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Optimization of Fermentation Process and Improvement of Nutritional and Functional Characteristics of Jujube-Wolfberry Compound Fruit Wine

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ABSTRACT

Ziziphus jujuba Mill. (Jujube) and Lycium barbarum L. (wolfberry), as traditional medicinal and edibleresources, are rich in various active components and exhibit significant antioxidant and immunomodulatory functions. Developing them into a fermented compound fruit wine represents an important direction for integrating their health benefits with innovative flavors. Traditional fruit wine research has mostly focused on limited physicochemical indicators, making it difficult to fully elucidate the material transformation rules during the fermentation of complex substrates. This study employed single-factor experiments and orthogonal experiments combined with untargeted metabolomics technology to systematically investigate the optimal fermentation process: and dynamic changes of metabolites in jujube-wolfberry compound fruit wine. Single-factor experiments analyzed the effects of yeast strain, fermentation temperature, inoculum size, solid-liquid ratio, and sulfur dioxide addition on fruit wine quality. Orthogonal experiments determined the order of influence of each factor on sensory evaluation as sulfur dioxide addition > fermentation temperature > solid-liquid ratio > inoculum size, with the fruit wine exhibiting optimal sensory quality under the optimal process conditions. Metabolomic detection revealed 1800 metabolites identified in positive ion mode and 1102 in negative ion mode. A total of 1214 differential metabolites were screened after fermentation, among which beneficial active components such as 6-hydroxyhexanoic acid (FC=349.42) were significantly upregulated, while adverse substances such as L-histidine and potentially toxic alkaloids were significantly downregulated. Furthermore, metabolic pathways including amino acid and lipid metabolism were remodeled. This study systematically mapped the metabolite transformation profile and quality formation mechanism of jujube-wolfberry compound fruit wine before and after fermentation, providing a solid scientific basis for quality control, functional exploration, and industrial upgrading of this characteristic fruit wine.

KEYWORDS

Ziziphus jujuba mill; Lycium barbarum L; Compound fruit wine; Fermentation process; Metabolomics; Differential metabolites; Quality regulation

1 Introduction

Red dates (Ziziphus jujuba Mill.) and barbarian wolfberry (Lycium barbarum L.) are traditional food and medicine foods. Modern research has confirmed that red dates are rich in polysaccharides, cyclic nucleotides, and phenolic acids, while barbarian wolfberry is renowned for its characteristic active components such as barbarian wolfberry polysaccharides, carotenoids, and betaine. Both have been proven to possess significant antioxidant and immunomodulatory functions [1]. Developing a fermented fruit wine by combining the two is an important approach to integrate their health attributes and create novel flavored beverages.

Fruit wine fermentation is a complex biochemical process in which microorganisms such as yeast convert sugars, proteins, and other components in the substrate into ethanol, organic acids, and a series of flavor compounds [2]. This transformation process is crucial in determining the final sensory quality, stability, and functional potential of the product. However, traditional studies often rely on conventional indicators such as alcohol content and total acidity, making it difficult to fully reveal the complete metabolic transformation profile of complex substrates like jujube barbary wolfberry during fermentation.

Metabolomics technology provides a powerful tool for systematically analyzing the patterns of material changes before and after fermentation [3]. It can unbiasedly identify and quantify all small-molecule metabolites in biological systems, and has been widely applied to the mechanistic studies of fermentation systems such as wine and beer. For example, studies have shown that fermentation can significantly alter the amino acid profile and phenolic composition in yellow rice wine [4]. However, for the specific system of jujube barbary wolfberry composite fruit wine, existing research still focuses mostly on process optimization or changes in a single category of components (such as polyphenols), lacking systematic studies that reveal the collaborative transformation patterns of multiple categories of substances (such as organic acid derivatives, characteristic peptides, and lipid molecules) during fermentation at the global metabolite level [5].

Therefore, this study aims to employ non-targeted metabolomics techniques to comprehensively analyze the changes in metabolites of jujube barbary wolfberry composite fruit wine before and after fermentation. The goal is to systematically identify differential metabolites, elucidate their transformation patterns, and explore the impact of these changes on the final product quality from the perspectives of flavor and function, in order to provide a solid scientific basis for quality control and product upgrading of this specialty fruit wine.

