International Journal of Applied Food Science and Nutrition

PRIME OPEN ACCESS

Volume 1, Issue 1

Research Article

Date of Submission: 01 March, 2025
Date of Acceptance: 28 March, 2025
Date of Publication: 07 April, 2025

Exploring Alternatives to Traditional Huangjing Processing Methods with Mixed-Culture Fermentation and its Mechanisms

Wenjing Gao[1,2], Yangchen Mao[1,2], Zhenzhen Wang[1,2],Ruyi Sha[1,2], Min Cai[1,2*] and Jianwei Mao[1,2*]

[1]School of Biological and Chemical Engineering, Zhejiang University of Science and Technology, Hangzhou 310023, China

[2]Zhejiang Provincial Key Laboratory for Chemical & Biological Processing Technology of Farm Product, Hangzhou 310023, China

[*]Corresponding Author
Min Cai and Jianwei Mao, School of Biological and Chemical Engineering, Zhejiang University of Science and Technology, Hangzhou 310023, China and Zhejiang Provincial Key Laboratory for Chemical & Biological Processing Technology of Farm Product, Hangzhou 310023, China.

Citation: Cai, M., Mao, J., Gao, W., Mao, Y., Wang, Z., et al. (2025). Exploring Alternatives to Traditional Huangjing Processing Methods with Mixed-Culture Fermentation and its Mechanisms. *Int J Appl Food Sci Nutr, 1*(1), 01-10.

Abstract
Huangjing, as the dried rhizome of Polygonatum sibiricum, is widely distributed and used in traditional medicine and food in China. However, it needs to be processed before direct consumption as it has a numbness and irritant sensation. In this study, a mixed-culture fermentation of huangjing with yeast and Rhizopus was performed and the supernatant of the broth was obtained for analysis. It was found that after 72h fermentation, the numbness totally disappeared and the sense of bitterness was also largely reduced. In addition, fermentation reduced the level of total saponins ($P < 0.05$), which was suggested to account for the numbness.

In terms of safety, the concentration of methanol in the FB group decreased to 0.5 mg/mL after fermentation at 25°C. There were only cadaverine (3.52 μg/mL) and tyramine (6.13 μg/mL) can be detected among biogenic amines in the FB group, indicating the good safety. The fermentation also exhibited a significant rise ($P < 0.05$) in the abundance of certain nutritional metabolites and three delicious amino acids (glycine, alanine and proline). Fermented huangjing also exhibited a better antioxidant effect in ABTS scavenging as well as regulating MDA content and SOD activity. These data indicated that a mixed-culture fermentation of huangjing with yeast and Rhizopus possessed large potential as an alternative method to replace the traditional consumption way.

Keywords: Huangjing, Fermentation, Biogenic Amines, Metabolites and Taste

Abbreviations
- FB, fermentation broth.
- SS, steaming and solarizing.
- AE, aqueous extract.
- GC-MS, gas chromatography–mass spectrometry.
- PCA, principal components analysis.
- OPLS-DA, orthogonal partial least squares-discriminant analysis).
- VIP, variable importance in the projection.
- KEGG, Kyoto Encyclopedia of Genes and Genomes.
- TCA, tricarboxylic acid cycle.
- HPLC, high-performance liquid chromatographic.
- Gly, glycine.

- Ala, alanine.
- Pro, proline.
- Asp, aspartate.
- Glu, glutamate.
- Val, valine.
- Leu, leucine.
- Lys, lysine.
- Arg, arginine.

Introduction

Huangjing in Chinese traditional medicine generally refers to the dried rhizome of polygonatum sibiricum, which belongs to the family lily. Huangjing is widely distributed in the middle and eastern regions of China. These rhizomes are also rich in various nutrients and bioactive compounds, thus they are fully exploited nutritional and health promoting properties from the ancient [1, 2]. Huangjing has several medical functions such as immunity enhancing, anti-tumor, antioxidant and depression prevention [1]. Due to the abundant content of polysaccharides, saponins, isoflavones and other components in huangjing, there is also a negative impact on the odor and taste [3].

Direct eating of huangjing has a numbness sensation. Thus, it needs to be processed to reduce its irritability before consumption [4]. From ancient times to the present, there are several recorded methods for processing huangjing, including steaming and solarizing nine times, alcohol processing, honey processing and fermentation. The most popular way to process huangjing was steaming and solarizing (SS), which could reduces the irritability and have an impact on the alteration of internal chemical composition [4]. However, the method of steaming and solarizing wastes energy and has a lack of standard. The huangjing processed by SS method still has a strong bitter taste when soaked in water or alcohol for consumption. In addition, a portion of nutritional components are difficult to utilize by human body. It is necessary to find a more palatable, effective, and energy-saving way to replace the traditional method.

Fermentation is an emerging method for processing the medicinal materials. It uses modern bioengineering techniques to reduce the hazardous ingredients of raw material and give it a unique taste and odor [5]. Certain kinds of probiotics have been largely applied in herb fermentation, such as Lactobacillus, yeast and Rhizopus [6]. Lactobacillus species was the most commonly used strains in the food industry, which were also utilized in the production of fermented herbal medicine. They added a favorable flavor to the fermented product and showed a high safety [7]. Yeasts (Saccharomyces cerevisiae) were largely used in the field of food as they could change sugars into ethanol and CO_2, thereby contributing unique flavors to the products. Adding yeast to grain products such as bread, cakes, cookies, and baked goods can not only increase its nutritional value and flavor, also promote gastrointestinal digestion through reducing the levels of anti-nutrients and toxins [8]. However, the application of yeasts in herbal fermentation was not fully exploited. Rhizopus is a common mold, with its spores present in air, soil and various surfaces of utensils. Rhizopus contains abundant amylase, generally including liquefying and saccharifying amylase, which made it an important role in brewing [9]. During fermentation, Rhizopus can produce lactic acid, succinic acid, trace amounts of alcohol and aromatic esters. Thus, it was widely used in producing edible sweet wine and saccharified food.

