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Improving the Quality and Flavor of *Monascus* Rice Wine Brewed by Pure Culture Using the Addition of *Trichosanthis Fructus*

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Abstract: *Trichosanthis Fructus* (TrF) is an edible medicinal fruit with a sweet taste and pleasant flavor. In this study, different concentrations of TrF were added into the media to brew *Monascus* rice wine using a pure culture method, and the physicochemical properties, volatile compounds, antioxidant activity, and sensory quality of the brewed samples were characterized. In addition, the effect of TrF on the growth and metabolism of *Monascus purpureus* and *Saccharomyces cerevisiae* was investigated. The results show that addition of TrF reduces the growth and metabolism of *M. purpureus* and *S. cerevisiae* in a dose-dependent manner, but also enriches the flavor components, in addition to increasing the total phenolic and flavonoid contents, and antioxidant activity of rice wine. Combined with the results of sensory evaluation, we find that 15% TrF is optimal for improving the flavor of *Monascus* rice wine. The results from this study can serve as a basis for improving the quality and flavor of rice wine brewed using pure strain culture.

Keywords: rice wine; pure culture; *Trichosanthis Fructus*; *Monascus purpureus*; volatile compounds



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1. Introduction

Rice wine is the oldest alcoholic beverage in countries of eastern Asia, including China, where its history dates back more than 5000 years [1]. Owing to the spacious territory, a variety of rice wines are brewed in China, and the typical representatives are Shaoxing rice wine, Jimo millet wine, and Hongqu rice wine [2]. Traditionally, rice wine has been brewed in an open fermentation system, and the microorganisms participating in the brewing process include the strains from koji, water, the container, and the environment [3]. A rich variety of microorganisms contribute to determining the unique flavor, taste, and nutrition of rice wine. However, the uncertainty of microbial species in rice wine brewing can result in unstable quality, thus limiting the standardization of rice wine [4]. In extreme cases, certain miscellaneous microorganisms in the rice wine present a food safety risk. Therefore, there are numerous studies aimed at determining the microbial composition during rice wine brewing [5,6], exploring the effect of different microbes on the quality of rice wine products [7], and attempting to produce rice wine using pure culture [3].

Rice wine brewing is a process of simultaneous saccharification and fermentation, in which molds are responsible for hydrolyzing starch into sugar, and yeasts play a key role in alcoholic fermentation and flavor formation. Compared to traditional multi-microbial fermentation, however, pure culture brewing with a single mold and yeast strain results in rice wine with fewer unique flavor substances and lower levels of bioactive components [7]. Some plant materials have a pleasant flavor and may be rich in nutritional and bioactive components. Thus, the quality and health function of rice wine can be enhanced by including these plants in brewing [8]. Therefore, we speculate that the incorporation of suitable plants into the brewing matrix may improve the flavor and bioactivity of the rice wine brewed with a pure culture of mold and yeast.

Trichosanthis Fructus (TrF) is the ripe fruit of *Trichosanthes kirilowii*, a plant belonging to the family Cucurbitaceae. TrF has a sweet taste and pleasant flavor. Chemical analysis

has shown that TrF is rich in saccharides, flavonoids, organic acids, terpenoids, phytosterols, lignans, and nitrogenous compounds, in addition to possessing multi-bioactivities, including antioxidant, hypoglycemic, hypolipidemic, and anti-inflammatory activity [9]. Based on its flavor, functional ingredients, and bioactivity, TrF may potentially be added to the media to increase the quality of rice wine brewed using pure culture.

In Fujian and Southern Zhejiang, China, Hongqu rice wine is primarily produced from glutinous rice using Hongqu (red koji) as a starter. The main microbe in red koji is *Monascus*, which can produce secondary metabolites, including red pigments, monacolin K, γ -aminobutyric acid, and flavone [10]. Therefore, Hongqu rice is well known for its red color, mellow flavor, and health functions, such as antioxidant, cholesterol-lowering, and hypolipidemic activities [11]. Based on MiSeq high-throughput sequencing, PCR denaturing gradient gel electrophoresis, and molecular biology combined with bioinformatics analysis, researchers have determined that the microorganisms involved in Hongqu rice wine brewing include *M. purpureus*, *Aspergillus oryzae*, *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera*, *Pichia guilliermondii*, *Lactobacillus plantarum*, *P. acidilactici*, *Bacillus subtilis*, and *B. megaterium* [5,6], where *M. purpureus* and *Saccharomyces cerevisiae* are the main fermentation microbes. In this work, pure *S. cerevisiae* and *M. purpureus* were used as a starter to brew Hongqu rice wine. To improve its flavor and bioactivity, TrF was incorporated into the brewing matrix. The effects of TrF on the growth and metabolism of *S. cerevisiae* and *M. purpureus* were investigated, and the physicochemical properties, antioxidant activity, and sensory quality of brewed samples were characterized. The results from this work can serve as a reference for the brewing of high-quality rice wine using pure culture.

