPLS-DA model was assessed by R²X, R²Y and Q². The metabolites with VIP value greater than 1 were selected as candidate differential metabolites by OPLS-DA model. The relative contents of candidate differential metabolites were subjected to t-test, and only metabolites with a p-value less than 0.05 were defined as differential metabolites.

To further elucidate the biological significance of the differential metabolites, the differential metabolites were imported into metaboanalyst 5.0 (https://www.metaboanalyst.ca) Middle, the key metabolic pathways were screened based on log (P) > 1 and impact > 0.01 (Pang et al., 2021).

3. Results and discussion

3.1. Analysis of the difference of two kinds of Huangjiu

3.1.1. Basic physicochemical characteristics

The dynamic changes of metabolite differences between traditional manual and mechanized Huangjiu during the post-fermentation stages are shown in Fig. 1. The variation trend of some metabolites in manual and mechanized Huangjiu was similar in post-fermentation. The metabolite differences between manual and mechanized Huangjiu in the post-fermentation stages were indicated by the significant differences in the contents of esters. A total of 26 esters were detected in manual Huangjiu, and 9 esters were detected in mechanized Huangjiu. This may
be one of the important reasons why mechanized Huangjiu mouthfeel lacks “flavor” compared with manual Huangjiu.

The contents of esters in the two kinds of Huangjiu were significantly different, which was mainly related to the different types and dosage of wheat Qu. By optimizing the wheat Qu formula, Zhang et al. (2016) significantly improved the content of esters in mechanized Huangjiu, thus improving the flavor quality of Huangjiu, and solved the bitter taste problem commonly reported in mechanized Huangjiu. The species and abundance of microorganisms in different fermented mash were significantly different due to the different types and dosage of wheat Qu. The preliminary study of our laboratory found that there were more bacterial species in well-fermented manual Huangjiu, while the bacterial species in mechanized Huangjiu were relatively few (Xie et al., 2021), and the quality of fermented mash was directly related to the content of Lactobacillus.

3.1.2. The difference of acids in two kinds of Huangjiu

At the post-fermentation stage, the content changes of organic acids in the two kinds of Huangjiu are shown in the Fig. 2. The content of tartaric acid was significantly different (P < 0.05), and the content of artificial yellow rice wine was higher. Since the solubility of tartaric acid in ethanol is less than that in water, and ethanol promotes the decomposition of tartaric acid, the rapid accumulation of ethanol content in mechanized rice wine at the early stage of fermentation accelerates the loss of tartaric acid (Amerine et al., 1980). Citric acid was more abundant in the fermentation after manual Huangjiu, and the fermentation ability of the naturally cultured yeast used in manual Huangjiu was stronger than that of the pure cultivated yeast in mechanized Huangjiu. The content of citric acid was higher in the post-fermentation of manual Huangjiu (Mao, 2008; Mao, 2004; Wang et al., 2012). Manual Huangjiu reached 93.216 (±14.8) on 72 days, while mechanized Huangjiu reached 91.4 (±16.6) on 21 days (Fig. 2 c.). A. G. Reynolds studies have shown that different yeasts vary in their ability to produce citric acid in the fermentation of wine (Reynolds et al., 2001).

3.1.3. The difference of amino acids in two kinds of Huangjiu

Amino acids are important flavor composition components in Huangjiu (Burin et al., 2015). The contents of amino acid nitrogen were increased slowly with the passage of fermentation time during the post fermentation of manual and mechanized Huangjiu (Fig. S2). The gradual death of yeast cells provides a steady source of feed for proteolysis to

Fig. 1. a. Changes of physical and chemical indicators in each stage of manual Huangjiu fermentation; b. Changes of physical and chemical indexes in each stage of mechanized Huangjiu fermentation; c. Content difference of Alcohols in two kinds of Huangjiu (1-manual Huangjiu 2d; 2-manual Huangjiu 4d; 3-manual Huangjiu 16d; 4-manual Huangjiu 16d; 5-mechanized Huangjiu 2d; 6-mechanized Huangjiu 4d; 7-mechanized Huangjiu 14d; 8-mechanized Huangjiu 24d).
amino acids (Lysine, arginine, serine, α-alanine, glutamic acid, etc.). The process produced contains long-chain fatty acids (C14-C1 ester 8), terpene alcohol (linalool-musk), isoamino alcohol (benzene 2-ethanol rose smell), aldehyde (methyl 3-butanol-grass smell), Lactone (α-delactone-peach, coconut) (Leskó et al., 2011).