2 Materials and Reagents

2.1 Materials and Reagents

2.1.1 Materials

Xinjiang Hetian jujube, Xinjiang barbarary wolfberry, white sugar, online shopping; pectinase, cellulase, Weifang Kangdien Biotechnology Co., Ltd.; RW yeast, Angel Yeast Co., Ltd.; K1 yeast, HG-1 yeast, L2323 yeast, Lallemand Inc; WB06, W34/70, Fermentis Yeast Company.

2.1.2 Reagent

Anhydrous ethanol, hydrochloric acid, sodium hydroxide, glycerol, copper sulfate, methylene blue, potassium sodium tartrate, glacial acetic acid, potassium ferrocyanide, anhydrous glucose, and potassium dihydrogen phosphate (analytical grade), provided by Chengdu Kelong Chemical Co., Ltd.; DPPH free radical scavenging rate detection kit, provided by Shanghai Yuanye Biotechnology Co., Ltd.; plant polyphenol (TP) content detection kit and plant flavonoid content detection kit, provided by Beijing Solarbio Co., Ltd.

2.2 Instruments and Equipment

Milli-QIQ7000 ultrapure water system, Sigma (Germany); Microfuge20R benchtop high-speed freezing centrifuge, Beckman (USA); MIR-554-PC low-temperature constant temperature incubator, Puruishi (Japan); 101-WA electric heating drying oven, Shanghai Yiheng; precision analytical balance and portable autoclave, Shanghai Lichen Instrument Technology Co., Ltd.; UV spectrophotometer, Shanghai Meipuda Co., Ltd.

2.3 Experimental Method

2.3.1 The Process Flow and Operational Key Points of Jujube-Barbary Wolfberry Composite Fruit Wine

Red dates, barbarary wolfberry → cleaning → removing the pits of red dates → hydration → crushing → enzymatic hydrolysis → juicing → adjusting the composition → inoculating yeast → pre-fermentation → post-fermentation → red dates barbarary wolfberry composite fruit wine

2.3.2 Operation Points

Raw material pretreatment: Wash the red dates and barbary wolfberry with running water 2-3 times to remove surface dust and impurities. Remove the pits from the red dates, while barbary wolfberry does not need to be seeded and can be used directly. Mix the raw materials in a ratio (red dates: barbary wolfberry=1: 1), then add purified water to achieve a material-to-water ratio of 1:3 (w/w), and soak for 30 minutes.

Crushing and enzymatic hydrolysis: The mixed raw materials were crushed to a homogenized particle size of ⩽2 mm using a pulper. A composite enzyme (pectinase 80 mg/L, cellulase 40 mg/L) was added and enzymatic hydrolysis was performed at 45° C for 2 hours. After the hydrolysis, the temperature was rapidly raised to 80° C and maintained for 5 minutes to inactivate the enzymes.

Juicing and Component Adjustment: Use gauze to extract the juice, collect the juice, and measure the initial sugar content. Calculate the required water volume based on the target alcohol content (11% vol), then add purified water to adjust the sugar content to 24 Brix. In the subsequent material-liquid ratio experiment, the material is the 24 Brix sugar content red date barbary wolfberry extract, and the liquid is a 24 Brix sugar content white sugar solution.

Yeast inoculation and fermentation: The yeast inoculation concentration was 1.5 g/L (viable count $\geqslant 1\times10^7$ CFU/mL). Prior to inoculation, the yeast was resuspended in a 5% glucose solution and activated at 30° C for 20 minutes. Pre-fermentation was conducted at 22° C for 7 days, with daily stirring twice. After pre-fermentation, the mixture was transferred to 4°C for 7 days of post-fermentation to promote clarification and flavor maturation of the wine.

Fermentation Monitoring and Termination: Monitor sugar content and temperature daily. When the sugar content drops to ⩽6 Brix and the alcohol content stabilizes, terminate the fermentation. Add 4‰ soapstone to the liquor, chill at 4℃ for 7 days, then filter through diatomaceous earth and age for 30 days to obtain the jujube barbary wolfberry composite fruit wine [6].

2.3.3 Experiment of Single Factor

The single-factor initial conditions were set as follows: temperature 28° C, inoculation amount 1.5 g/L, feed-to-liquid ratio 10: 0, and initial sugar content 24 Brix. Based on this, single-factor experiments were conducted on five process parameters: yeast varieties (W-34/70, WB-06, HG-1, D254, L2323, RW yeast), fermentation temperature (16°C, 18°C, 20°C, 22°C, 24°C), inoculation amount (0.05%,0.1%,0.15%,0.2%,0.25%), feed-to-liquid ratio (10:0, 9:1, 8:2, 7:3, 6:4), and sulfur dioxide addition (20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L, 60 mg/L). The effects of these single factors on the physicochemical indicators and sensory evaluation of the composite fruit wine from red dates and barbarian wolfberry were investigated.