2. Materials and Methods

2.1. Materials and Microorganisms

Fresh TrF was obtained from Siyang County, Jiangsu Province, China. *M. purpureus* CGMCC 16790 was screened from Hongqu koji by the fermentation laboratory of Wenzhou University and preserved in the China General Microbiological Culture Collection Center (CGMCC, Beijing, China). Active dry yeast (*S. cerevisiae* intended for rice wine brewing) and glucoamylase were purchased from Angel Yeast Co., Ltd. (Yichang, China).

2.2. Fermentation Process of Rice Wine

M. purpureus was inoculated into malt media (malt 20%, glucose 2%, MgSO_4 0.03%, and KH_2PO_4 0.06%) and incubated in a rotary shaker at 28 °C and 150 rpm for 5 days to obtain liquid inoculum. The rice was soaked, washed, drained, cooked by steam, and cooled. The liquid inoculum was mixed with cooked rice at a ratio of 1:5 (*v/w*) and cultured at 28 °C for 3 days to obtain a fermentation starter.

Fresh TrF was washed, crushed into pulp by a juicer, and autoclaved at 115 °C for 15 min. The rice wine was brewed according to our previously published methods [8] with some modifications. Briefly, the glutinous rice was soaked, cooked by steam, and cooled. Then, the cooked glutinous rice, fermentation starter, active dry yeast, glucoamylase, and distilled water were mixed at a ratio of 100:12:0.05:0.2:100, and 3 kg of mixture was transferred into a 5 L sterilized container. Following this, 150 g, 450 g, and 750 g of sterilized TrF pulp were added, i.e., 5% (AYW), 15% (BYW), and 25% (CYW) of TrF pulp weight to mixture weight, respectively. The sample without added TrF was used as a control. After fermenting at 28 °C for 10 days, the resulting fermentation mash was filtered, and the filtrate was transferred into 500 mL bottles and heated at 100 °C for 30 min. Afterward, the samples were stored for three months for maturation.

2.3. Physicochemical Analysis

The physicochemical indexes, including alcohol content, pH, total acid, amino nitrogen, reducing sugar, and soluble protein, were determined as described in our previous work [8]. Briefly, alcohol content was determined using an alcohol meter (Yuyao Glass Instrument Factory, Ningbo, China) after distillation. pH was determined using a Mettler Toledo pH

meter. Total acidity and amino acid nitrogen were determined using NaOH and NaOH-formaldehyde titration, respectively [12]. The reducing sugar and soluble protein contents were colorimetrically determined using the 3,5-dinitrosalicylic acid (DNS) method and the Coomassie bright blue method, respectively.

2.4. Measurements of Glucose Uptake and Vitality of *S. cerevisiae*

TrF was dried, pulverized, extracted with 70% ethanol, and concentrated under vacuum conditions to remove ethanol and obtain TrF extract. The effects of the TrF extract on the glucose uptake and vitality of yeast cells were determined as described by Chen et al. [13] with some modifications.

For the glucose uptake analysis, *S. cerevisiae* was cultured in YPD media at 28 °C for 3 days, and 10% yeast suspension was subject to centrifugation, repeated washing, and resuspension in physiological saline. Yeast suspension (0.5 mL) was added into 5 mL of 8% glucose solution containing TrF extracts equivalent to 0%, 5%, 15%, and 25% TrF pulp, mixed, and incubated at 30 °C for 30 min. The glucose concentration in the mixture was then determined using the DNS method after first removing the yeast cells by centrifugation, and the glucose uptake of yeast was calculated and expressed in mg/g/min.

2.5. Measurements of Biomass and Amylase Activity of *M. purpureus*

M. purpureus was cultured in medium (peptone 0.5%, yeast extract 0.5%, soluble starch 3%, CaCl₂ 0.05%, MnCl₂ 0.05%, MgSO₄ 0.05%, KH₂PO₄ 0.1%; pH 7.0) supplemented with TrF extracts equivalent to 0%, 5%, 15%, and 25% TrF pulp in a shaking incubator at 30 °C and 150 r/min for 6 d [14].

The *M. purpureus* mycelia were collected by filter, washed with deionized water, dried at 60 °C for 24 h, and weighed to obtain the biomass. The amylase activity was determined using the DNS method, as described by Hu et al. [15]. One unit of amylase activity is defined as the quantity of enzyme that hydrolyzed soluble starch to produce 1.0 µg glucose per minute.