The content of amino acids in the two kinds of Huangjiu is significantly different (P < 0.05), especially the amino acids with umami taste (Serine, glutamic acid, proline, valine, isoleucine, leucine, tyrosine, alanine, lysine, histidine, arginine), as shown in Fig. S2.

3.2. Chemometric analysis of two kinds of Huangjiu

3.2.1. PCA topographic plots of two kinds of Huangjiu

SIMCA 14.1 software was used for data conversion and centralized formatting, and then modeling and analyzing the data of two kinds of Huangjiu samples (Fig. 3). Q^2 > 0.5 indicated good fitting degree of PCA model. Q^2 < 0.5, indicating poor fitting degree of PCA model. The manual Huangjiu sample model showed that the values of R^2X and Q^2 were 0.928, 0.958, respectively (Fig. 3 a); the mechanized Huangjiu sample model showed that the values of R^2X and Q^2 were 0.947, 0.916, respectively (Fig. 3 b); the models of all Huangjiu samples at the start and end of post-fermentation stage showed that the values of R^2X and Q^2 were 0.975, and 0.961, respectively (Fig. 3 c).

PCA analysis based on the contribution of metabolites showed that the metabolites of the both Huangjiu were significantly different (Fig. 3 a), the internal metabolites of the mechanized Huangjiu were quite different (Fig. 3 b), but the difference was smaller than that of the manual Huangjiu (Fig. 3 c).

PCA analysis based on metabolite contribution showed that the metabolites of the two kinds of Huangjiu were significantly different (Fig. 3 a), while the internal metabolites of mechanized Huangjiu were significantly different (Fig. 3 b), but the difference was smaller than that of manual Huangjiu (Fig. 3 c).

3.2.2. Metabolite difference analysis of two kinds of Huangjiu

The OPLS-DA model was used to analyze the metabolite data in Huangjiu samples (Fig. S3). Q^2 > 0.5 indicated good fitting degree of OPLS-DA model. Q^2 < 0.5, indicating poor fitting degree of OPLS-DA model. The model of manual Huangjiu sample showed that the values of R^2X, R^2Y and Q^2 were 0.968, 0.949, 0.928, respectively (Fig. S3 a); the model of mechanized Huangjiu sample showed that the values of R^2X, R^2Y and Q^2 were 0.969, 0.919, 0.9 (Fig. S3 b); the models of all Huangjiu samples at the start and end of post-fermentation stage showed that the values of R^2X, R^2Y and Q^2 were 0.969, 0.735, and 0.703, respectively (Fig. S3 c). Based on the results of OPLS-DA model analysis, metabolites (VIP > 1, P < 0.05) were regarded as differential
metabolites in the post-fermentation stage. The results showed that there were 25 different metabolites (VIP > 1, P < 0.05) in the whole post-fermentation stage (prophase vs telophase) of manual Huangjiu, as shown in Table 1; there were 14 different metabolites in the whole post-fermentation stage (prophase vs telophase) of mechanized Huangjiu (VIP > 1, P < 0.05), as shown in Table 2. There were 21 different metabolites between the two kinds of Huangjiu in the whole post-fermentation stage, as shown in Table 3. The s-plots and heat maps clearly showed that there was a significant difference between the metabolites of manual and mechanized Huangjiu, and the metabolites of manual Huangjiu were significantly different in the post-fermentation stage (prophase vs telophase) (Fig. S4, Fig. 4). A total of 25 metabolites in the whole post-fermentation stage of manual Huangjiu were identified as differential metabolites (VIP > 1, P < 0.05); the metabolic pathways with significant effects on the differential metabolites (−log(P) > 1, impact > 0.01) were phenylalanine, tyrosine and tryptophan biosynthesis pathway, alanine, aspartic acid and glutamate metabolism pathway, and arginine biosynthesis pathway (Fig. 5). A total of 14 metabolites in the whole post-fermentation stage of mechanized Huangjiu were identified as differential metabolites (VIP > 1, P < 0.05); the metabolic pathways with significant effects on differential metabolites (−log(P) > 1, impact > 0.01). A total of 21 metabolites of the two kinds of Huangjiu were identified as differential metabolites during the whole post-fermentation stage (VIP > 1, P < 0.05); the metabolic pathways with significant effects on the differential metabolism content of manual Huangjiu at different post-fermentation stages (+ indicates that after the end of post-fermentation period, the substance content of manual Huangjiu increases; – indicates that the substance content of manual Huangjiu decreases after the end of post-fermentation period).