Compared with single-strain fermentation, mixed-culture fermentation was able to utilize collaborative advantages of multiple microorganisms and enzymes. In the mixed-culture fermentation, there was enhanced fermentation efficiency, more abundant products, and less toxic residual in the fermentation broth [10]. In this study, the supernatant of huangjing fermented with yeast and Rhizopus (FB) was performed to investigate the possibility of replacing the traditional way of consumption. The flavor, nutrition, and safety characteristics of the FB were also fully tested and compared with the water and alcohol extract of huangjing.

Materials and Methods

Preparation of Huangjing Aqueous Extract

Huangjing (polygonatum sibiricum) was harvested on October 9, 2023. It was 20 months after seeding in Hubei, China. The rhizome of huangjing were cleaned with distilled water and cut into pieces. The sample was then placed into baking oven for drying in 65°C for 3h. After that, the sample was added to a blender for crushing. To obtain the aqueous extract (AE) of huangjing, the sample was performed ultrasound water extraction for 30min and filtered to remove the sediment. To obtain the alcohol extract of steaming and solarizing (SS) sample, the cleaned rhizome of huangjing was steamed in a steamer under normal pressure and water for 6h. The sample was then laid flat in sunlight until the water content is under 10%. After that, the sample was added to a blender for crushing and performed ultrasound 40% ethanol extraction.

Preparation of Huangjing Fermentation Broth

The crushing powder of huangjing mentioned above was subjected to UV sterilization for 60min. Commercial wheat starter (Saccharomyces cerevisiae RV171: Rhizopus oryzae Q303 = 1:1, Hubei Angel Yeast Co., Ltd.) was dissolved in a water bath (ratio of 6% v/v) at 35°C for 30min. Subsequently, the sterilized powder of huangjing was added to the mixed bacteria suspension at a ratio of 1: 25 (v/v) and fermented at 35°C for 72h. After centrifugation, the supernatant was obtained and sterilized as fermentation broth (FB).

Sensory Analysis

A group of specialists (14 members, 7 men and 7 women) were invited to evaluate the taste of the huangjing samples. All the specialists were aged between 28 to 39 and employed in the biotechnology and food engineering department of ZUST. They had abundant experience of this analysis and assessed the colour, aroma, sweetness, acidity, bitterness, numbness and off-flavour. The 7 aspects attributed to the sensory characters of three groups and the final result converted to the percentage of the maximum value). During the analysis process, all members tested independently. The samples were tested randomly at room temperature in 50 mL cups. The study was reviewed and approved by the Zhejiang university of science and technology IRB under approval (n.10237/CAI 49827034.0.0000.0032) and informed consent was obtained from each subject prior to their participation in the study.

Detection of Volatile Components with GC-MS

The detection of volatile components in three groups were applied using the gas chromatography mass spectrometry (GC-MS) with GCMS-TO8040 NX (Shimadzu, Kyoto, Japan) [11]. As for sample preparation, 150μL of samples were taken into an EP tube and added 300μL of methanol and 10μL of ribosyl alcohol (25μg/mL). The samples were mixed well and treated with ultrasound at 0°C (ultrasound power=400W) for 10 min. After that, they were centrifuged at 12000r/min for 15 min to collect the supernatant. 50μL of the supernatant was taken and dried with a nitrogen blower. Then the samples performed a derivatization (amination) reaction with 100μL methoxyamine salt reagent and 100μL of bis (trimethylsilyl) trifluoroacetamide. The solution was incubated at 70 °C for 1.5h and cooled to room temperature before detection.

PCA (principal components analysis) and OPLS-DA (Orthogonal Partial Least Squares-Discriminant Analysis) analysis were performed using SIMCA 14.1 [12]. The stability and predictive ability of the OPLS-DA model were evaluated according to R2Y and Q2. The differential metabolites were obtained in the light of OPLS-DA (VIP ≥1.0) and t-test ($P < 0.05$).

Test of Amino Acids

Samples (1 mL) were taken into a hydrolysis tube and 10mL of 6 mol/L HCL was added. After evaporating 3-4 drops of phenol and cooled for 5min, the samples were then filled with high-purity nitrogen gas on a nitrogen blower and placed in a drying oven at 110°C for 22h. After cooling, the hydrolysis tube was carefully opened and all the hydrolysis solution was transferred to a 25mL volumetric flask. After rinsing the hydrolysis tube multiple times with deionized water, 1ml of filtrate was taken and dried at 45±5°C in a vacuum dryer. The sample was dissolved in 1mL of mobile phase and passed through a 0.22um aqueous microporous filter membrane. The final solution of 20μL was injected into the amino acid analyzer. Proline is measured at 440nm, and the remaining 17 amino acids are measured at 570nm.

RP-HPLC Analysis of Amines

Biogenic amines were extracted from the samples using 5% trichloroacetic acid and degreased with hexyl hydride. The samples were then derivatized with 1,7-diaminetetrane as the internal standard and dansyl chloride as the derivatizing agent. The analysis used a Shimadzu C18 (200mm×4.6 mm,5μm) chromatographic column with 0.01 mol/L ammonium acetate solution (A) - acetonitrile (B) containing 0.1% acetic acid as the mobile phase for gradient elution at a flow rate of 0.8 mL/min. Detection wavelength is 254 nm and column temperature is 35°C. 5 different biogenic amines standards including β- phenylethylamine, putrescine, cadaverine, histamine and tyramine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China).