2.6. Antioxidant Activity Analysis

The antioxidant activity was evaluated by scavenging ability against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, as described in our previous work [8], and the results are expressed as vitamin C equivalent per liter of rice wine (mg VcE/L).

2.7. Measurements of Total Phenolics and Total Flavonoid Contents

The total phenolics and total flavonoid contents were determined using the Folin-Ciocalteu method and the aluminum nitrate method, as previously described [8], and the results are expressed as the gallic acid (mg GAE/L) or rutin (mg RE/L) equivalent per liter of rice wine, respectively.

2.8. γ -Aminobutyric Acid (GABA) Content Analysis

The GABA content was determined using high-performance liquid chromatography (HPLC) after derivatization with *o*-phthalaldehyde (OPA) [8]. Briefly, 1 mL of rice wine was mixed with 2 mL of 4% sulfosalicylic acid and left to stand at 37 °C for 30 min. Subsequently, 200 µL of sample was thoroughly mixed with 1 mL of OPA derivatization solution for 2 min and filtered through a 0.45 µm membrane. The derived GABA was separated on an Agilent AavanceBio AAA column by gradient elution using sodium acetate solution (0.025 mol/L, pH 5.9) and acetonitrile as mobile phases A and B, respectively. The HPLC conditions were as follows: UV detector (Agilent Science Technology, Santa Clara, CA, USA), 332 nm; column temperature, 40 °C; injection volume, 10 µL; flow rate, 1 mL/min. The gradient elution procedure is shown in Table 1.

Table 1. HPLC gradient elution procedure for GABA determination.

Step	Time (min)	A (%)	B (%)
1	0	90	10
2	30	40	60
3	35	40	60
4	40	90	10
5	45	90	10

2.9. Volatile Composition Analysis

The volatile components of rice wine were examined with reference to the method of Zhao et al. [16] using solid-phase microextraction–gas chromatography–mass spectrometry (SPME-GC-MS). Briefly, sample (6 mL), NaCl (2 g), and 2-octanol (10 µL, 100 mg/L in methanol; internal standard) were added to a 15 mL SPME vial in succession. Following a 15 min period of equilibrium, the PDMS/CAR/DVB SPME extraction fiber (50/30 µm; Supelco Inc., Bellefonte, PA, USA)—which had been aged at 250 °C beforehand—was introduced, and the extraction was carried out for 40 min at 70 °C. The extraction fiber was then rapidly inserted into the GC injection port and desorbed at 250 °C for three minutes. The 7890A-5975C GCMS apparatus (Agilent Science Technology, Santa Clara, CA, USA) fitted with an HP-INNOWAX (60 m × 250 µm, 0.25 µm) was used for GC-MS analysis. Helium was the carrier gas, flowing at a rate of 1 mL min^{−1}. The MS conditions were as follows: electron energy, 70 eV; ionization source temperature, 230 °C; quadrupole temperature, 150 °C; scan range, 35–500 *m/z*. The gradient temperature program is shown in Table 2.

Table 2. SPME-GC-MS gradient temperature program for determination of volatile compounds.

Step	Time (min)	Temperature (°C)
1	5	40
2	21	120
3	33	240
4	43	240

The volatile compounds were identified by comparing their mass spectra with those in the NIST11 database, with qualitative determination according to the retention time and quantitative calculation based on comparing the peak area with that of the internal standard.

2.10. Sensory Analysis

The sensory analysis was conducted by ten experienced tasters with reference to GB/T 13662-2018 (Huangjiu) [12]. The color, aroma, flavor, taste, and overall impression of the samples were evaluated and scored. Quantification of each sensory index was expressed using values 1–10 (1, none; 2–3, very weak; 4–5, ordinary; 6–7, moderate; 8–9, intensity; 10, high intensity).

2.11. Statistical Analysis

Data are expressed as the mean ± standard deviation of three replicates. One-way ANOVA with Duncan’s multiple range test was used to determine significant differences at the 0.05 level using SPSS25 statistical software.

3. Results and Discussion

3.1. Effect of TrF Addition on the Physicochemical Properties of Rice Wine

The basic physicochemical properties (i.e., alcohol content, pH, total acid, amino nitrogen, reducing sugar, and soluble protein) of rice wine brewed using different concentrations of TrF were determined. As shown in Table 3, the control sample (YW) had the highest pH, alcohol content, and reducing sugar content, whereas the total acidity, amino nitrogen, and

soluble protein content in rice wine increased with the addition of TrF. This is due to the rich amino acids, organic acids, and proteins in TrF [17], which may be released into the rice wine. Compared with the control, the addition of TrF led to a decrease in alcohol content and an increase in total acidity, similar to a previous study on cordyceps rice wine [18].

Table 3. Basic physicochemical characteristics of rice wine.