| Var ID (Var. Sec. ID:1) | M10. VIPpred | Probability Fermentation change trend |
|-------------------------|--------------|-------------------------------------|
| Butanol                 | 1.06307      | 0.000382375 | + |
| Hexanol                 | 1.14137      | 1.13519E-05 | – |
| Octanol                 | 3.86508      | 1.06451E-27 | + |
| 2,3-Butanediol          | 1.15063      | 2.11185E-11 | + |
| Propanol                | 1.14995      | 3.40687E-05 | + |
| Ethyl benzoate          | 1.07946      | 3.6587E-08  | – |
| Ethyl phenylacetate     | 1.1903       | 1.67303E-07 | + |
| Ethyl lactate           | 1.03623      | 0.000109722 | + |
| Ethyl Myristate         | 1.04684      | 0.000443796 | + |
| 2-phenethyl acetate     | 1.32564      | 4.939293E-07 | + |
| Ethyl butyrate          | 1.33323      | 1.9035E-06  | + |
| Ethyl propionate        | 1.54982      | 3.64852E-10 | – |
| Ethyl Valerate          | 2.14518      | 3.66139E-08 | + |
| Furfural                | 1.05775      | 2.18988E-07 | – |
| Nonanal                 | 2.35651      | 3.4388E-16  | – |
| Decanal                 | 2.68285      | 2.0444E-12  | – |
| Capric acid             | 2.07368      | 1.58668E-10 | – |
| 3-methylbutyric acid    | 1.50799      | 7.4952E-07  | – |
| Aspartic acid           | 1.63616      | 1.10282E-13 | + |
| Isoleucine              | 1.75483      | 2.11589E-19 | + |
| Tyrosine                | 1.40572      | 2.63338E-29 | + |
| Phenylalanine           | 1.2445       | 8.35209E-23 | + |
| Lysine                  | 1.75266      | 5.80359E-23 | + |
| Serine                  | 1.79176      | 1.11464E-18 | + |
| Glutamate               | 1.06404      | 1.3974E-24  | – |

| Var ID (Var. Sec. ID:1) | M11. VIPpred | Probability Fermentation change trend |
|-------------------------|--------------|-------------------------------------|
| 2-methyl-1-propanol     | 1.33808      | 0.00199426 | + |
| Butanol                 | 1.43532      | 5.77938E-05 | + |
| Octanol                 | 3.95865      | 4.27997E-23 | + |
| 2,3-Butanediol          | 1.23102      | 6.82137E-10 | + |
| 4-methyl-2-pentanol     | 1.10731      | 0.000565835 | – |
| Propanol                | 1.37468      | 1.90317E-06 | + |
| Methyl octadecanoate    | 2.01571      | 0.00290336 | + |
| Ethyl Myristate         | 1.68022      | 3.70571E-08 | + |
| 2-phenethyl acetate     | 1.58522      | 4.09498E-06 | + |
| Benzaldehyde            | 1.17072      | 0.00719877 | – |
| Nonanal                 | 2.32914      | 4.07877E-09 | + |
| Decanal                 | 2.38281      | 3.4179E-08  | + |
| Capric acid             | 1.82434      | 1.33252E-05 | + |
| 3-methylbutyric acid    | 2.44974      | 1.91206E-09 | + |