Determination of Total Saponins and Phenolics

The total saponins content was tested by a spectrophotometric method according to the specific reaction of vanillin and sulfuric acid with the saponins [13]. The detection wavelength of the samples was 544 nm and the standard curve of oleanolic acid was $y = 5.0239x + 0.0183$, $R2 = 0.9942$. The results were exhibited in w/w.

The total phenolic content in the extract was determined using the folin phenol method and represented with gallic acid equivalent (mg/g). A series of gallic acid standard solutions with different concentrations were prepared using deionized water. Taking 100μL each concentration of gallic acid solution and add it to 500μL of 10% phenol reagent. The solution was then shaken thoroughly and mixed well. After 3 minutes of reaction, 400μL of $Na2CO3$ solution with a mass fraction of 7.5% was added. The reaction was under shaken condition in the dark at 25°C and 120 r/min for 1 h. The absorbance value of the solution was measured at a wavelength of 765 nm using a microplate reader.

Cells Culture Conditions and Biochemical Studies

Human intestinal epithelial cell lines (HIEC) were purchased from the National Center for Medical Culture Collection (Shanghai, China). Cells were cultured in complete medium containing 10% imported fetal bovine serum (FBS, Gibco) and 1% P/S (100 U/mL penicillin G and 100 μg/mL streptomycin, Gibco). Cells were cultured in an incubator in 5% CO2 and 37 °C condition. MTT cytotoxicity assay was performed according to the manufacturer's instructions. H2O2 (H112515, 50μM for modeling) and MTT (CT01–5) Assay Kit was purchased from Aladdin (Shanghai, China).

Levels of MDA in HIEC cells were determined by malondialdehyde assay kit (A003-4-1) according to the manufacturer's protocols. Levels of SOD were determined by superoxide dismutase (A001-3-1, WST-1 method) assay kit according to the manufacturer's protocols. All assay kits were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The absorbance of samples was measured with VERSA max tunable microplate reader (Molecular Devices, California, USA).

Statistical Analysis

All the data was calculated and analyzed with SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) presented as the mean value ± Standard Error of Mean (SEM). One- way ANOVA or t-test was used to analyze the significance of the differences. LSD method was applied for multiple comparisons. P value <0.05 was considered significant, P value <0.01 was considered extremely significant.

Results

Mixed-Culture Fermentation of Huangjing Improved its Taste and Reduced the Numbness

To relieve the numbness and bitterness of huangjing, a mixed-culture fermentation was performed. In brief, the mixture of strains contains yeast and Rhizopus, which called distiller's yeast in Chinese millet wine industry. After 72h fermentation, the numbness was totally disappeared in the supernatant of the fermentation broth (FB) group (Fig.1A), while the alcohol extract of steaming and solarizing sample (SS) still remains a bit of numbness. On the other side, fermentation also largely reduced the sense of bitterness. In addition, fermentation enhanced the acidity and slightly improved the sweetness and aroma of the broth, which brings it highest overall rating (Fig.1A).

Several studies indicated that the substances causing numbness in huangjing might be phenolics, flavanones or saponins [4]. After fermentation, the content of alcohols increased rapidly compared to the AE and SS groups (Fig.1B), indicating that the major transformation of substance in the fermentation might be the breakdown of carbohydrates and the generation of alcohols. In addition, the content of total phenolics and saponins were tested subsequently. The level of total saponins were also significantly declined in the FB group and the level of total phenolics decreased in the SS group (Fig.1C-D), which may account for the vanishment of numbness.

Huangjing Fermentation Broth Exhibited a High Safety in Terms of Methanol and Biogenic Amines

To further explore the safety of the fermentation broth, several safety related indicators were tested. Methanol is a by-product of Rhizopus when it performed pectin degradation of huangjing [14]. Methanol content in the FB group was significantly higher ($P < 0.05$) than that in the AE and SS groups. The concentration of methanol in the broth reached 2.1 mg/mL, which would have a risk of poisoning (Fig.2A). However, the methanol produced in fermentation could be controlled by reducing the temperature of incubation. The methanol content decreased to 0.5 mg/mL after fermentation in 25°C (FB-25) instead of 35°C, which is much lower than the level of China national standard (GB2757-2012). As for ethanol production, the content in the FB-25 group was reduced to 25 mg/mL (Fig.2B). As an index monitoring the fermentation efficiency of the yeast, the result suggested that the fermentation activity in 25°C reached 64.3% of that in 35°C.

Biogenic amines were another kind of potentially risky substances produced by microorganisms, including β-phenylethylamine, putrescine, cadaverine, histamine and tyramine [15]. Thus, the level of biogenic amines during the fermentation of huangjing was detected by HPLC, using precolumn derivatization with DNS-Cl. There was only tyramine (9.95 μg/mL) detected in AE group, while there were no biogenic amines mentioned above detected in SS group (Fig.2C). On the contrary, fermentation by yeast and Rhizopus produced a relative low content of cadaverine (3.52 μg/mL) and tyramine (6.13 μg/mL), which was quite lower than that of commercial Huangjiu and would not be harmful to health (Table 1). In comparison, commercial Huangjiu contains putrescine (24.47 μg/mL), cadaverine (3.83 μg/mL) and tyramine (44.28 μg/mL).