Sample	pH	Total Acidity (g/L)	Amino Nitrogen (g/L)	Alcohol (% v/v)	Reducing Sugar (g/L)	Soluble Protein (mg/L)
YW *	4.18 ± 0.02 ^a	4.84 ± 0.02 ^c	0.73 ± 0.00 ^c	16.35 ± 0.24 ^a	63.48 ± 0.42 ^a	61.54 ± 1.14 ^d
AYW	3.73 ± 0.01 ^c	8.20 ± 0.05 ^b	0.83 ± 0.01 ^b	15.33 ± 0.94 ^{ab}	13.34 ± 0.95 ^b	65.43 ± 1.14 ^c
BYW	3.87 ± 0.01 ^b	8.26 ± 0.01 ^b	0.89 ± 0.01 ^a	14.67 ± 0.28 ^b	6.96 ± 0.51 ^c	80.31 ± 0.86 ^b
CYW	3.90 ± 0.02 ^b	8.56 ± 0.05 ^a	0.90 ± 0.03 ^a	11.70 ± 0.49 ^c	1.97 ± 0.07 ^d	85.61 ± 0.54 ^a

* YW, AYW, BYW, and CYW are rice wine brewed by adding 0, 5, 15, and 25% TrF pulp, respectively. Different letters within each column indicate statistically significant differences at $p < 0.05$.

TrF is also rich in sugar, with a sugar content in the range of 16.17–32.65% based on the different habitats [9]. On the other hand, rice wine brewing is a process of simultaneous saccharification and fermentation, and the sugar content is low in the initial stage. During the fermentation, the starch in the rice is hydrolyzed into sugar by mold, and the sugar is then metabolized into alcohol by yeast. Therefore, the addition of *Trichosanthis Fructus* would not dilute the sugar in the fermentation media. However, TrF addition is negatively correlated to the reducing sugar content ($r = -0.788$, $p < 0.01$) and alcohol content ($r = -0.929$, $p < 0.01$) of rice wines. As reported previously, TrF contains a series of secondary metabolites, such as flavones, lignans, and triterpenes [19]. These constituents might inhibit the growth and metabolism of *M. purpureus* and yeast to some extent, resulting in low reducing sugar and alcohol contents in the TrF addition samples. An effect on microbial fermentation from the addition of plant additives was also observed by Cai et al. in *Cynanchum bungei* Decne rice wine brewing [8].

3.2. Effect of TrF Addition on the Growth and Amylase Activity of *M. purpureus*

The brewing of rice wine is a process of simultaneous saccharification and fermentation [20], and in this work, *M. purpureus* is responsible for the hydrolyzation of rice starch into sugar. To explore the influence of TrF addition on the hydrolyzation process of rice starch, TrF extract was prepared, and its effects on the growth and amylase activity of *M. purpureus* were studied. As shown in Table 4, the biomass and amylase activity of *M. purpureus* all decreased with the addition of TrF extract. Compared with the control (TF0), the biomass of *M. purpureus* was not significantly lower in TF5 but significantly ($p < 0.05$) lower by 30.01% and 32.03% in TF15 and TF25, respectively. Correspondingly, the amylase activity of *M. purpureus* was significantly ($p < 0.05$) decreased by 9.67%, 20.64%, and 33.95% in TF5, TF15, and TF25, respectively. TrF has been shown to have anti-microbial activity [9], which may thereby present an inhibitory effect on *M. purpureus*. Plant materials are rich in compounds, some of which can inhibit the growth of microorganisms [21]. Anti-microbial activity has also been reported for other plant matrices, such as *Hydrocotyle asiatica*, *Chrysanthemum coronarium*, and *Paederia scandens* [22].

Table 4. Effects of TrF extraction on the growth and amylase activity of *M. purpureus*.

Sample	Biomass (g/L)	Amylase Activity (U/mL)
TF0	8.43 ± 0.64 ^a	477.46 ± 10.38 ^a
TF5	7.47 ± 0.25 ^a	431.30 ± 4.49 ^b
TF15	5.90 ± 0.72 ^b	378.90 ± 12.99 ^c
TF25	5.73 ± 0.60 ^b	315.36 ± 21.62 ^d

TF0, TF5, TF15, and TF25 are samples containing TrF extracts equivalent to 0%, 5%, 15%, and 25% TrF pulp, respectively. Different letters within each column indicate statistically significant differences at $p < 0.05$.

