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# Quantitative analysis of organic acid metabolic pathways during fermentation of clear white wine lees spirits

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**SUMMARY:** *Organic acid is an important flavor presenting substance in liquor, and its content directly affects the acidity of the spirits. In this paper, in order to further understand the content of organic acids and their metabolic pathways in the spirits samples of Qingxiang-type baijiu during the period of spirits production, based on the production process of Qingxiang-type baijiu and the requirements of the control, we used the principal component analysis to analyze the types and contents of organic acids contained in Qingxiang-type baijiu. The partial least squares regression model was used to establish a prediction model for the quantitative analysis of PLSR of organic acids in clear-flavored liquor. The quantitative method of GC-MS was combined with HS-SPME to detect the volatile flavor substances in the wine spirits and analyze the metabolism of the main organic acids during the fermentation process. The average concentration of various non-volatile organic acids in clear-flavored liquor was characterized by BSTFA derivatization combined with GC-MS analysis. Solving the PLSR quantitative analysis model for organic acids in clear-flavored white wine yielded that* $R^2$ *of the major organic acids in white wine, acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid, and isovaleric acid, were all above 0.9. The RMSEP of the model ranged from 0.086 to 0.425, and the RPD values ranged from 3.895 to 12.007. With good model fitting and high prediction accuracy, the model can be promoted and applied to the quantitative detection of trace organic fractions in baijiu or other important foodstuffs, and it has a wide applicability.*

***KEYWORDS:*** *organic acid metabolism; partial least squares regression; GC-MS method; fermentation process of wine spirits; clear-flavored white wine*

## 1 Introduction

Baijiu is a traditional liquor with a long history of brewing in China, and its open production method involves the participation of multiple microorganisms in the fermentation process [1]. Studies have shown that baijiu contains nearly 1,000 flavor substances, which are inextricably linked to its fermentation involving multiple microorganisms [2]. Baijiu uses raw materials such as sorghum and barley to make the brewing process of steaming and clear burning and clear ballast, solid-state fermentation in underground earthenware tanks, and clear distillation, and its standard style features reveal a long aftertaste and a long aftertaste in the mouth and sweetness in the mouth [3, 4].

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Baijiu process is characterized by “steaming and clearing hawthorn, fermentation in the ground tank, steaming and clearing for two times” [5]. “Steaming Qingzha” refers to the fermentation of sorghum after the removal of impurities and other treatment of crushing for separate steaming. The “ground jar fermentation” refers to the use of white wine as a brewing container, liquor spirits mixed in the pottery jar buried in the ground after the fermentation (the mouth of the jar and the ground level). “Steaming second clear” refers to each batch of raw materials put into the liquor spirits, sorghum and auxiliary materials have to be steamed separately once, so that the starch in the raw materials paste, to facilitate the saccharification and fermentation of microorganisms and enzymes of the large song, the production of wine into the aroma, and at the same time, volatilization of the raw materials in the evil taste. Steam pasteurization of sorghum after the second fermentation, distillation of alcohol twice. The first is pure grain fermentation, and the distilled wine is called Dazhaji wine. The second time is pure grains fermentation, not with new grain, the distilled liquor is called two hawthorn liquor, and the lees are thrown as dregs. This process makes full use of the starch in the raw materials to produce wine and aroma, and at the same time avoids the evil and miscellaneous flavors in the raw materials from being brought into the wine, so that the steamed wine will be fresh and clean. The uniqueness of the liquor brewing process, so that its production process presents a relatively stable, simple state, by the outside world relatively few interference factors, unique technology combined with the complex structure of the microbial flora created a pure aroma of white wine, the body of sweet and soft and smooth, natural coordination, the aftertaste of the long and refreshing style [6-8].

The flavor characteristics of liquor come from some of the aroma components of the raw materials themselves, the aromatic components brought by the products formed by the growth and metabolism of different microorganisms, the aroma produced during the steaming process, and the environment and other microorganisms produce the aroma, etc. [9, 10]. It is mainly due to the growth of its many types and numbers of microorganisms, the products formed by metabolism and the interactions between various microbial species, which interact to produce a variety of compounds with special flavors [11, 12]. These microorganisms are swept throughout the brewing system, all types of microorganisms are intertwined and complementary, forming a unique system of brewing microorganisms, also known as liquor brewing microecology [13]. All kinds of liquor have their own flavor characteristics are due to the microecological composition of their brewing is very different. The microecology of white wine is mainly dominated by microorganisms of the macro-quartz and wine spirits, supplemented by environmental microorganisms [14]. The fermentation mechanism of baijiu is complex, covering not only the physiological and biochemical metabolism of the microbial strains themselves, but also touching on the interactions between microorganisms from different sources, which together constitute the ecological community of baijiu microorganisms [15, 16]. The analysis of the composition, function and interrelationship of these microbial communities can help to artificially manipulate the brewing process, optimize the production process, improve the quality and efficiency of production, reduce the cost of raw materials and produce more and better safe and high-quality baijiu [17, 18].

Organic acids are a very important class of flavor components in baijiu, which can eliminate the bitterness of baijiu, inhibit and mask the aroma of baijiu, and are the most important flavor sensors in baijiu [19, 20]. For example, lactic acid can increase the mellowness of baijiu, but it has astringent flavor when it is too strong and makes the wine rancid and sour [21]. Suitable acetic acid can make the taste of baijiu refreshing and sweet, but in excess, it increases irritation [22]. Up to 70 organic acids have been reported in baijiu [23]. However, there are fewer studies on the organic acids of wine spirits during the fermentation process of baijiu. Researchers analyzed the content of organic acids in wine spirits during fermentation in the cellar in some rounds and found that the organic acids in wine spirits were mainly lactic acid, acetic acid and succinic acid [24]. During the fermentation process of white wine, the high content of organic acids in wine spirits not only inhibits the growth and metabolism of microorganisms such as Saccharomyces cerevisiae and Clostridium capricornutum, but also inhibits the production of alcohols in wine spirits [25, 26]. High lactic acid content was also found to inhibit the growth of Saccharomyces cerevisiae and its ethanol production in pure culture level studies [27]. There is a lack of research on the metabolic pattern of organic acids during the stacked fermentation of baijiu, and their effects on the structure and succession pattern of microbial communities are not clear. Fermentation abnormalities due to high acidity frequently occur in baijiu production, resulting in low wine yield and poor product quality [28]. Therefore, it is necessary to elucidate the metabolic pattern of organic acids in the fermentation process of grains, which can provide a starting point for further investigation of the causes of production abnormalities caused by high acidity, and it is an important theoretical basis for revealing the flavor formation and production regulation of sake [29, 30].