Mixed-Culture Fermentation of Huangjing Improved its Nutritional Value

To evaluate the metabolites of mixed-culture fermentation and provide a more intuitive explanation of the relationship between samples and different patterns of metabolites, PCA analysis were performed based on the content of metabolites acquired by GC-MS test in each group (Fig.3A). The results showed that FB group samples were tightly clustered near the origin of the axis (Fig.3A), suggesting that the established GC-MS method had good repeatability and stability. In addition, the metabolites produced in FB group have a significant cluster trend, which indicated that the metabolic activity of yeast and Rhizopus during fermentation was intense. To further explore the metabolic differences between AE, SS and FB groups, OPLS-DA analysis was performed. As two important score values, R2Y and Q2 can explain the quality of the model (Fig.3B). R2Y represents the interpretability of the model, which means a great difference between the two groups when it is close to 1. The model also generated a Q2 value through cross validation to assess its predictability [16]. The score of 0.966 proved its reliability. The sample points from the FB group were separated from the AE and SS group, indicating that fermentation of huangjing largely regulated the metabolites in yeast.

To further elucidate the specific changes of the metabolites between different groups, heatmaps with hierarchical clustering were performed according to the expression level of metabolites in AE, SS and FB groups. The results exhibited that the abundance of certain metabolites were highly raised in FB group compared to the AE and SS groups, such as galactose, fructose, acetamide and ethylene glycol (Fig.3C-E). Besides, the content of other metabolites has decreased in the FB group, suggesting the specific patterns of differences in the metabolites. Analysis was also focused on the changes in amino acids content between the FB and other groups of huangjing. Fermentation increased the level of Gly, Ala and Pro, while a significant decrease of Asp, Glu, Val, Leu, Lys and Arg was also observed compared to the AE and SS groups (Table.2). However, the mechanism on how each of the two microorganisms metabolize amino acids needs further exploration.

Fermentation Process of Huangjing Had Better Antioxidant Effects

The free radical scavenging potential of AE, SS and FB groups was valued by DPPH and ABTS scavenging assays. SEP extract exhibited concentration gradient dependent pattern in the percentage inhibition of free radicals. The SS and FB groups showed a rising DPPH scavenging ability than the AE group, while the ability between SS and FB groups was not significant (Fig. 4A). As for the ABTS assay, the FB group exhibited the best scavenging potential among the samples, which proved its enhanced antioxidant capacity (Fig. 4B). FB treatment of less than 10 times dilution will not impair the cell viability (Fig. 4C), thus the concentrations of 10- and 50-times dilution were selected for the next test. FB treatment decreased cell MDA level and increased cell SOD content as compared with the model group in a dose dependant manner (Fig 4D-E).

Discussion

Huangjing is a traditional Chinese medicinal herb that has been used for hundreds of years in China. However, raw huangjing can cause numbness in the tongue and throat, as well as lead to itching when in contact with the skin. Therefore, huangjing has to be well processed before used as medicine [17]. There are various components in huangjing, while there are currently no researches reporting on the specific ingredient giving rise to the numbness. After processing, the chemical composition of huangjing undergoes significant changes, with an increase in active ingredients and enhanced pharmacological activity. Exploring the detoxifying and enhancing effects of processed huangjing is of great significance for the safety and elucidating the processing mechanism.

It was reported that the stimulating components of huangjing are mainly concentrated in the ethylacetate fraction, according to the cytotoxicity of different polarity fractions. Thus, the author indicated that the main type of stimulating component is total phenols [18]. There are also other studies supposed that the substances causing numbness in huangjing could be flavanones or saponins [19]. It was reported that a single strain of Lactobacillus plantarum was used to ferment huangjing distilled water extract under static dark conditions [20]. The difference in polyphenol content between huangjing extract and fermentation broth is not significant, while the polysaccharide and the total acid content in the fermentation broth are largely increased.

It can be inferred that the rising level of polysaccharides and total acids might be the source of the sour and sweet taste of huangjing fermentation broth. In this study, the sensation of numbness was totally disappeared in the FB group. The content of certain compounds, including flavanones and saponins, diminished severely compared to the AE and SS groups after fermentation. These results revealed the advantage of the mixed-culture fermentation in reducing the toxicity and improving the taste. It indicated the potential to replace the traditional consumption way of soaking processed huangjing in water and alcohol.

Huangjing has a relatively high content of polysaccharides, which was 15.23% - 12.90%. In addition, the content of starch in huangjing was up to 3.62 g/100g [18]. In order to better decompose and utilize these substances, strains with saccharification ability were introduced in the fermentation. Polysaccharides in huangjing have large amount of α-(1→4) glycosidic bonds, which could be degraded by some kinds of molds efficiently [21]. Since Rhizopus was the major source of saccharification in huangjiu production, which was fermented by starch- rich rice. Thus, the mixed-culture fermentation of huangjing by yeast and Rhizopus could improve the utilization of biomass material.

Biogenic amines are a class of nitrogen-containing alkaline organic compounds that exist in various foods. The level was especially high in fermented foods such as sausages, wine, beer, and yellow wine [22]. Putrescine and cadaverine are precursors of carcinogens and nitroso compounds, while histamine and tyramine can cause headaches and digestive problems. Histamine is the most harmful to health among biogenic amines. Alcohol inhibits the activity of monoamine oxidase, which can decompose biogenic amines, thereby increasing the sensitivity of the human body to histamine. Therefore, the safety of biogenic amines in fermented broth especially the issue of histamine limit, is highly valued.

In this study, there were only very limited levels of cadaverine (3.52 μg/mL) and tyramine (6.13 μg/mL) detected in the fermentation broth, while the content of histamine was lower than the limit of detection. Therefore, the risk of excessive biogenic amines after mixed-culture fermentation was excluded. Another risk of safety originates from methanol. It could cause severe damage to the human body as a strong neurotoxin [23]. By using scientific methods in the fermentation process, methanol production could be reduced as much as possible. Choosing high-quality materials with less pectin and no spoilage was the first key point.