2.3.4 Statistical Analysis

Statistical analysis was performed using Excel 2021, SPSS 22.0, Origin 2021, and Orthogonal Design Assistant v3.1. The identification of non-volatile metabolites was based on the in-house database of Beijing Novogene Biotech Co., Ltd., with comprehensive comparisons conducted against public spectral databases including mzCloud, HMDB, MoNA, LIPID MAPS, and NIST 2020 MS/MS.

3 Results and Analysis

3.1 Single-Factor Experiment Optimization of Fermentation Conditions

3.1.1 Effect of Yeast Strain Fermentation on the Quality of Jujube and Barbarian Wolfberry Composite Fruit Wine

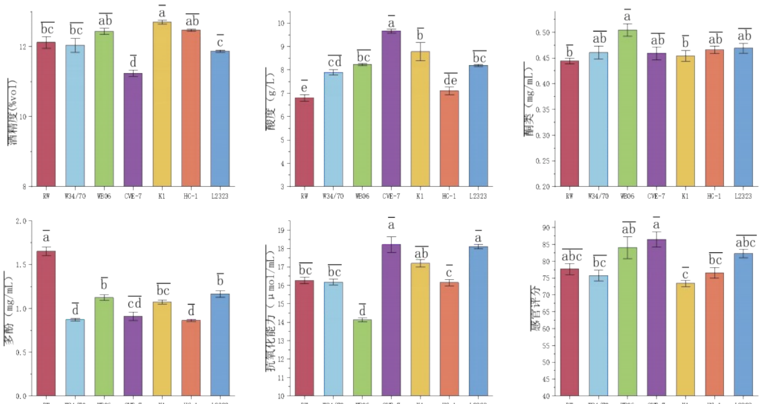


Figure 1 Effects of Different Fermentation Yeast Strains on the Quality of Jujube-Wolfberry Compound Fruit Wine Physicochemical differences among different groups are indicated by lowercase letters, and significant differences are indicated by different letters ($p<0.05$)

The influence of fermentation strains on the quality of jujube barbary wolfberry composite fruit wine is shown in Figure 1. K1, CVE-7, WB-06, and RW each have their own advantages in regulating the physicochemical properties of the wine. Among them, yeast K1, WB-06, and HG-1 exhibit the strongest wine production capacity, with the alcohol content of the fermented wine significantly higher than that of the other yeasts ($p<0.05$). CVE-7 demonstrates the strongest acid production capacity, significantly higher than the other yeasts ($p<0.05$). WB-06 produces the highest ketone content in the fermented wine, significantly higher than that of RW and K1 ($p<0.05$). RW yields the highest polyphenol content in the fermented wine, significantly higher than that of the other yeast fermented wines ($p<0.05$). CVE-7 exhibits the strongest antioxidant capacity in the fermented wine, significantly higher than that of RW, W34/70, WR, and HG-1 ($p<0.05$). Additionally, CVE-7 achieves the highest sensory score in the wine, significantly higher than that of W34/70, K1, and HG-1 ($p<0.05$). CVE-7 stands out in acid production and antioxidant capacity but has the weakest wine production, indicating its metabolic preference for generating acids and glycerol products. High acidity helps balance the high sugar and greasiness of jujube barbary wolfberry raw materials, enhancing the freshness and complexity of the wine. The ketone compounds in the fermented liquor form the foundation for complex aroma development. Although the polyphenol content of CVE-7 (0.882 mg/g) was lower than that of RW and WB06 strains, its outstanding antioxidant capacity (18.21 μmol/L) suggests the enrichment of polyphenol-contribution-limited but highly active antioxidant components (e. g., specific aglycones, short-chain phenolic acids, or glutathione). In sensory evaluation, CVE-7 achieved the highest score (86.4 points), indicating optimal harmony in color, aroma, and taste. Consequently, CVE-7 was selected as the strain for subsequent process optimization.