In this paper, according to the requirements of the production process control of clear-flavored liquor, it is pointed out that the volatile flavor substances in liquor spirits are detected by using the method of combining HS-SPME and GC-MS. Combined with the partial least squares regression analysis method, a prediction model for quantitative analysis of organic acids PLSR in liquor was established. The quantitative analysis model of organic acid PLSR was solved by $R^2$, RMSEP and RPD. On the basis of the production process of clear-flavored white wine, stainless steel fermentation tanks, stone cellars, and ceramic vats were used as fermentation vessels to analyze the fermentation temperature changes of Dazhajia and Erzha under different fermentation vessels and their effects on the brewing of clear-flavored white wine. We analyzed the metabolism of major organic acids and the average concentration of non-volatile organic acids during the fermentation of clear-flavored white wine spirits.

# 2 Materials and methods

## 2.1 Sample collection

Three batches of samples were collected from Taiyuan Distillery, Fenjiu Distillery and Zhongfen Distillery respectively. The samples collected from Taiyuan distillery included the big currant, including the clear-crop currant, red heart currant, and back-fire currant, low-temperature big currant powder, and the spirits of wine, respectively. Among them, 1, 7, 14, 21 and 25 days of fermentation were observed for the large-crop fermentation and 1, 4, 17 and 25 days for the second-crop fermentation in the wine spirits.

Samples of wine spirits were collected from Fenjiu distillery for different fermentation periods, 1, 4, 7, 10, 15, 21 and 28 days for large-crop fermentation, and 1, 4, 7, 10, 15, 21 and 28 days for second-crop fermentation.

Samples were collected from the Fenjiu factory for the clear cropping, the red heart cropping, the backfire cropping, and the different fermentation periods of the spirits. The big crop fermented for 4, 7, 15, 21 and 28 days, and the second crop fermented for 0, 4, 7, 15, 21 and 28 days.

The above samples were taken by the five-point sampling method, and about 600g of each sample was put into a processed aseptic sealed bag. After the samples were taken back to the laboratory, the samples were processed as soon as possible to extract the genome. After that, the samples will be put into the refrigerator at -20°C or -80°C for storage, and the refrigerator at -80°C is more durable.

## 2.2 Materials and reagents

The experimental reagents used in this experiment are shown in Table 1.

2-Octanol standard (chromatographically pure), purchased from Shanghai Anpu. Sodium chloride, sodium hydroxide, glucose, hydrochloric acid, copper sulfate pentahydrate, potassium sodium tartrate (all analytically pure), purchased from Polymerization Chemical.

*Table 1: Experimental reagent used in this experiment*

| Name | Specification | Producer |
|---|---|---|
| 2-sinol standard | Chromatogram | Polymerization chemical |
| Sodium chloride | Analytical purity | Polymerization chemical |
| Sodium hydroxide | Analytical purity | Polymerization chemical |
| Glucose | Analytical purity | Polymerization chemical |
| Hydrochloric acid | Analytical purity | Polymerization chemical |
| Copper sulfate | Analytical purity | Polymerization chemical |
| Sodium tartrate | Analytical purity | Polymerization chemical |
| Tryptone | Analytical purity | Bioengineering (Shanghai) co., LTD |
| Yeast powder | Analytical purity | Bioengineering (Shanghai) co., LTD |
| AGAR powder | Analytical purity | Bioengineering (Shanghai) co., LTD |
| AGAR sugar | Superior purity | Shiko biotech |
| Tris | Superior purity | Bioengineering (Shanghai) co., LTD |
| EDTA | Superior purity | BBI |
| SDS | Superior purity | Aladdin |
| Tris saturated phenol | Analytical purity | Aladdin |
| Chloroform | Analytical purity | Aladdin |
| Isopropyl alcohol | Analytical purity | Aladdin |
| Anhydrous ethanol | Analytical purity | Bioengineering (Shanghai) co., LTD |
| Ampicillin | Superior purity | Solais |
| Acrylamide | Superior purity | Van der biko technology co., LTD |
| Diacrylamide | Superior purity | Mforest |
| Acetic acid | Analytical purity | Aladdin |
| Formamine | Analytical purity | Aladdin |
| Urea | Analytical purity | Aladdin |
| 10000×Gelstain | 1ml | Full gold |
| Ammonium persulfate | Superior purity | Solais |
| TEMED | Superior purity | Solais |
| AGAR gel recovery kit | B518131-0100100PREPS | Bioengineering (Shanghai) co., LTD |
| Plasmid extraction kit | B518131-0100100PREPS | Bioengineering (Shanghai) co., LTD |
| Plasmid connection kit | 60 pcs | Shiko biotech |

## 2.3 Instruments and equipment

The instrumentation utilized in this paper is shown in Table 2.

*Table 2: Instrument and equipment used in this article*

| Instrument name | Instrument type | Producer |
|---|---|---|
| Analytical electronic balance | ME20A | Mettler torrido instrument co., LTD |
| Thermostat | DZKWD-4 | Beijing yongguang medical instrument co., LTD |
| Drying oven | DHG-9245A | Shanghai yiheng science instrument co., LTD. |
| Automatic sterilizing pan | MLS-375L | Hitachi |
| Top empty solid phase extraction head | 50/30 $\mu m$ DVB/CAR/PDMS | Japan island |
| Gas chromatography-mass spectrometer | GCMS-QP2010 Plus | Japan island |
| PCR meter | T100 Thermal Cycler | Bio-Rad |
| Electrophoresis meter | DYY-6C | Beijing six life technology co., LTD |
| Transition gradient electrophoresis meter | DcodeTM | BioRad |
| Ultraviolet analyzer | UV-3000 | Shanghai jieng technology co., LTD |
| Small high speed centrifuge | 5424 | Eppendorf |
| Large high speed centrifuge | LD5-10 | Beijing medical centrifuge plant |
| Multi-function shaking bed | ZWY-200D | Shanghai zhicheng analyzer manufacturing company |
| Biochemical incubator | HPX-11-200 | Kangqiao town, pudong new area, Shanghai |
| Electronic balance | Scout SE-SE602F | Orhouse instrument co., LTD |
| -80oc refrigerator | U410 | Eppendorf |