The molds in the fermentation are closely related to the generation of methanol. In this study, Rhizopus was supposed as the source of methanol during the fermentation. To limit the methanol level, the fermentation temperature was reduced to 25°C and the methanol content decreased to 0.5 mg/mL subsequently. It was also inferred that reducing the proportion of Rhizopus in the mixed-culture fermentation will further decrease the methanol production.

Amino acids, as decomposition products of proteins, are important indicators for evaluating the nutritional value and flavor of the fermentation broth [24]. According to the Table 2, the fermentation broth contains 17 types of amino acids, of which essential amino acids account for 47%. The proportion of essential amino acids is close to the recommended level (40%) by the World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO) [25]. The total amount of three delicious amino acids (umami amino acids, sweet amino acids, and aromatic amino acids) in the fermentation broth contributed about 56% of the total amino acid content. The levels of umami and sweet amino acids were higher than aromatic amino acids, indicating that the unique odor and taste of the fermentation broth may be related to high levels of delicious amino acids.

Conclusions

In conclusion, the results of the present study suggested that the mixed-culture fermentation of huangjing with yeast and Rhizopus was a new way of procession and consumption. It was better than traditional consumption way of soaking in water or alcohol in some aspects. It made the fermentation broth a sweet-and-sour taste with the aroma of alcohol. Meanwhile, it also completely eliminated the numbness of raw huangjing. In addition, fermented huangjing performed quite well in terms of safety and nutrition, as well as exhibiting a better antioxidant effect. Therefore, fermented huangjing possessed large potential as a functional food and health supplement. The specific mechanism of the mixed-culture fermentation and the improved proportions of yeast and Rhizopus need to be further studied.

Data Availability

All data presented in this study are available on reasonable request from the corresponding author.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

This research was funded by the Fundamental Research Funds for the Zhejiang University of Science and Technology (2023JLYB007).

	SS	FB	commercial Huangjiu
β - phenylethylamine	-	-	-
Putrescine	-	-	24.47
Cadaverine	-	3.52	3.83
Histamine	-	-	-
Tyramine	9.95	6.13	44.28

Table 1: Biogenic Amines Content Between SS, FB Groups and Commercial Huangjiu (Mg/Ml)

	AE	SS	FB
Asp	35.51 ± 6.38^{b}	55.63 ± 1.11^{c}	18.72 ± 4.58^{a}
Thr	6.66 ± 2.87^{a}	11.13 ± 0.89^{a}	13.21 ± 3.84^{a}
Ser	19.60 ± 3.23^{a}	25.31 ± 6.01^{a}	18.19 ± 6.82^{a}
Glu	359.36 ± 64.40^{b}	306.40 ± 66.1^{ab}	186.01 ± 48.3^{a}
Gly	16.08 ± 2.23^{a}	18.20 ± 4.09^{ab}	26.16 ± 4.97^{b}
Ala	33.35 ± 5.34^{a}	33.52 ± 8.22^{a}	59.99 ± 9.82^{b}
Cys	29.82 ± 4.42^{a}	43.82 ± 9.30^{a}	33.26 ± 4.79^{a}
Val	17.89 ± 2.39^{b}	17.75 ± 3.26^{b}	5.51 ± 0.53^{a}
Met	21.81 ± 1.90^{a}	26.53 ± 5.78^{a}	23.31 ± 3.52^{a}
Ile	14.21 ± 1.98^{a}	14.79 ± 3.32^{a}	9.63 ± 1.48^{a}
Leu	20.21 ± 3.15^{b}	20.63 ± 4.98^{b}	10.06 ± 1.74^{a}
Tyr	41.61 ± 1.70^{a}	46.49 ± 3.28^{a}	46.63 ± 2.32^{a}
Phe	32.30 ± 4.21^{a}	31.78 ± 6.62^{a}	27.34 ± 8.23^{a}
Lys	22.64 ± 3.87^{b}	18.57 ± 4.70^{b}	-
His	9.56 ± 1.66^{b}	9.20 ± 2.28^{ad}	4.86 ± 1.31^{a}
Arg	142.83 ± 23.00^{b}	121.99 ± 29.2^{b}	25.69 ± 4.06^{a}
Pro	-	9.37 ± 4.01^{c}	57.09 ± 1.77^{a}

Table 2: Changes in Amino Acids Content Between Fermentation and Other Groups of Huangjing (Mg/G DW)

Values are means ± SEM (n=4). Different superscripts in the same raw indicate significant differences ($p < 0.05$).

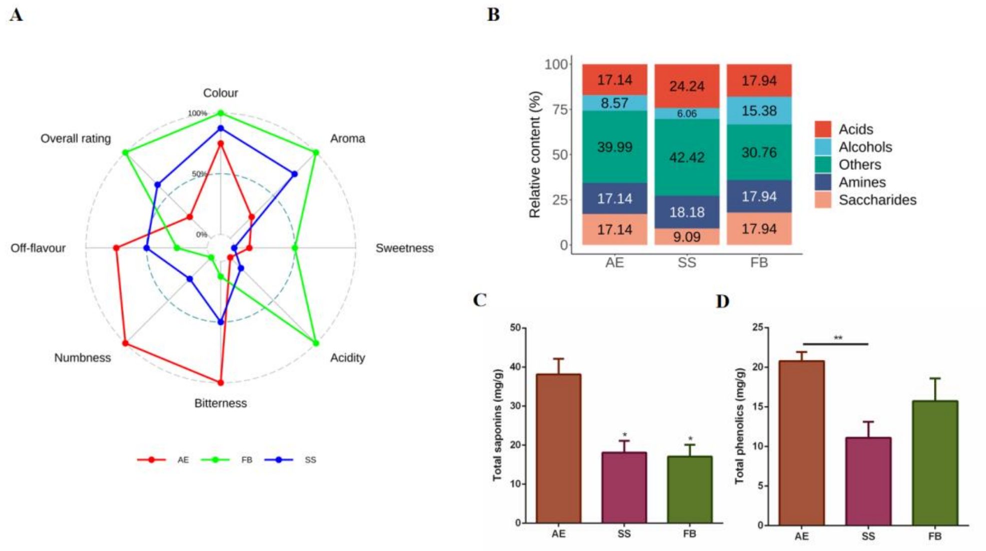

Figure 1: Mixed-Culture Fermentation of Huangjing Improved its Taste and Reduced the Numbness