3.1.2 Effect of Fermentation Temperature on the Quality of Barbary Wolfberry Composite Fruit Wine

The effect of fermentation temperature on the quality of red date barbary wolfberry in fruit wine is shown in Figure 2:

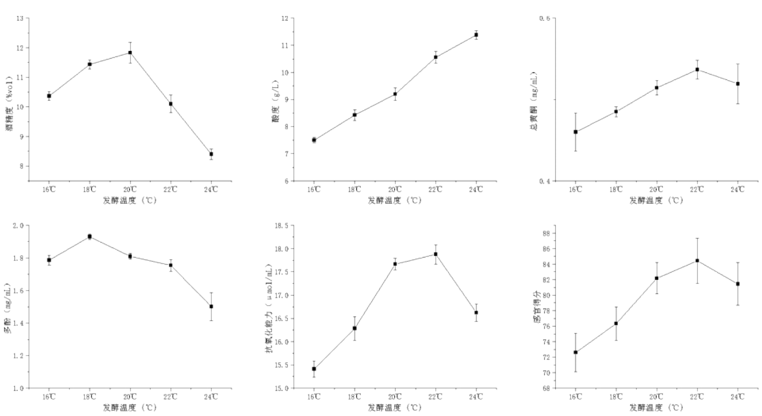


Figure 2 Effects of Different Fermentation Temperatures on the Quality of Jujube-Wolfberry Compound Fruit Wine

within the range of 16~20°C, the alcohol content of CVE-7 yeast increases with rising temperature but decreases after exceeding 20°C. The acid production continues to increase with temperature, which may promote certain acid-producing metabolic activities of the yeast. Acid is the fundamental framework of fruit wine flavor, but excessive acidity can lead to sensory disharmony, resulting in a sharp and pungent taste that affects the quality of the fruit wine [7]. The total flavonoids, polyphenols, antioxidant capacity, and sensory scores of the fruit wine all exhibit a trend of first increasing and then decreasing with rising temperature, peaking at 22°C, 18°C, 22°C, and 22°C, respectively. The optimal temperature not only promotes the extraction of polyphenols but also converts bound polyphenols into free forms through yeast β -glucosidase, enhancing their biological activity and flavor contribution. Meanwhile, stable metabolism facilitates the coordinated formation of pleasant flavor compounds such as esters, contributing to the development of typical aromas. Excessively high or low temperatures can induce yeast stress, leading to excessive production of higher alcohols and reducing sulfides, which disrupt flavor balance [8].

3.1.3 Effect of Material-to-liquid Ratio Fermentation on the Quality of Jujube-barbary Wolfberry Composite Fruit Wine

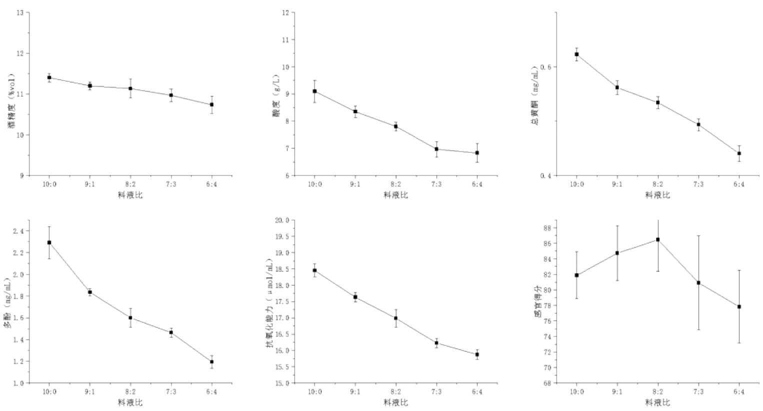


Figure 3 Effects of Different Solid-Liquid Ratios on the Quality of Jujube-Wolfberry Compound Fruit Wine

The effect of the material-liquid ratio on the quality of jujube-barbary wolfberry composite fruit wine is shown in Figure 3: The optimal process condition is a material-liquid ratio of 8: 2, under which the highest sensory score (86.4 points) and the best body harmony are achieved. The alcohol content of 11.14% vol avoids the limitation of insufficient substrate at low material-liquid ratios while preventing the inhibition of yeast activity by osmotic pressure at high material-liquid ratios. The acidity of 7.80 g/L effectively balances the high-sugar and greasy taste of the raw materials, outperforming other ratios (10:0 has excessively high acidity, while 7:3 and 6:4 have excessively low acidity). Although the ketone content of 0.53 mg/g is slightly lower than that of 10:0 and 9: 1, the yeast metabolism is more stable, and the proportions of esters, higher alcohols, and other flavor compounds are more harmonious, with more prominent typical aromas . Additionally, under the 8:2 ratio, although the polyphenols and antioxidant capacity are slightly lower than those of 10:0 and 9:1, the synergistic effects of alcohol content, acidity, and flavor compounds are better, resulting in the best overall sensory experience.