# 3 Experimental methods

## 3.1 Standard solution preparation

Precisely weigh the appropriate amount of 0.70 $g$ organic acid standard, with 0.2% aqueous phosphoric acid solution as solvent, valeric acid, hexanoic acid formulated into 6000.00 $mg/L$ single standard solution. The rest of the organic acids were prepared into a single standard solution of 10,000.00 $mg/L$. Take 11 $mL$ each of valeric acid and hexanoic acid single standard solution, and 3 $mL$ each of the remaining single standard solution in a 50.00 $mL$ volumetric flask, and then obtain the mixed solution of the standard with the mass concentration of 2000.00mg/L of each organic acid by solvent determination, and then dilute them proportionally to formulate them into the mass concentration of 0.3mg/L, 1.00 $mg/L$, and 5.00 $mg/L$ and other standard mixed solutions.

## 3.2 Pre-treatment of samples

Aspirate 1 $mL$ sample of wine with an alcohol content of <0% $voL$, pass it through a 0.3 $\mu m$ microfiltration membrane and feed it directly into the sample. Pipette 1 $mL$ of wine sample with alcohol >10% $voL$, dilute it with 0.1% aqueous phosphoric acid solution ( $pH$ =2.30) until alcohol <10% $voL$, fix the volume and shake well, calculate the dilution factor, pass it through

0.3 $\mu m$ microfiltration membrane, and then inject into the sample for testing.

## 3.3 GC-MS Quantitative Methods

A 50% *voL* aqueous alcoholic solution was used as the matrix, and suitable gradient concentration solutions were prepared according to the concentration of each organic acid in white wine. The sample was analyzed after the pretreatment and derivatization methods consistent with those of the samples were performed for each gradient solution. The ratio of the peak area of the substance and the internal standard was taken as the horizontal coordinate, and the concentration of the substance was taken as the vertical coordinate, and the standard curves of the corresponding compounds were established to calculate the content of non-volatile organic acids in white wine. The above experiments were carried out in three parallel experiments.

## 3.4 Detection of flavor substances in wine spirits

The control requirements of the production process of clear-flavored white wine include the following steps:

Step1: raw material crushing

The most important raw materials in the brewing process of fragrant white wine are sorghum and rice wine, and sorghum is required to be full of seeds, with thin skin and fewer shells. Liquorice is divided into three kinds: clear stubble, red heart and backfire, which need to be crushed and usually mixed according to the ratio of 30% clear stubble, 30% red heart and 40% backfire. When using liquorice, we need to check the appearance quality of the liquorice, and also pay attention to the biochemical characteristics of its liquefaction power, saccharification power and fermentation power.

Step2: Run the grits

Sorghum raw material is crushed to become red grits, which are moistened with water at a higher temperature before steaming. The operation requirements of high temperature grits moistening are strict, if the water temperature is too high, it will easily cause the raw material to form lumps, if the water temperature is low, the raw material will be drenched after it is put into the vat.

Step3: Steaming

Steaming is also known as steaming grits, which adopts the steaming process. The first step of steaming material needs to boil the bottom pot of water, and then sprinkle a layer of rice husk or grain husk on the castor of the retort, load the retort on the material, the requirements of the steam withdrawal, loaded evenly on the flat. After the round steam, in the material surface splash water for raw materials 1.5% to 3.0%, the temperature of 60 ℃ of hot water.

Step4: Add water, raise the cold, add the song.

After steaming the red grits should be hot out of the cauldron, spread into a rectangle, splash 18 ~ 20 ℃ of well water, the amount of water for the amount of raw materials about 30%, splash of water while stirring, so that the particles are fully dispersed to absorb water, followed by mixing and ventilation of cool residue. Cooling residue requires winter cooling to a higher temperature than the cylinder temperature of 2 ~ 3 ℃, other seasons cooling to the same temperature as the cylinder can be added to the song. Strictly control the amount of added quartz, usually 9% to 12% of the amount of raw materials, specific need to be adjusted in combination with the temperature and fermentation cycle of different production periods.

Step5: Vatting and fermentation of dregs

Typical clear-flavored wine is fermented in floor tanks, which are earthenware tanks buried in the ground with their mouths flush with the ground. Before the dregs are put into the tank, it is necessary to clean the inner wall of the tank and the lid, and scrub the surface of the inner wall of the tank with a rag moistened with a trace amount of 4% of pepper water to disinfect the tank and leave the aroma in the tank.

Step6: Discharge the vat and distill

At the end of fermentation, scoop out the large dregs of wine spirits and mix them with 18% to 20% filler to loosen them.

Step7: Second dregs fermentation

The dregs of wine spirits still contain starch, in order to make full use of the need for secondary fermentation, that is, two dregs of fermentation, the cycle is about 25 d, the operation is similar to the dregs of fermentation, is a pure dregs of fermentation, do not add new materials, to be fermentation is completed out of the cylinder mixed with a small amount of grain chaff, on the retort to steam the two dregs of wine, lees as a throw lees discharged.

Step8: Aging and storage

Aging is the aging process of the wine, a process that makes the clear liquor softer and improves its quality.

Step9: Blending

Blending is the last step in the brewing of liquor, blending is not simply mixing water into the wine, but the previous stages of brewing the base wine in proportion to the matching and blending together, the wine hook wine, aroma, taste and flavor, so that the liquor “color, aroma, taste, style” to achieve a certain degree of coordination and balance.

Combined with the above description of the production process control requirements of clear liquor, this paper adopts headspace solid-phase microextraction (HS-SPME) combined with the GC-MS method to detect the volatile flavor substances in the wine spirits.

## 3.5 Data analysis

Qualitative analysis was performed against a standard spectral library (NIST14). Semi-quantitative analysis of all volatiles in the wine spirits samples was performed by internal standard (2-octanol). SPSS22 and origin2021 were used for data processing and graphing.