- The sensory rating of samples in AE, SS and FB groups.
- Stacking histogram of volatile substances (derivatized) in AE, SS and FB groups.
- The level of total saponins.
- The level of total phenolics. Error bars were presented as Standard Error of Mean (SEM). *P < 0.05, **P <0.01 compared to the AE group.

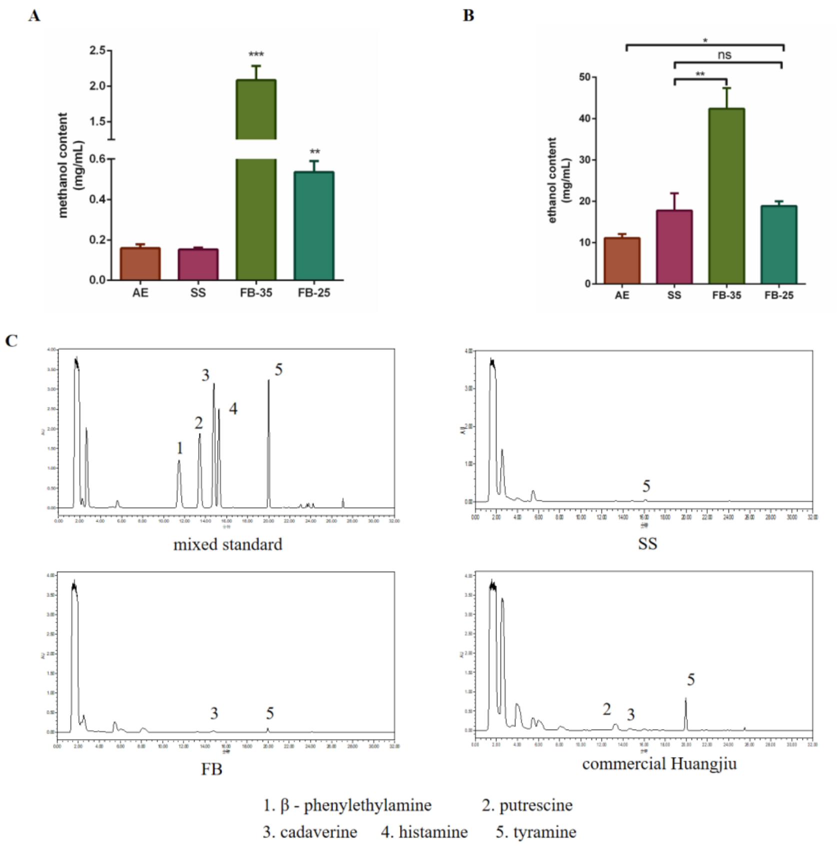

Figure 2: Huangjing Fermentation Broth Exhibited a High Safety in Terms of Methanol and Biogenic Amines

- The level of methanol in AE, SS FB (25 & 35°C fermentation) groups.
- The level of ethanol in AE, SS FB (25 & 35°C fermentation) groups.
- HPLC chromatogram of the different solutions.

Major compounds of biogenic amines were identified and compared to the standards by the HPLC method. Error bars were presented as Standard Error of Mean (SEM). *P < 0.05, **P <0.01 compared to the AE group.