3.3. Effect of TrF Addition on the Glucose Uptake and Viability of *S. cerevisiae*

In rice wine brewing, *S. cerevisiae* is key for metabolizing sugar into alcohol and other flavor constituents. As shown in Figure 1A, TrF extract reduces the glucose uptake capacity of yeast in a dose-dependent manner. Compared with the control (TF0), the glucose uptake capacity of the yeast was significantly ($p < 0.05$) reduced by 28%, 37%, and 46% in TF5, TF15, and TF25, respectively. Similar results were also obtained by Chen et al. [13], who reported that *Dendrobium officinale* extracts have an inhibitory effect on the glucose uptake of *S. cerevisiae*. Similar to the results of the glucose uptake experiment, the cell viability of yeast was also inhibited by the addition of TrF extract (Figure 1B). Further, the cell viability of yeast was examined by fluorescent staining. The results from fluorescence microscopy visually demonstrate that the fluorescence intensities of the samples with TrF extract (Figure 2B–D) are weaker than those of the control (Figure 2A). The inhibitory effect of TrF on the yeast can be attributed to its anti-microbial activity [9].

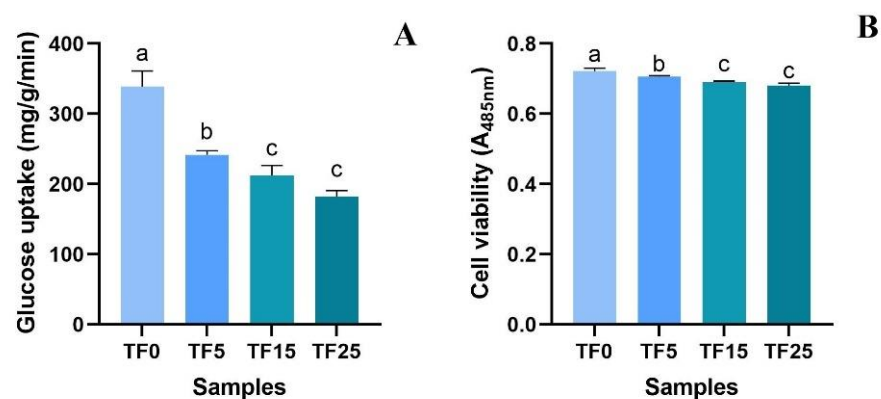


Figure 1. Effects of TrF on the glucose uptake (A) and cell viability (B) of yeast. TF0, TF5, TF15, and TF25 are samples containing TrF extracts equivalent to 0%, 5%, 15%, and 25% TrF pulp, respectively. Different letters indicate statistically significant differences at $p < 0.05$.

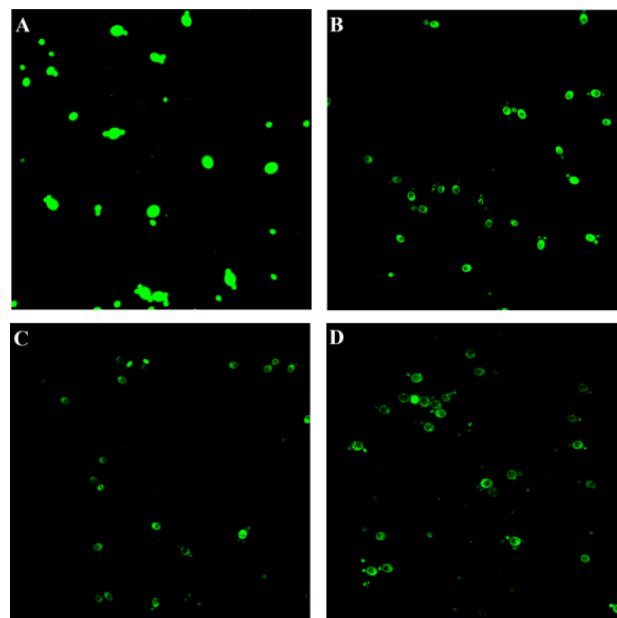


Figure 2. Fluorescence images indicating cell viability of *S. cerevisiae*. (A–D) represent the samples containing TrF extracts equivalent to 0%, 5%, 15%, and 25% TrF pulp, respectively.

3.4. Effect of TrF Addition on the Total Phenolics, Total Flavonoids, and Antioxidant Activity of Rice Wine

The content of phenolic substances has an important influence on the antioxidant activity of rice wine, and their main sources are the raw and auxiliary materials [23]. As shown in Table 4, the total phenolic and total flavonoid contents in rice wine were increased by the addition of TrF. Compared with the control (YW), the total phenolics increased 1.12-, 1.13-, and 1.20-fold, while the total flavonoids increased 1.20-, 1.64-, and 2.24-fold in rice wines brewed by adding 5, 15, and 25% TrF pulp, respectively. TrF contains quercetin, isoquercitrin, rutin, kaempferol, and other phenolic compounds [17], which may be released into the rice wine during fermentation, resulting in a significant increase in its total phenolic and total flavonoid contents.