### 3.5.1 Principal Component Analysis

The historical data generated from operation under normal operating conditions are selected to construct the sample matrix $\mathbf{X} = \left( X_{ij} \right)_{n\times m}$. where $m$ is the number of variables in the sample and $n$ is the number of samples. The data were standardized using the $z$-score method and its covariance matrix $S$ was calculated using the formula:

$$\mathbf{S} = \frac{1}{n-1}\mathbf{X}^{\mathrm{T}}\mathbf{X} \tag{1}$$

The number of principal elements is determined by the cumulative contribution to variance (CPV), which is expressed as:

$$\mathrm{CPV} = \frac{\sum_{j=1}^{k} \lambda_j}{\sum_{j=1}^{m} \lambda_j} \tag{2}$$

In equation (2): $\lambda_j$ is the eigenvalue of $S$. Namely:

$$\mathbf{X}_i = \mathbf{X}_p + \mathbf{E} = \mathbf{T}_i \mathbf{P}_i^{\mathrm{T}} + \mathbf{E} \tag{3}$$

$$\mathbf{T}_i = \mathbf{X}_i \mathbf{P}_i \tag{4}$$

where: $\mathbf{X}_P$ is the projection of the sample vector $\mathbf{X}$ in the principal subspace. $\mathbf{T}_i$ is the score matrix, $\mathbf{P}_i$ is the loading matrix, and $\mathbf{E}$ is the residual subspace.

The $T^2$ and SPE statistics are two important metrics for fault diagnosis by PCA. The control limit of the $T^2$ statistic $\mathrm{CL}_{T^2}$ can be expressed as:

$$\mathrm{CL}_{\tau^2} = \frac{\alpha(n+1)(n-1)}{n(n-\alpha)} F_{\alpha}(\alpha, n-\alpha) \tag{5}$$

In equation (5), $\alpha$ is the significance level and $1-\alpha$ is the confidence level. The $F_{\alpha}(\alpha, n-\alpha)$ is the $F$ distribution with $\alpha$ first degree of freedom and $n-\alpha$ second degree of freedom.

The control limit of the SPE statistic $\mathrm{CL}_{\mathrm{SPE}}$ can be expressed as:

$$\mathrm{CL}_{\mathrm{SPE}} = \theta_1 \left[ \frac{c_{\alpha} h_0 \sqrt{2\theta_2}}{\theta_1} + 1 + \frac{\theta_2 h_0 \left(h_0 - 1\right)}{\theta_1^2} \right]^{\frac{1}{n_0}} \tag{6}$$

$$\theta_i = \sum_{j=k+1}^{m} \lambda_j^i, i = 1, 2, 3 \tag{7}$$

$$h_0 = \frac{1 - 2\theta_1\theta_3}{3\theta_1^2} \tag{8}$$

where: $c_{\alpha}$ is the confidence limit of the standard normal score at a cloth confidence level of $\alpha$. $h_0$ is the threshold, $\theta_1$ and $\theta_3$ are the quartiles and trinomials of the dataset, $\theta_i$ is defined as shown in Equation (7), and $\lambda$ is the eigenvalue.

### 3.5.2 Partial Least Squares Regression Models

Linear regression is a commonly used regression algorithm that is characterized by simplicity and efficiency, as well as being the basis for many nonlinear algorithms. It essentially assumes a linear relationship between the response variable (dependent variable) and the explanatory variable (independent variable), and solves for the parameter with the smallest loss function by constructing a loss function.

Partial least squares regression (PLSR) combines the advantages of three analytical methods, including principal component analysis, typical correlation analysis and multiple linear regression analysis, which is very suitable for regression modeling with a large number of variables and multiple covariates, and can be applied to this paper [31, 32]. The specific realization process is as follows:

Determine the independent variable matrix X, dependent variable matrix Y of this paper. Firstly, the 2 matrices are centered to get $X_0$ and $Y_0$, and iteratively solve the transformation weights and transformation factors of $X$, $Y$, and the solution formula is as follows:

$$
\begin{aligned}
w_1 &= \frac{X_0^T Y_0}{Y_0^T Y_0} \\
t_1 &= X_0 w_1
\end{aligned}
\tag{9}
$$

$$
\begin{aligned}
c_1 &= \frac{Y_0^T t_1}{t_1^T t_1} \\
u_1^* &= Y_0 c_1
\end{aligned}
\tag{10}
$$

In Eq. (9), $w_1$ is called the transformation weight of X, which is utilized to approximate the information of $X_0$ by $t_1$, and $t_1$ is called the transformation factor of X. Similarly, in Eq. (10), $c_1$ is called the transformation weight of Y, and $u_1^*$ is called the transformation factor of Y.

The above two equations alternately establish the regression relationship between X and Y. Usually, multiple iterations are needed until convergence, and the maximum number of iterations is set to 300 in this paper.The formula to verify whether convergence has been reached is as follows:

$$
\begin{aligned}
v &= u^* - Y_0 \\
\Delta u &= v^T v
\end{aligned}
\tag{11}
$$

In Eq. (11), the calculated value of $\Delta u$ is compared with the pre-set threshold, if it is less than the inter-value, it indicates that the alternating regression has reached convergence, at this time, the principal components can be extracted and proceed to the next step. Otherwise, make $u^* = Y_0$ and repeat the above steps until convergence. In this paper, the convergence threshold is set to 1e-6. After confirming the convergence, the residual matrices of X and Y are calculated:

$$
\begin{aligned}
p_1 &= \frac{X_0^T t_1}{t_1^T t_1} \\
X_1 &= X_0 - t_1 p_1^T
\end{aligned}
\tag{12}
$$

$$
\begin{aligned}
q_1 &= \frac{Y_0^T u_1}{u_1^T u_1} \\
Y_1 &= Y_0 - u_1 q_1^T
\end{aligned}
\tag{13}
$$

In Eq. (12), $p_1$ is called the loading of X, reflecting the relationship from $t_1$ to $X_0$, and $X_1$ denotes the residual matrix between the real value $X_0$ and the predicted value $t_1 p_1^T$.

Similarly, in Eq. (13), $q_1$ is called the loading of Y, reflecting the relationship from $u_1$ to $Y_0$, $Y_1$ denotes the matrix of residuals between the real value $Y_0$ and the predicted value $u_1 q_1^T$ and $u_1$ is the column vector of $Y_0$.