3.2 Orthogonal Test on Fermentation Process of Composite Fruit Wine of Red Date and Barbary Wolfberry

The results and analysis of the orthogonal experiment on the fermentation process of jujube-barbary wolfberry composite fruit wine are shown in Table 3. As can be seen from Table 3, the main and secondary order of the influencing

Table 3 Results and Analysis of Orthogonal Test for Optimization of Fermentation Process of Jujube-Wolfberry Compound Fruit Wine

test number	factor				Taste evaluation
	A	B	C	D	
Experiment 1	1	1	1	1	67.4
Experiment 2	1	2	2	2	75.6
Experiment 3	1	3	3	3	64. 1
Experiment 4	2	1	2	3	91.3
Experiment 5	2	2	3	1	92. 1
Experiment 6	2	3	1	2	81.9
Experiment 7	3	1	3	2	77.5
Experiment 8	3	2	1	3	72.7
Experiment 9	3	3	2	1	69.3
mean value1	69.03	78.73	74.00	76.27	
mean value 2	88.43	80. 13	78.73	78.33	
mean value 3	73. 16	71.76	77.90	76.03	
range	19.40	8.36	4.73	2.30	

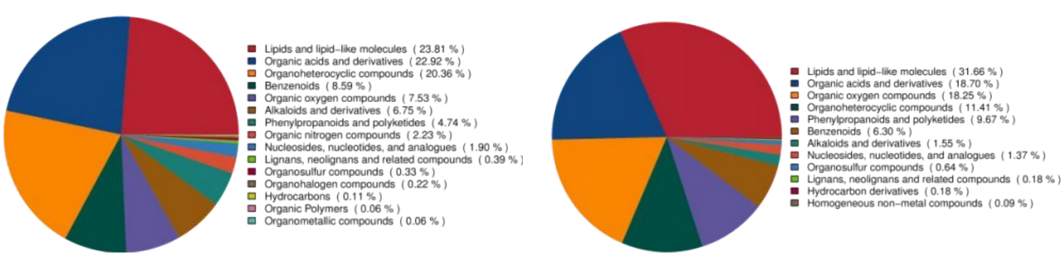
factors on the quality of jujube-barbary wolfberry composite fruit wine obtained through range analysis is inoculation amount> fermentation temperature> material-liquid ratio> sulfur dioxide addition amount. The optimal fermentation process parameters are A2B2C2D2, namely inoculation amount 1.5g/L, fermentation temperature 20℃, material-liquid ratio 8:2, and sulfur dioxide addition amount 30mg/L. As can be seen from Table 4, the variance of inoculation amount has a highly significant impact on the sensory score of the fruit wine ($P<0.01$), while the variance of fermentation temperature and material-liquid ratio has a significant impact ($P<0.05$). However, the addition amount of sulfur dioxide has no significant impact. Under this optimal fermentation process, the sensory quality of the fruit wine is the best, with the best coordination of alcohol content, acidity, flavor compounds, and antioxidant capacity, verifying the expected results.

Table 4 Analysis of Variance for Orthogonal Test Results

origin	Sum of Squares of Deviations (SS)	Degrees of Freedom(df)	mean square (MS)	F value	P value	significance
calibration model	952.68	6	158.78	13.05	0.002	**
nodal increment	52216.39	1	52216.39	4300.52	0.000	***
inoculum concentration	654.22	2	327. 11	26.93	0.001	**
fermentation temperature	136.55	2	68.28	5.63	0.0032	**
liquid to solid ratio	45.72	2	22.86	1.88	0.041	*
sulfur dioxide addition amount	56. 19	2	28. 10	2.31	0. 159	
deviation	97.24	8	12. 15			
total	53266.31	9				
Corrected total	1050.00	14				