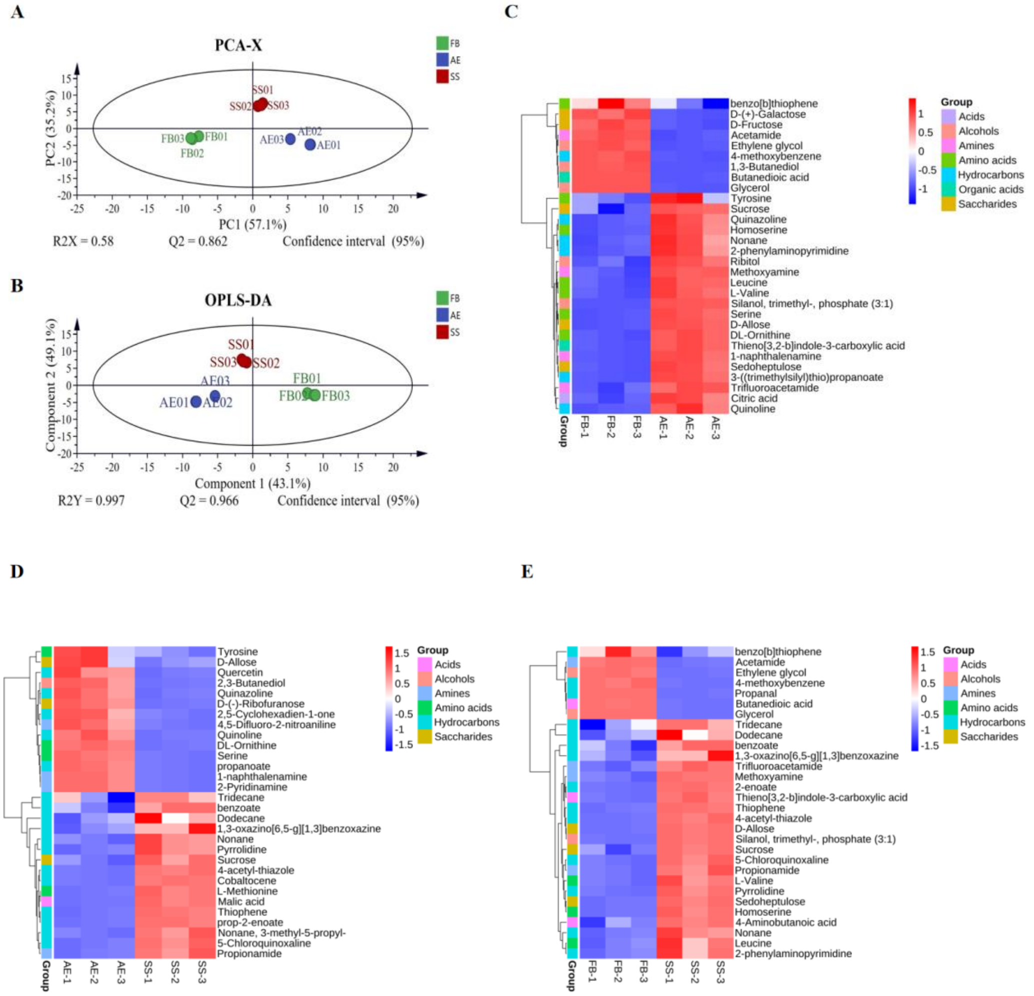

Figure 3: Mixed-Culture Fermentation of Huangjing Improved its Nutritional Value

- Principal components analysis (PCA) analysis score plots of the AE and FB groups based on the data acquired in GC-MS.
- Orthogonal partial least squares discriminant analysis (OPLS-DA) score plots of the AE and FB groups based on the data acquired in GC-MS.
- Analysis of differential metabolites in the FB versus AE.
- AE versus SS.
- FB versus SS.
- (VIP > 1).

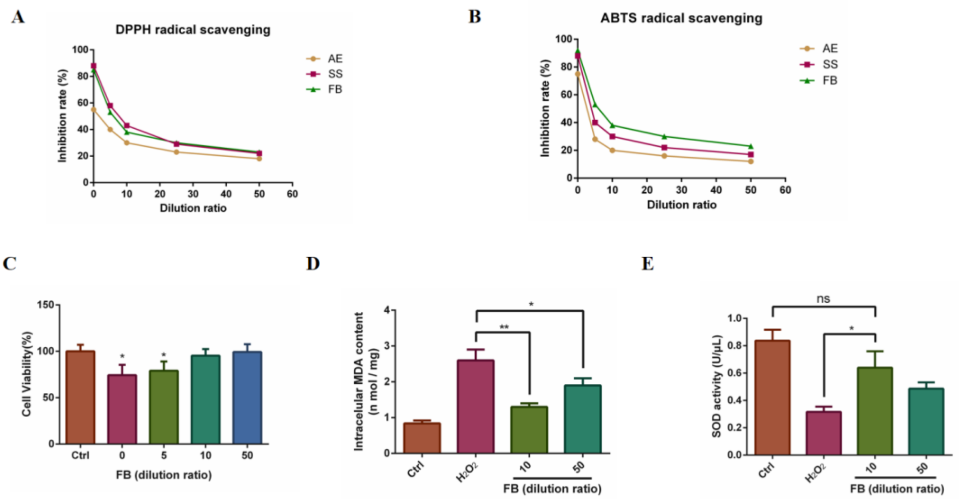

Figure 4: Fermentation Process of Huangjing Had Better Antioxidant Effects

- 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.
- 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) radical scavenging activity. X axis: Scales presented 0×, 5×, 10×, and 50× dilution of the sample.
- Cell viability tested by MTT assay with different FB treatment dilution ratio.
- Quantitative date of cell malondialdehyde (MDA) content from H2O2 and FB treated cells.

• Quantitative date of cell SOD content from H2O2 and FB treated cells.