Thereafter, $X_1$ and $Y_1$ are used instead of $X_0$ and $Y_0$, respectively, and the extraction of the principal components is repeated from Eq. (9) until the completion of the preset goal, and the number of the extracted principal components preset in this paper is 5. It can be seen that in the PLSR algorithm, when a principal component is computed, the next principal component is computed from the current residual matrix and is weakly associated with the original matrix. Define $b_1$ as follows:

$$b_1 = \frac{u_1^T t_1}{t_1^T t_1} \tag{14}$$

Then we have $u_1 = b_1 t_1$. After the above steps are repeated a times, the $w_1$ to $w_a$ are sequentially arranged as matrix W. Similarly, matrix Q is obtained, and then we can get the regression coefficients matrix R from X to Y:

$$R = Wdiag(b)Q^T \tag{15}$$

Such that $Y = XR$.

# 4 Results and discussion

## 4.1 Quantitative analysis of organic acids and aldehydes

### 4.1.1 Selection of Master Component Fractions in PLSR Models

The selection of the number of principal components is key to PLSR modeling. If too many principal components are selected, the background noise generated by variables unrelated to the response will lead to overfitting of the model, affecting the recognition of statistical trends and reducing the prediction ability. If the number of principal components selected is too small, the sample information cannot be adequately reflected, resulting in inadequate model fitting and reduced prediction accuracy.

In this paper, the number of principal components to establish PLSR was selected by the cumulative contribution of principal components to each variable and the corrected standard deviation (RMSECV) of each principal component in leave-one-out cross-validation.

Taking hexanoic acid as an example, a number of principal components were selected for PLSR modeling separately, and the cumulative contribution rate of principal components is shown in Figure 1. Combined with the change rule of RMSECV with the number of principal components, it can be seen that with the increase of the number of principal components, the value of RMSECV gradually decreases, and the cumulative explained variance gradually increases. When the number of principal components is 14, the RMSECV value is relatively smallest, the cumulative contribution of principal components to both independent and dependent variables is greater than 85%, and the cumulative explained variance of the independent variable X matrix remains basically unchanged. Therefore, considering the cumulative contribution of principal components and RMSECV, the optimal number of principal components, 14, was selected for PLSR modeling.

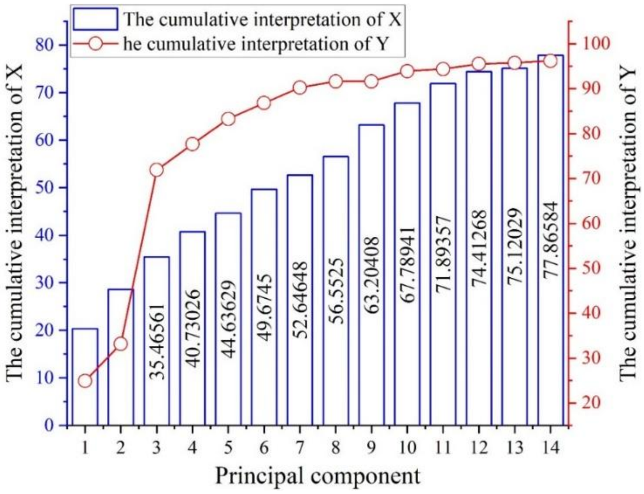

*Figure 1: Cumulative contribution rate of main component*

Based on the above principle, the PLSR quantitative analysis model was established by selecting the principal components of organic acids (acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid, and isovaleric acid) and aldehydes (acetaldehyde, acetaldehyde, and isovaleric aldehyde) in baijiu, and the results of the PLSR quantitative analysis model for the principal components are shown in Fig. 2.

From the PLSR quantitative analysis model, the principal components of the main organic acids in white wine were obtained. The fractions of acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid, and isovaleric acid were 12, 8, 13, 16, 13, and 10, respectively, and the fractions of valeric acid were more than those of the other organic acid analytes.

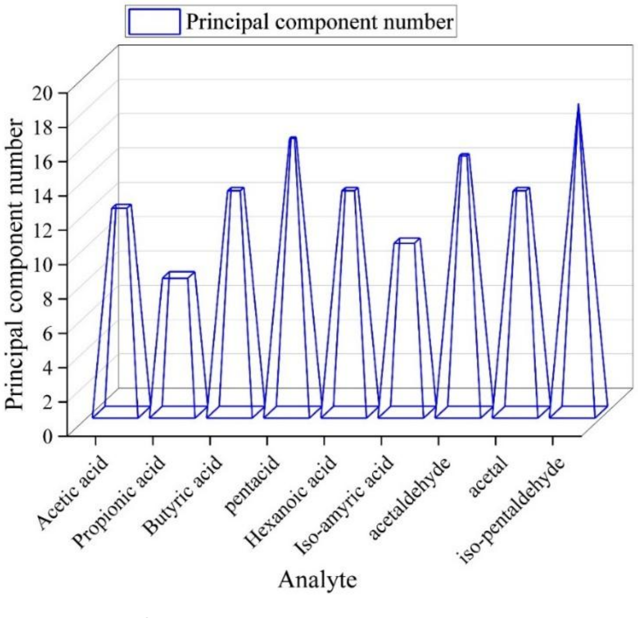

*Figure 2: The main component of the PLSR quantitative analysis model*

### 4.1.2 Evaluation of prediction models for organic acids and aldehydes in white wine

Based on the above experimental methods, the PLSR prediction models of organic acids and aldehydes in white wine were established respectively. The measured and predicted points of each component were distributed diagonally, and the linear relationship between predicted and measured values was good, indicating that the model had a good fitting and prediction effect.

The quality of the prediction model usually needs to be evaluated by establishing the relationship between the predicted values and the measured values, and the commonly used parameters for model evaluation are the coefficient of determination ( $R^2$ ), the standard deviation of prediction (RMSEP), and the ratio of the range of error (RPD), etc., which were calculated by the following formulas:

$$R^2 = 1 - \frac{\sum_{i=1}^{n} \left( y_i - y_i \right)^2}{\sum_{i=1}^{n} \left( y_i - \overline{y_i} \right)^2} \tag{16}$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n} \left( y_i - y_i \right)^2}{n}} \tag{17}$$

$$RPD = \frac{SD}{RMSEP} \tag{18}$$

where $n$ is the number of samples. $y_i$ is the measured value of the $i$th sample. $y_i$ is the predicted value of the $i$th sample. $\overline{y_i}$ is the average of the measured values of all samples. SD is the standard deviation of all samples. The smaller the RMSEP is, the higher the prediction accuracy of the model. When RPD > 3, the model is considered to have a high predictive ability.