Table 1

Table 2

The results showed that there were 25 different metabolites (VIP > 1, P < 0.05) in the whole post-fermentation stage (prophase vs telophase) of manual Huangjiu, as shown in Table 1; there were 14 different metabolites in the whole post-fermentation stage (prophase vs telophase) of mechanized Huangjiu (VIP > 1, P < 0.05), as shown in Table 2. There were 21 different metabolites between the two kinds of Huangjiu in the whole post-fermentation stage, as shown in Table 3. The s-plots and heat maps clearly showed that there was a significant difference between the metabolites of manual and mechanized Huangjiu, and the metabolites of manual Huangjiu were significantly different in the post-fermentation stage (prophase vs telophase) (Fig. S4, Fig. 4). A total of 25 metabolites in the whole post-fermentation stage of manual Huangjiu were identified as differential metabolites (VIP > 1, P < 0.05); the metabolic pathways with significant effects on the differential metabolites (−log(P) > 1, impact > 0.01) were phenylalanine, tyrosine and tryptophan biosynthesis pathway, alanine, aspartic acid and glutamate metabolism pathway, and arginine biosynthesis pathway (Fig. 5). A total of 14 metabolites in the whole post-fermentation stage of mechanized Huangjiu were identified as differential metabolites (VIP > 1, P < 0.05); the absence of metabolic pathways had a significant effect on differential metabolites (−log(P) > 1, impact > 0.01). A total of 21 metabolites of the two kinds of Huangjiu were identified as differential metabolites during the whole post-fermentation stage (VIP > 1, P < 0.05); the metabolic pathways with significant effects on the differential metabolites were...
proline metabolism pathway (Fig. 6).

Huangjiu increases after the end of post-fermentation period). Aspartic acid is synthesized by addition reaction of manual Huangjiu, aspartic acid is metabolized to produce oxaloacetate to complete a cycle, or generate arginine, fumarate, etc. Arginine and proline are involved in the metabolism of glutamic acid and α-ketoglutaric acid. Glutamic acid, acetic acid, and citric acid are metabolized to glyoxylate and dicarboxylic acid. In the post-fermentation stage, the contents of tyrosine, alanine, arginine, glutamic acid, proline, acetic acid and citric acid in manual Huangjiu were higher than those of mechanized Huangjiu, but the contents of glycine, aspartic acid and methionine were lower. Since glycine is involved in the synthesis of inosinic acid (IMP) and the synthesis of nucleotide is involved in the growth and metabolism of yeast, it is speculated that the active pathways of arginine biosynthesis and arginine and proline metabolism are higher in manual Huangjiu than those in mechanized Huangjiu, but the active levels of glyoxylate acid and dicarboxylic acid metabolism are lower. Genes such as CARI, CAN1, ALP1 and GAP1 are involved in the regulation of this process. The strong metabolism of arginine in manual Huangjiu may be related to the expression levels of DAL80, DUR1,2, DUR3, DARS2, and GAP1 (Alberto et al., 2012; Elberry et al., 1993; Jiao, 2016; Zhang et al., 2012; Zhang et al., 2016).

In the post-fermentation stage, the contents of ethyl acetate and isoamyl acetate in manual Huangjiu were higher than those of mechanized Huangjiu, while the contents of higher alcohols such as isoamyl alcohol were lower. Lilly et al. (2006) showed that overexpression of ATF1 gene in wine yeast S. cerevisiae VIN13 could significantly increase the production of ethyl acetate and isoamyl acetate, while the production of higher alcohol was significantly decreased (Lilly et al., 2006). Zhang et al. overexpressed the ATF1 gene in the Huangjiu yeast S. cerevisiae RY1 with low isoamyl acid production and knocked out the IAH1 gene. The production of isoamyl alcohol was reduced by 49.0%, and the production of ethyl acetate was increased by 20.9 times (Zhang et al., 2012). In the post-fermentation stage, the content of ethyl acetate, isoamyl acetate and isoamyl alcohol showed significant differences in the two kinds of Huangjiu, which may be closely related to these genes. In the post-fermentation stage, the total amount of higher alcohols in the two kinds of Huangjiu is different. Many genes can affect the production of higher alcohols. In Saccharomyces cerevisiae, aldehydes are hydrogenated to alcohols under the catalysis of gene-coded (ADH1, ADH2, ADH3, ADH4, ADH5, ADH6, ADH7, SFA1, etc.) alcohol dehydrogenase. In addition, there are also differences in the roles of genes encoding decarboxylase and alcohol dehydrogenase in the metabolic pathways of higher alcohols (Hazelwood et al., 2008). The difference of wheat Qu, especially the difference of microorganism in fermented mash, is the main reason that causes the difference between manual and mechanized Huangjiu in post-fermentation stage. The wheat Qu of mechanized Huangjiu is cultivated by pure breed. The wheat Qu of manual Huangjiu is naturally cultivated and mixed with pure breeds. Due to the different types and dosages of wheat Qu, there are significant differences in the types and abundance of microorganisms in different fermented mash (Xie et al., 2021), resulting in differences in metabolic pathways between manual Huangjiu and mechanized Huangjiu in the post-fermentation stage.