The antioxidant capacity of samples is directly related to the content of phenolic compounds obtained externally [24]. The scavenging abilities of the ABTS and DPPH free radicals of the rice wines were determined, and increasing trends were observed with the addition of TrF (Table 5). The ABTS radical scavenging capacity showed significant ($p < 0.05$) differences between the control (YW) and samples with different concentrations of TrF addition (AYW, BYW, and CYW), 1.06-, 1.19-, and 1.24-fold higher, respectively, than those of the control. For DPPH radical scavenging ability, there were no significant differences between YW, AYW, and BYW but a significant ($p < 0.05$) increase in CYW, indicating that at low concentrations, TrF had no significant effect on the improvement of DPPH scavenging ability of rice wine. Edible plant matrices can increase the phenolic content and antioxidant ability of rice wine, which has been achieved by the addition of bamboo extract [25], *Cynanchum bungei* [8], *Eucommia ulmoides* leaf [26], and cordyceps [18].

Table 5. Total phenolics, total flavonoids, and antioxidant activity of rice wine.

Sample	Total Phenolics (mg GAE/L)	Total Flavonoid (mg RE/L)	ABTS Radical Scavenging (mg VcE/L)	DPPH Radical Scavenging (mg VcE/L)
YW	624.07 ± 6.14 ^c	49.35 ± 1.35 ^d	139.83 ± 3.98 ^d	77.71 ± 0.97 ^b
AYW	700.43 ± 4.64 ^b	59.07 ± 1.35 ^c	148.30 ± 1.23 ^c	80.82 ± 0.89 ^b
BYW	705.79 ± 10.11 ^b	81.17 ± 3.35 ^b	166.30 ± 0.97 ^b	87.65 ± 10.55 ^b
CYW	747.33 ± 12.06 ^a	110.64 ± 3.10 ^a	174.04 ± 3.44 ^a	110.53 ± 1.44 ^a

YW, AYW, BYW, and CYW are rice wine brewed by adding 0, 5, 15, and 25% TrF pulp, respectively. Different letters within each column indicate statistically significant differences at $p < 0.05$.

3.5. Effect of TrF Addition on GABA Content of Rice Wine

GABA has neurotransmitter, anti-hypotension, long-term memory enhancement, and sedative effects [27]. Numerous studies have found that GABA-rich foods such as tea [28,29], rice germ [30], and fermented soybeans [31] can reduce the incidence of insomnia and depression and improve symptoms associated with chronic alcoholism [32]. Studies have shown that yellow wine is rich in GABA and can be used as a source of food with high GABA content [33]. In this work, we determined the GABA content in rice wines brewed by adding different concentrations of TrF, and the results (Figure 3) show that TrF significantly enhances the GABA content in rice wine, with a positive correlation ($r = 0.913$, $p < 0.01$). Similar results were also obtained by Cai et al. [8] that the GABA content in rice wine shows a dose-dependent increase with the addition of *C. bungei* Decne. The GABA contents were 1.32-, 1.63-, and 2.06-fold higher in AYW, BYW, and CYW than in YW. During the fermentation process, microorganisms convert the glutamic acid of raw material into GABA via glutamate decarboxylase [33], resulting in an increase in the concentration of GABA in rice wine. The addition of TrF was effective in enhancing the GABA content in rice wine, probably due to the rich amino acid content in TrF.

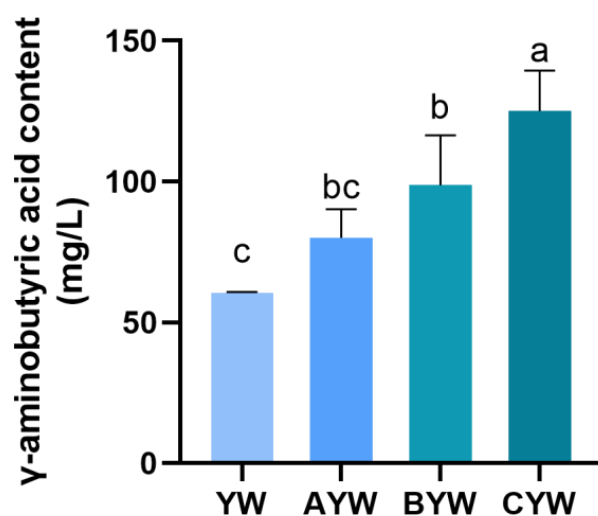


Figure 3. γ -Aminobutyric acid concentration in rice wine. YW, AYW, BYW, and CYW are rice wine brewed by adding 0, 5, 15, and 25% TrF pulp, respectively. Different letters indicate statistically significant differences at $p < 0.05$.