The effective region of the full H NMR spectrum of white wine was divided by every 0.03 ppm as an interval, and this was used as the independent variable. The PLSR prediction model for organic acids and aldehydes in white wine was established using the results of component concentration measurement in white wine obtained by gas chromatography as the dependent variable.

The PLSR quantitative analysis model for organic acids and aldehydes in white wine is shown in Table 3. The $R^2$ of the main organic acids in white wine, acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid and isovaleric acid, were all above 0.9. The $R^2$ of valeric acid was 0.989, and the value of $R^2$ was the largest. The range of RMSEP was 0.086~0.425. The minimum value of RPD was 3.895, which showed good regression effect and high prediction accuracy.

*Table 3: Quantitative analysis model of organic acid and aldehyde PLSR in baijiu*

| Analyte | $R^2$ | RMSEP | RPD |
|---|---|---|---|
| Acetic acid | 0.912 | 0.425 | 3.895 |
| Propionic acid | 0.975 | 0.086 | 8.133 |
| Butyric acid | 0.983 | 0.152 | 9.524 |
| pentacid | 0.989 | 0.176 | 11.285 |
| Hexanoic acid | 0.974 | 0.163 | 6.694 |
| Iso-amyric acid | 0.963 | 0.251 | 7.206 |
| acetaldehyde | 0.938 | 0.365 | 6.895 |
| acetal | 0.985 | 0.124 | 12.007 |
| iso-pentaldehyde | 0.927 | 0.528 | 4.239 |

## 4.2 Analysis of temperature change of grains during fermentation process

Clear-flavored liquor, also known as Fen-flavored liquor, represented by Shanxi Fen Liquor and Fenyang Wang Liquor, belongs to the category of large-quotient liquor, which has the characteristics of pure fragrance, mellow, sweet and soft, natural coordination, and refreshing aftertaste, etc. Most of the clear-flavored liquors require fermentation twice. Most clear-flavored white wines need to be fermented twice. The first fermentation results in what is known as Da Zha Jiu, and the second fermentation results in Er Zha Jiu.

The aroma and flavor of white wine is inextricably linked to the composition of microbial flora and its community succession law during the fermentation process.

In the process of fermentation, the rise and fall of temperature is the visualization of the difference between the energy released by microbial reproduction and metabolism in the cellar and the heat dissipation in the cellar. The speed of temperature rise and the magnitude of the magnitude reflects the degree of microbial reproduction and metabolism in the cellar. The changes of fermentation temperature of dazhaji in different containers are shown as follows.

The temperature change curve of hawthorn fermentation in different fermentation containers is shown in Figure 3. During the 30 days of hawthorn fermentation, the ambient temperature was always maintained at 18℃, and the maximum temperature did not exceed 22℃. The fermentation temperature of bad hawthorn in stone cellar was higher than that in stainless steel tank and ceramic tank.

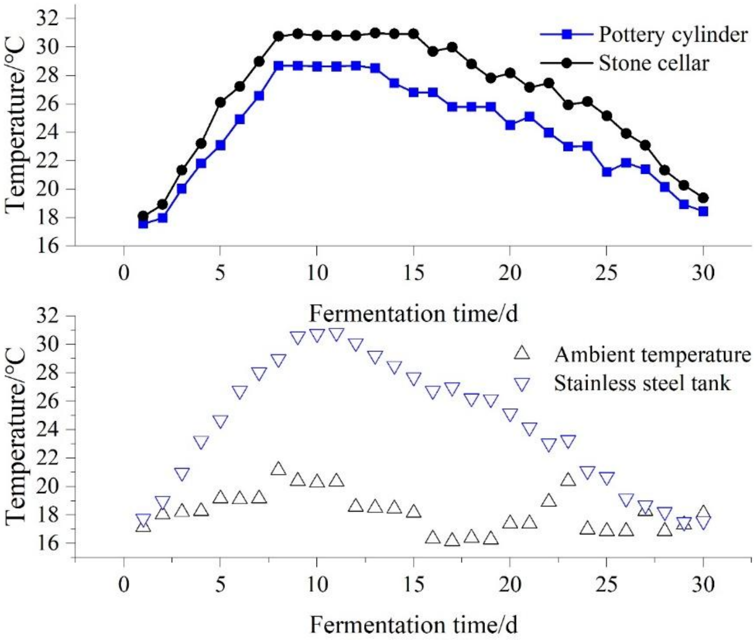

*Figure 3: The fermentation temperature of the big hawthorn*

The fermentation time of 30 days was also used to compare the fermentation temperature change of Erzha under different fermentation vessels. The change curve of fermentation temperature of Erzha under different fermentation containers is shown in Figure 4.

The fermentation temperature in different fermentation containers showed the trend of rapid increase in the early stage, maintaining stability in the middle stage, and decreasing slowly in the late stage. The top fermentation temperature of the second hawthorn in different fermentation containers was reached after about the 7th day of the vatting.

Comprehensively, due to the high starch content of hawthorn and low acidity in the cellar, the microbial fermentation of hawthorn was obviously higher than that of second hawthorn, and its intuitive manifestation was that the fermentation temperature of hawthorn was higher than that of second hawthorn.

Under different fermentation containers, stone cellar has the largest volume, followed by stainless steel tank and ceramic tank. Due to the large volume of stone cellar itself, the quantity of grain in the cellar is large, plus the stone material itself has poor heat dissipation. Therefore, in the fermentation process of hawthorn and second hawthorn, the temperature of fermentation of grains in the stone cellar was higher than that of stainless steel tank and ceramic jar in terms of warming amplitude and neutralization time. The volume of ceramic tank is the smallest, and the warming amplitude and the neutralization time of dregs in the fermentation process are the smallest and the shortest.

According to the curve analysis of the temperature change of lees spirits during the fermentation process, it can be found that under the same natural environment, the fermentation equipment has a greater influence on the fermentation temperature of hawthorn and erhawthorn, in which the volume size of the fermentation equipment may be the main factor leading to the difference.