3.3 Analysis of Flavoring Substances and Metabolomics

To analyze metabolic patterns and further elucidate the intrinsic mechanisms of flavor changes, based on the self-built database of Beijing Novogene Biotech Co., Ltd., integrated public databases, AI prediction databases, and other databases, the compound fruit wine of red dates and barbarian wolfberry was analyzed and detected. A total of 1,800 positive ion mode metabolites and 1, 102 negative ion mode metabolites were identified in 10 samples. The chemical classification of the identified metabolites was statistically analyzed, and a pie chart was drawn to reflect the classification of detected metabolites and the number of each classification. All metabolite classifications are shown in Figure 6. Under the positive ion mode, 427 types of lipids and lipid-like molecules, 411 types of organic acids and their derivatives, 365 types of organic heterocyclic compounds, 154 types of benzene compounds, 135 types of organic oxides, 121 types of alkaloids and their derivatives, 85 types of phenylpropanoids and polyketones, and 102 types of other substances were identified. Under the negative ion mode, 347 types of lipids and lipid-like molecules, 205 types of organic acids and their derivatives, 200 types of organic oxides, 125 types of organic heterocyclic compounds, 69 types of benzene compounds, 17 types of alkaloids and their derivatives, 85 types of phenylpropanoids and polyketones, and 32 types of other substances were identified.



(a) ESI+ (b) ESI-

Figure 4 Types and Proportions of Non-volatile Metabolites in Jujube-Wolfberry Compound Fruit Wine 2.3.2 Screening of non-volatile metabolite differences

Table 5 Screening Results Table of Differential Metabolites

project	Total identification results of metabolites	Total number of metabolites with significant differences	The total number of metabolites that were significantly upregulated	The total number of metabolites that were significantly downregulated
positive ion	1800	769	353	416
anion	1102	445	167	278

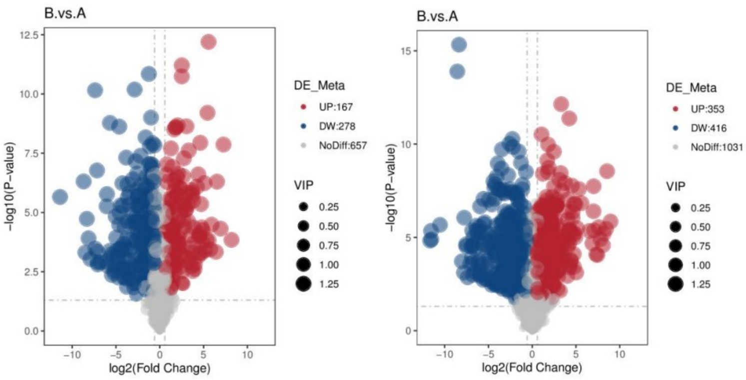


Figure 5 Volcano Plot of Non-volatile Metabolites in Jujube-Wolfberry Compound Fruit Wine Before and After Fermentation (a)ESI+ (b) ESI-

4 Conclusion and Discussion

This study optimized the fermentation process of jujube-barbary wolfberry composite fruit wine through single-factor and orthogonal experiments, determining the optimal conditions as follows: CVE-7 yeast, inoculation rate 1.5 g/L, feed-to-liquid ratio 8:2, fermentation temperature 20°C, and SO_2 addition amount 30 mg/L. Under these conditions, the resulting fruit wine exhibited the best sensory quality, with optimal coordination of alcohol content, acidity, flavor compounds, and antioxidant capacity. Non-targeted metabolomics analysis revealed that 2, 902 metabolites were identified post-fermentation, including 1,214 differential metabolites. Beneficial active components such as 6-hydroxyhexanoic acid were significantly upregulated, while adverse substances like L-histidine were markedly downregulated, simultaneously reshaping key metabolic pathways such as amino acids and lipids. This study provides a scientific basis for quality regulation and functional enhancement of the fruit wine from both process and metabolic perspectives.

The innovation of this study lies in combining the optimization of traditional fermentation techniques with non-targeted metabolomics technology, systematically revealing the material transformation and metabolic pathway regulation patterns of the fermented fruit wine composed of red dates and barbarian wolfberry. The results indicate that the optimal process not only improves the sensory characteristics of the fruit wine but also enhances its nutritional functional potential by promoting the accumulation of beneficial components and reducing harmful substances. Although the current study comprehensively analyzes the changes in metabolite composition, the specific biological effects and molecular mechanisms of key active components still require further validation through cell or animal experiments.

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