3.6. Effect of TrF Addition on the Volatile Compounds of Rice Wine

The volatile components directly affect the flavor of rice wine. A variety of volatile compounds have been identified in Hongqu rice wine brewed using traditional techniques. Chen et al. [34] identified a total of 81 volatile compounds in Gutian and Wuyi rice wines. In another study by Yuan et al. [35], 102 volatile compounds were detected and identified in the brewing samples of Hongqu rice wine. To study the effect of TrF addition, the volatile compounds of the control (YW) and samples brewed with 25% TrF pulp (CYW) were determined using SPME-GC-MS, and the results are shown in Figure 4. A total of 29 volatile compounds were identified in YW and CYW, with esters (10 compounds), alcohols (6 compounds), and aldehydes (4 compounds) being the main components. Compared with previous research [34,35], notably fewer volatile compounds were detected in this study, indicating that the pure culture results in a decrease in the volatile components of rice wine. However, CYW had more volatile components than YW, and moreover, the contents of esters, aldehydes, and alcohols were enhanced by the addition of TrF.

Esters provide floral and fruity aromas, and ethyl acetate, which is responsible for the pineapple flavor of rice wine [36], was found to be the dominant ester in all the tested samples. Ethyl lactate, methyl heptanoate, and butyrolactone were unique components in CYW that were not detected in YW. Ethyl lactate, characterized by fruity and creamy flavors, reached 12% of the total ester content in CYW. Methyl heptanoate is characterized by vegetal, fruity, and iris aromas, and butyrolactone has a caramel, sweet aroma. The addition of TrF resulted in the biosynthesis of ethyl lactate, methyl heptanoate, and butyrolactone, thereby enhancing the flavor of rice wine. Similar results were also reported in a study by Zou et al. [37], that supplementing with *Castanea mollissima* Blume could increase the contents of ethyl acetate, ethyl hexanoate, and ethyl hexadecanoate of rice wine, i.e., provide fruity flavors.

Alcohols are important volatiles in rice wine, and the influence of alcohols on the flavor of rice wine mainly depends on their type and content. 3-Methyl-1-butanol was produced by yeast metabolism, and its concentration was the highest of all the volatiles of the tested rice wines. This result is similar to the study of Wang et al. [38], who brewed rice wine with the addition of pyracantha power. The effect of 3-methyl-1-butanol on the flavor and aroma of rice wine varies with the concentration [39], and its content is increased by the addition of TrF and reaches 36.08 $\mu\text{g/mL}$ in CYW. Phenylethyl alcohol is characterized by a rose fragrance [40], and a higher content (8.71 $\mu\text{g/mL}$) was found in CYW than in YW. The contents of other alcohols, including 2-methyl-1-propanol and 1-propanol, both sensory compounds associated with wine aroma, were also changed by the addition of TrF.

Aldehydes have a fruity flavor at low concentrations, and benzaldehyde is a compound with almond flavor and caramel flavor. The benzaldehyde content in CYW was increased, possibly resulting from microbial utilization of unsaturated fatty acids and amino acids in TrF during the fermentation [41].