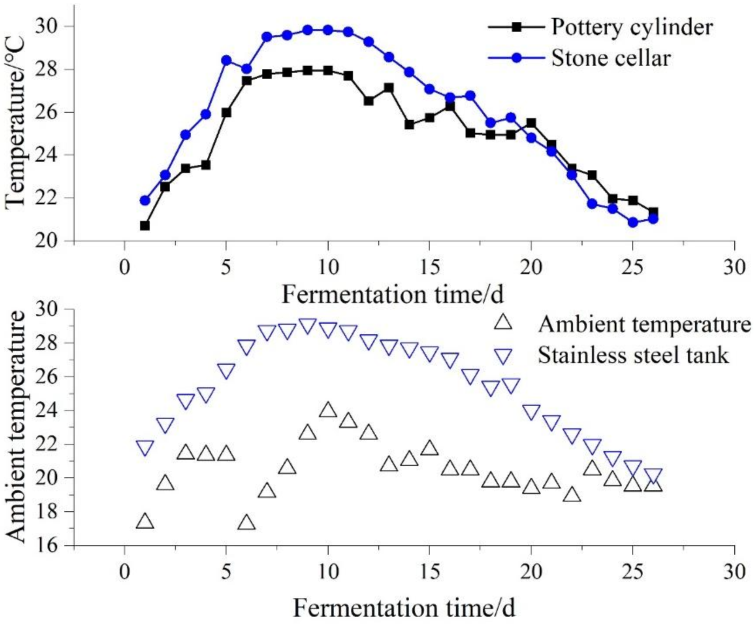

*Figure 4: The temperature change curve of the haw fermentation temperature*

## 4.3 Metabolism of major organic acids during fermentation of wine spirits

In the fermentation process of clear-flavored white wine spirits, the organic acids in the spirits are dominated by lactic acid with the highest content, followed by acetic acid. Therefore, lactic acid and acetic acid are the main organic acids constituting the flavor of wine, and the appropriate acid content in the spirits during the period of spirits formation has a very important role in the subsequent production of clear-flavored white wine.

The changes of lactic acid during the fermentation process of the grains of wine as shown in Fig. 5, lactic acid in the fermentation process of the grains of wine takes 37 days and 27 days respectively. In the fermentation stage of DaZhaZha, the lactic acid content gradually increased in the early stage of fermentation from 0 to 6 days, and then gradually stabilized after 6 to 7 days with no significant change in the lactic acid content until the time of vatting.

In the second hawthorn fermentation stage, the initial content of lactic acid was basically the same as that of the main acid content when the wine spirits were released from the vat during the period of big hawthorn, which indicated that the steps of distilling the spirits when they were released from the vat had a small impact on the loss of organic acid and the metabolism of the organic acid was slow, which was consistent with the actual situation of the production process of clear-flavored white wine.

The content of lactic acid increased gradually from 0 to 16 days in the early stage of fermentation but the growth rate slowed down, and the content of lactic acid decreased slowly from 16 days to the time of vatting.

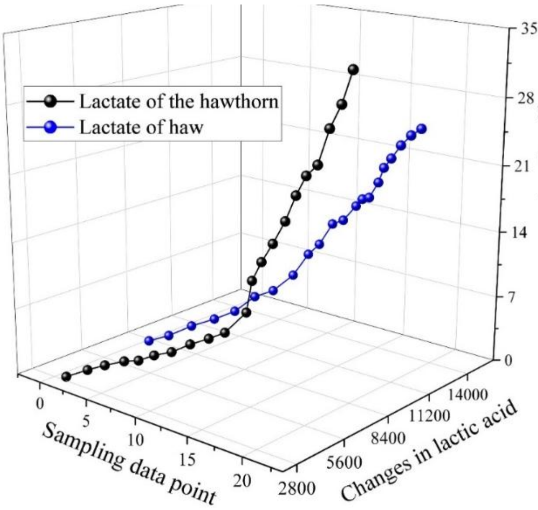

*Figure 5: Changes in lactic acid in the fermentation of big hawthorn and haw*

The changes of acetic acid during the fermentation of wine spirits of Dazha and Erzha are shown in Fig. 6.

Analyzing the changes of acetic acid in different fermentation stages of Dazha and Erzha of clear-flavored white wine, it was found that acetic acid increased significantly from 0 to 16 days in the early stage of fermentation, and the growth rate slowed down after 16 to 17 days.

On the whole, the content of acetic acid in wine grains was in a rising state from the time of vatting to the time of vatting out, the growth rate of acetic acid content was higher in the early stage of fermentation from 0 to 5 days, the growth rate of acetic acid was slowed down after 5 to 20 days, and the growth rate of acetic acid was significantly increased again from 21 days to the time of vatting out.

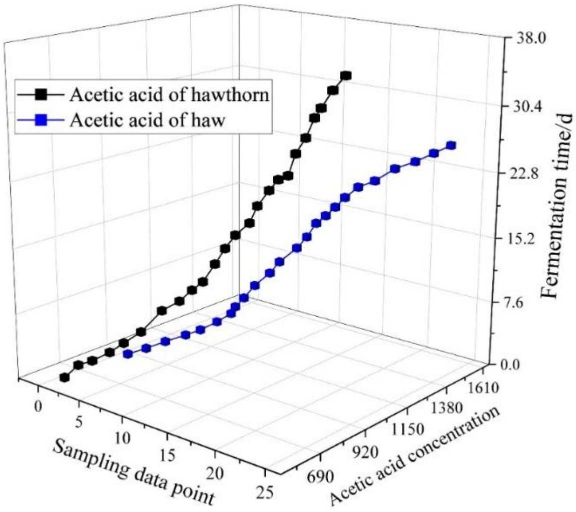

*Figure 6: Changes in acetic acid during fermentation of large hawthorn and haw wine*

Combining the changes of lactic acid during the fermentation of wine spirits in the above graph, the lactic acid content in large hawthorn wine spirits during the period of spirits formation was about 3.00~8.00 $g / L$, and the acetic acid was about 0.62~0.95 $g / L$. The lactic acid content in the second hawthorn wine spirits was 7.50~14.32 $g / L$, and the acetic acid content was 0.87~1.67 $g / L$.