4. Conclusion

The metabolites of Huangjiu produced by different fermentation processes were studied in this paper. The key metabolic pathways of different metabolites in the post-fermentation process of Huangjiu with two brewing methods were explored. In the whole post-fermentation stage, there are three metabolic pathways which had significant effects on the differential metabolites in the manual Huangjiu. During the whole post-fermentation stage of mechanized Huangjiu, the related metabolic pathways had no significant effect on the differential metabolites. Comparing the two kinds of Huangjiu, a total of 21 metabolites were identified as differential metabolites during the whole post-fermentation stage; four metabolic pathways had a significant impact on the differential metabolites.

In the manual pressing, the moisture and air in the raw wheat Qu are higher (Xie et al., 2021), which is conducive to the early growth of yeast and fungi, and Aspergillus enzyme in the manual Qu quickly established the growth advantage. Manual operation also brought more bacterial

| Var ID (Var. Sec. ID:1) | M14.VIPred | Probability | Content comparison |
|-------------------------|------------|-------------|--------------------|
| Ethyl acetate           | 1.34111    | 3.61796E-18 | +                  |
| Ethyl lactate           | 1.46777    | 1.89278E-22 | +                  |
| Ethyl butyrate          | 1.4756     | 2.91E-23    | +                  |
| Isocitric acid          | 1.377      | 5.246E-14   | +                  |
| Leucine                 | 2.77925    | 7.16145E-32 | +                  |
| Tyrosine                | 2.4368     | 1.21258E-31 | +                  |
| Phenylalanine           | 2.16063    | 9.89335E-28 | +                  |
| Lysine                  | 2.57151    | 2.67207E-27 | +                  |
| Histidine               | 1.74284    | 2.28398E-17 | +                  |
| Arginine                | 2.20517    | 2.81916E-19 | +                  |
| Serine                  | 1.57135    | 1.38331E-13 | +                  |
| Glutamate               | 1.66034    | 1.00779E-14 | +                  |
| Proline                 | 2.4321     | 1.26658E-24 | +                  |
| Glycine                 | 1.2428     | 4.91504E-10 | -                  |
| Valine                  | 1.93658    | 1.03105E-21 | +                  |
| Methionine              | 1.20071    | 2.9255E-14  | -                  |
| Acetic acid             | 1.55697    | 5.23613E-07 | +                  |
| Lactic acid             | 2.3334     | 0.0163673   | -                  |
| Citric acid             | 1.34154    | 1.54733E-15 | +                  |
| Malic acid              | 2.05118    | 1.16062E-27 | +                  |
| Tartaric acid           | 2.42603    | 6.25122E-37 | +                  |
Fig. 4. Heat map of different metabolites in Huangjiu at the end of fermentation. The mechanized Huangjiu is in the lower-adjusted interval on the left, and manual Huangjiu is in the upper-adjusted interval on the right. The horizontal axis corresponds to the sample number, and the vertical axis corresponds to the substance number (refer to the supplementary materials for the substance type corresponding to the number).
infection to raw wheat Qu, resulting in higher microbial diversity in finished manual wheat Qu. Mechanized ripe wheat Qu had a more uniform fungal community, but less water and air, resulting in slower early mold growth (Zhou, 2021). These factors lead to significant differences in the expression levels of many genes in the fermented flora of the two kinds of Huangjiu. The main reason for the significant difference in the metabolite content of the two kinds of Huangjiu in the post-fermentation stage is mainly due to the different strains in the fermentation process. The content of metabolites between the two kinds of Huangjiu in the post-fermentation stage is significantly different, mainly due to the differences in the expression and regulation of arginine biosynthesis pathway and its associated pathways. These differential metabolic pathways are regulated by genes such as CAR1, CAN1, ALP1, GAP1, DAL80 and ATF1.

The exploration of the differences in metabolites in Huangjiu during the post-fermentation process will help to understand the potential mechanism of flavor formation in the post-fermentation process of Huangjiu, and provide a theoretical basis for the industrial development of Huangjiu.

CRediT authorship contribution statement

Qi Peng: Writing – review & editing, Supervision, Project administration, Funding acquisition. Kai Meng: Writing – review & editing, Investigation, Data curation. Huajun Zheng: Conceptualization, Writing – original draft, Visualization, Investigation. Hefeng Yu:
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of interest

There are no conflicts of interest in this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfoch.2022.100324.

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