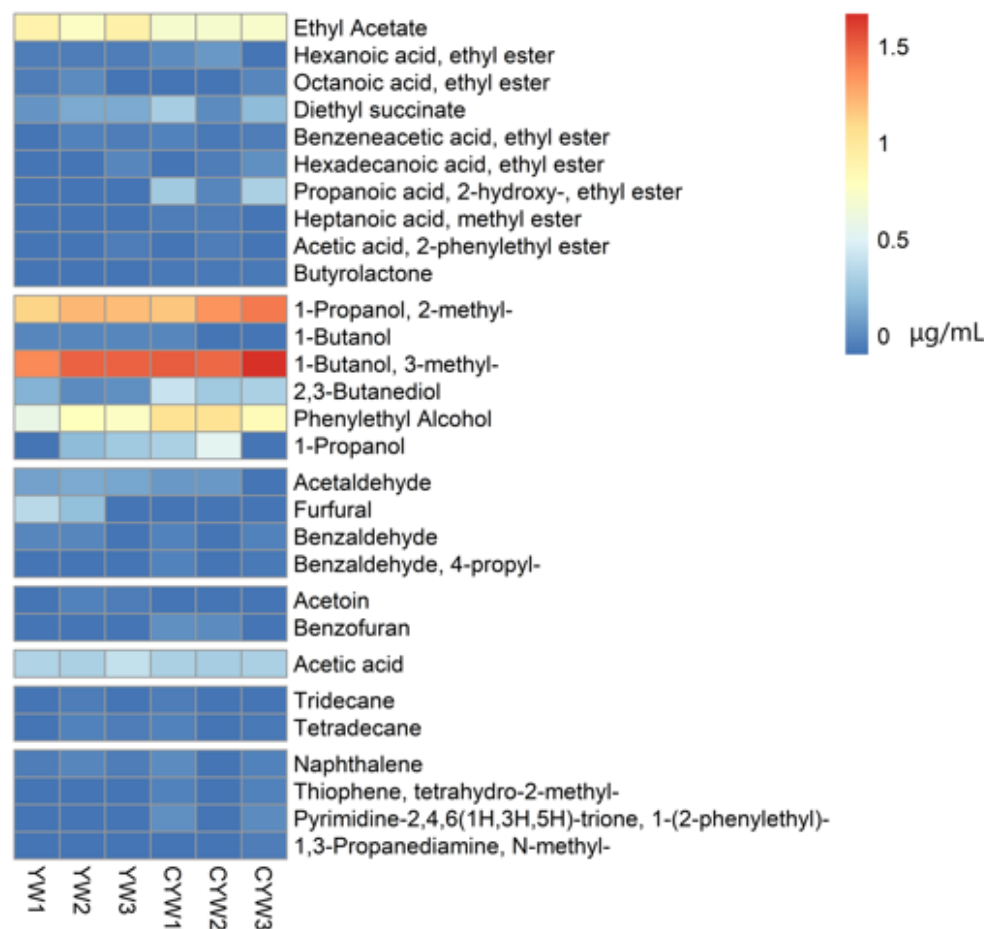


Figure 4. Heatmap of volatile substance composition of rice wine. YW1-3, control rice wine; CYW1-3, rice wine brewed by the addition of 25% TrF pulp.

3.7. Effect of TrF Addition on the Sensory Index of Rice Wine

The sensory quality of a drink directly affects its acceptance by consumers. TrF contains a series of phytochemicals [9], which may influence the smell and flavor of rice wine. As shown in Figure 5, the YW sample is pale yellow, clear, and transparent with a simple smell and taste, being slightly bitter. The color intensified with the addition of TrF, and CYW is darker and brownish yellow. The smell and taste of AYW are also simple, less astringent, and have some TrF flavor, while CYW is slightly bitter, astringent, and has a strong TrF flavor. Based on the scores obtained from the tasters, CYW had a significantly ($p < 0.05$) lower value in color and taste than other groups (no significant difference among YW, AYW, and BYW). For aroma, mouth-feel, and overall impression, the scores were significantly ($p < 0.05$) higher in AW and BYW (no significant difference between them), followed by AYW and CYW. Overall, BYW is less bitter and astringent, has a good balanced taste, aroma, and fragrance, and was scored highly by experienced tasters.

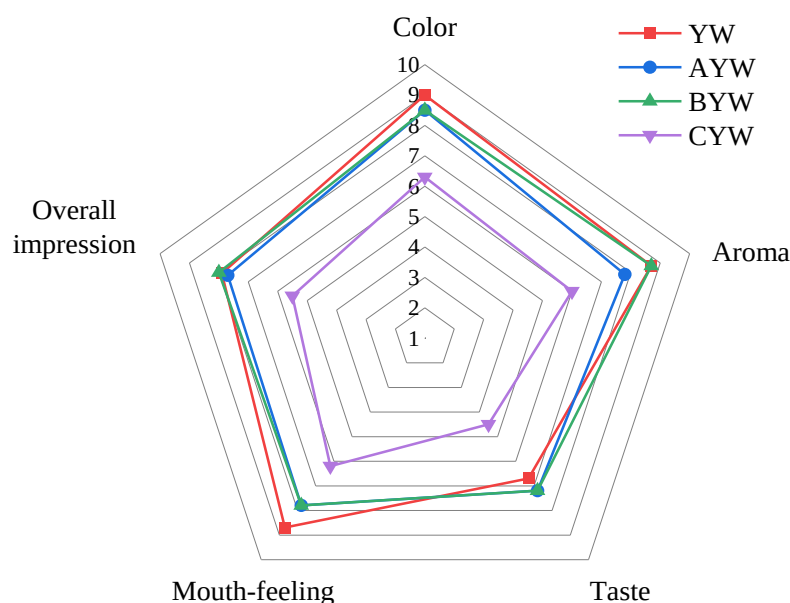


Figure 5. Sensory analysis of rice wine. YW, AYW, BYW, and CYW are rice wine brewed by adding 0, 5, 15, and 25% TrF pulp, respectively.

4. Conclusions

In summary, TrF can be added during brewing to increase the contents of total phenolics, total flavonoids, and GABA, thereby increasing the antioxidant activity of rice wine. Appropriate amounts of TrF can also improve the smell, taste, and flavor, and 15% TrF pulp was found to be optimal according to the consumer sensory evaluation. The results from this work indicate a new way to improve the quality of rice wine brewed using pure culture.

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