It can be seen that the main acid content of the second hawthorn spirits is significantly higher than that of the big hawthorn spirits. This is because when the second hawthorn distillation is carried out, auxiliary materials will be added again, and the second hawthorn spirits are more loose compared with the big hawthorn spirits. And at this time, the spirits already contain various metabolic substances produced in the fermentation stage of hawthorn, so the microorganisms in the second hawthorn stage multiply rapidly, and the temperature rises in advance. At the same time, because of the mixing of the second hawthorn, the currant is fine, the moisture is big, the temperature of the tank is high, the high temperature time is significantly earlier than that of the big hawthorn. The high temperature and the mixing process of the second hawthorn were suitable for the growth of acid-producing bacteria, so the acid content of the main body of the second hawthorn wine was significantly higher than that of the big hawthorn wine.

## 4.4 Average concentration of non-volatile organic acids in clear-flavored white wine

A variety of nonvolatile organic acids in Qing Xiang Baijiu were characterized by BSTFA derivatization combined with GC-MS analysis, with a view to understanding the content of nonvolatile organic acids in Qing Xiang Baijiu. The average concentrations of nonvolatile organic acids in clear-flavored white wine are shown in Table 4. A total of lactic acid, hexanoic acid, hydroxyacetic acid, 2-hydroxybutyric acid, 2-hydroxy-2-methylbutyric acid, furoic acid, monoethyl malonate, 3-hydroxybutyric acid, 2-hydroxy-3-methylbutyric acid, hydroxyisovaleric acid, 2-hydroxy-4-methylglutaric acid, monoethyl butanedioic acid, benzoic acid, maleic acid, succinic acid, 2,3-dihydroxypropionic acid, fumaric acid, glutaric acid, 3-benzenepropionic acid, and malic acid were involved in the table, Adipic acid, cinnamic acid, 2-hydroxy-3-phenylpropionic acid, heptanedioic acid, 4-hydroxybenzoic acid, tartaric acid, dodecanoic acid, octanedioic acid, vanillic acid, nonanedioic acid, citric acid, tetradecanoic acid, palmitoleic acid, hexadecanoic acid, linoleic acid, oleic acid, trans-oleic acid, octadecanoic acid.

The highest average concentrations were found in lactic acid and octadecanoic acid, which were $1155.26 \pm 563.98\ \mu g / L$ and $1145.26 \pm 501.69\ \mu g / L$, respectively.

*Table 4: The average concentration of non-volatile organic acids in fragrant liquor*

| Compound/ $\mu g / L$ | Fragrance | Compound/ $\mu g / L$ | Fragrance |
|---|---|---|---|
| Lactic acid | 1155.26 ± 563.98 | Furfurlic acid | 125.47 ± 45.05 |
| Hexanoic acid | 65.89 ± 2.71 | Monoethyl malonate | 30.45 ± 32.51 |
| Hydroxyacetic acid | 653.98 ± 52.48 | 3-hydroxybutyric acid | 168.96 ± 89.64 |
| 2-Hydroxybutyric acid | 204.65 ± 21.75 | 2-hydroxyn-3-methylbutyric acid | 175.24 ± 70.22 |
| 2hydroxyl-2-methyl butyric acid | 230.65 ± 40.28 | Isovalyl acid | 132.47 ± 75.59 |
| 2hydroxyl-4-Tetramethyl pentacid | 1256.11 ± 81.03 | 2,3-Dihydroxypropionate | 316.85 ± 124.62 |
| Ethyl butyrate | 304.87 ± 221.57 | Fumaric acid | 78.94 ± 31.25 |
| Benzoic acid | 460.62 ± 408.61 | Glutaric acid | 32.59 ± 32.66 |
| Maleic acid | 235.77 ± 102.42 | 3-Triphenyl acid | 59.85 ± 57.14 |
| Succinic acid | 159.86 ± 90.33 | Apple acid | 80.07 ± 51.25 |
| Adipate | 37.14 ± 21.24 | Tartaric acid | 7.45 ± 5.18 |
| Cinnamic acid | 39.72 ± 22.45 | Diacid | 1733 ± 721.65 |
| 2-hydroxyl -3-Benzyl acid | 62.59 ± 60.85 | Sinic acid | 125.65 ± 20.84 |
| Heptanic acid | 482.62 ± 66.31 | Vanillin acid | 35.91 ± 12.56 |
| 4-hydroxybenzoic acid | 25.79 ± 22.08 | Nonazic acid | 118.62 ± 47.32 |
| Fourlic acid | 121.52 ± 41.57 | Oleic acid | 65.71 ± 10.25 |
| Palm oil acid | 132.95 ± 60.42 | Antioleic acid | 40.64 ± 14.23 |
| VPH | 90.57 ± 70.14 | Octolic acid | 1145.26 ± 501.69 |

# 5 Conclusion

In this paper, the effects of organic acids on volatile flavor substances in wine spirits and their dynamic changes were detected, and a prediction model for the quantitative analysis of organic acids in white wine was established by combining partial least squares regression analysis. The accurate quantification of organic acids in liquor was verified by model fitting. Combined with GC-MS analysis, we analyzed and characterized the concentration of various non-volatile organic acids in clear-flavored liquor.

(1) The RMSEP range of the PLSR quantitative analysis model for organic acids and aldehydes in baijiu was 0.086~0.425. The RPD values ranged from 3.895 to 12.007, with a high prediction accuracy. The resulting $R^2$ values for the major organic acids in white wine: acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid, and isovaleric acid, were 0.912, 0.975, 0.983, 0.989, 0.974, and 0.963, respectively.

(2) The fermentation temperatures of hawthorn and second hawthorn in different fermentation vessels all showed the trend of rapid increase in the early stage, maintaining stability in the middle stage, and decreasing slowly in the late stage. The top fermentation temperature of the second hawthorn in different fermentation containers was reached about the 7th day after entering the tank. And the fermentation temperature in the stone cellar is higher than that in the stainless steel tank and ceramic tank.

(3) During the fermentation stage of the second hawthorn of clear-flavored white wine, the initial content of lactic acid is basically the same as that of the main acid content when the wine

comes out of the vat during the period of big hawthorn, which is consistent with the actual situation of the production process of clear-flavored white wine. Acetic acid, on the other hand, increased significantly from 0 to 16 days at the beginning of fermentation, and the growth rate slowed down after 16 to 17 days.

(4) GC-MS analysis showed that lactic acid and octadecanoic acid among the nonvolatile organic acids had the highest average concentration in the clear-flavored white wine.

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