References

1. Cui, X. Y., Wu, X., Lu, D., & Wang, D. (2022). Network pharmacology-based strategy for predicting therapy targets of Sanqi and Huangjing in diabetes mellitus. *World Journal of Clinical Cases, 10*(20), 6900.
2. Hu, Y., Yin, M., Bai, Y., Chu, S., Zhang, L., Yang, M., ... & Peng, H. (2022). An evaluation of traits, nutritional, and medicinal component quality of Polygonatum cyrtonema Hua and P. sibiricum Red. *Frontiers in Plant Science, 13,* 891775.
3. Bi, S. J., Yuan, A. L., Chen, Z. J., Ren, Y., Liu, K. Y., Liu, C. Q., ... & Zhang, Y. L. (2024). Quantitative predictive model for screening optimal processing methods of Polygonati rhizoma. *Journal of Asian Natural Products Research*, 1-19.
4. Cheng, X., Ji, H., Cheng, C., Sun, Y., Cheng, H., Wang, D., ... & Liu, X. (2022). Comprehensive determination of the processing level of rhizome of Polygonatum sibiricum by macroscopic, micromorphological, and microscopic characterizations. *Microscopy research and technique, 85*(7), 2669-2678.
5. Quaresma, L. S., Santos, R. C. V., Gomes, G. C., Américo, M. F., Campos, G. M., Laguna, J. G., ... & de Jesus, L. C. L. (2024). Multidrug resistance profile in Lactobacillus delbrueckii: a food industry species with probiotic properties. *World Journal of Microbiology and Biotechnology, 40*(8), 235.
6. Yang, H. Y., Han, L., Lin, Y. Q., Li, T., Wei, Y., Zhao, L. H., & Tong, X. L. (2023). Probiotic fermentation of herbal medicine: progress, challenges, and opportunities. *The American Journal of Chinese Medicine, 51*(05), 1105-1126.
7. Liang, Z., Zhi, H., Fang, Z., & Zhang, P. (2021). Genetic engineering of yeast, filamentous fungi and bacteria for terpene production and applications in food industry. *Food Research International, 147*, 110487.
8. Tangyu, M., Muller, J., Bolten, C. J., & Wittmann, C. (2019). Fermentation of plant-based milk alternatives for improved flavour and nutritional value. *Applied microbiology and biotechnology, 103*, 9263-9275.
9. Zhao, C., Su, W., Mu, Y., Luo, L., Zhao, M., Qiu, S., ... & Jiang, L. (2023). Effects of Jiuqu inoculating Rhizopus oryzae Q303 and Saccharomyces cerevisiae on chemical components and microbiota during black glutinous rice wine fermentation. *International Journal of Food Microbiology, 385*, 110012.
10. Liu, Q., Liu, M., Chen, W., Yuan, H., Jiang, Y., Huang, D., ... & Wang, T. (2024). Recent Advances in 2-Keto-l-gulonic Acid Production Using Mixed-Culture Fermentation and Future Prospects. *Journal of Agricultural and Food Chemistry, 72*(3), 1419-1428.
11. Arilla, E., Martínez-Monzó, J., Chiş, M. S., Fărcaş, A. C., Socaci, S. A., Codoñer-Franch, P., ... & Igual, M. (2023). Sensory Evaluation, Physico-Chemical Properties, and Aromatic Profile of Pasteurised Orange Juice with Resistant Maltodextrin. *Foods, 12*(21), 4025.
12. Cheng XL et al. (2022b). Characterization, Classification, and Authentication of Polygonatum sibiricum Samples by Volatile Profiles and Flavor Properties. *Molecules, 27*. doi: 10.3390/molecules27010025
13. Mursaliyeva, V. K., Sarsenbek, B. T., Dzhakibaeva, G. T., Mukhanov, T. M., & Mammadov, R. (2023). Total content of saponins, phenols and flavonoids and antioxidant and antimicrobial activity of in vitro culture of Allochrusa gypsophiloides (Regel) Schischk compared to wild plants. *Plants, 12*(20), 3521.
14. Petrasch, S., Silva, C. J., Mesquida-Pesci, S. D., Gallegos, K., Van Den Abeele, C., Papin, V., ... & Blanco-Ulate, B. (2019). Infection strategies deployed by Botrytis cinerea, Fusarium acuminatum, and Rhizopus stolonifer as a function of tomato fruit ripening stage. *Frontiers in Plant Science, 10*, 223.
15. El-Ghareeb, W. R., Elhelaly, A. E., Abdallah, K. M. E., El-Sherbiny, H. M. M., & Darwish, W. S. (2021). Formation of biogenic amines in fish: Dietary intakes and health risk assessment. *Food Science & Nutrition, 9*(6), 3123-3129.
16. Peng, B., Li, J., Shan, C., Cai, W., Zhang, Q., Zhao, X., ... & Tang, F. (2023). Exploring metabolic dynamics during the fermentation of sea buckthorn beverage: comparative analysis of volatile aroma compounds and non-volatile metabolites using GC–MS and UHPLC–MS. *Frontiers in Nutrition, 10,* 1268633.
17. Cui, X., Wang, S., Cao, H., Guo, H., Li, Y., Xu, F., ... & Han, C. (2018). A review: the bioactivities and pharmacological applications of Polygonatum sibiricum polysaccharides. *Molecules, 23*(5), 1170.
18. Zhang, Q., Yang, Z., & Su, W. (2024). Review of studies on polysaccharides, lignins and small molecular compounds from three Polygonatum Mill.(Asparagaceae) spp. in crude and processed states. *International Journal of Biological Macromolecules, 260*, 129511.
19. Zhao P et al. (2018). The genus Dioscorea L. (Dioscoreaceae): A review of ethnopharmacology, phytochemistry and pharmacology. *Journal of Ethnopharmacology, 214*, 274-291. doi: 10.1016/j.jep.2017.12.00
20. Wang, Z. L., Lao, J., Xie, Z. N., He, W., Zhong, C., Zhang, S. H., & Jin, J. (2024). Fermentation of Polygonati Rhizoma aqueous extract using Lactiplantibacillus plantarum under the condition of eutrophication. *Archives of Microbiology, 206*(8), 359.
21. Chen, Z., Zhu, B., Chen, Z., Cao, W., Wang, J., Li, S., & Zhao, J. (2022). Effects of steam on polysaccharides from Polygonatum cyrtonema based on saccharide mapping analysis and pharmacological activity assays. *Chinese Medicine, 17*(1), 97.
22. Mah, J. H., Park, Y. K., Jin, Y. H., Lee, J. H., & Hwang, H. J. (2019). Bacterial production and control of biogenic amines in Asian fermented soybean foods. *Foods, 8*(2), 85.
23. Kim, Y., Shim, Y. S., & Lee, K. G. (2022). Determination of alcohols in various fermented food matrices using gas chromatography-flame ionization detector for halal certification. *Food science and biotechnology, 31*(13),

1639-1646.

24. Zhao JB, Wei J, Wang XL, Cao H (2023b). Coupling fermentation of glutamic acid and γ-polyglutamic acid and preparation of poly (amino acid) superabsorbent polymers. *Bmc Biotechnology, 23*. doi: 10.1186/s12896-023-00819-0
25. Li, L., Wang, L., Fan, W., Jiang, Y., Zhang, C., Li, J., ... & Wu, C. (2020). The application of fermentation technology in traditional Chinese medicine: A review. *The American journal of Chinese medicine, 48*(04), 899-921.