Effects of tandem fermentation of edible mushroom and *L. plantarum* on sensory, polysaccharide, vitamin C, and γ-aminobutyric acid of *Rosa roxburghii Tratt* and coix seed beverage

Mengqi Liu [a,b], Xueyi Tian [a,b], Laping He [a,b,*], Cuiqin Li [a,c], Han Tao [a,b], Xiao Wang [a,b,*], Shunbin Qiao [d], Xuefeng Zeng [a,b]

[a] *Key Laboratory of Agricultural and Animal Products Store & Processing of Guizhou Province, Guizhou University, Guiyang 550025, PR China*
[b] *College of Liquor and Food Engineering, Guizhou University, Guiyang 550025, PR China*
[c] *School of Chemistry and Chemical Engineering, Guizhou University, Guiyang 550025, PR China*
[d] *Guizhou Industry Polytechnic College, Guiyang 550025, PR China*

ABSTRACT

A new *Rosa roxburghii* Tratt (RRT) and coix seed (CS) beverage rich in multi-active ingredients was developed. Edible mushrooms and *L. plantarum* were selected for fermentation in stages. Some physicochemical properties, γ-aminobutyric acid (GABA), polysaccharides and sensory were studied during the *T. versicolor* and *L. plantarum* fermentation. *T. versicolor* increased the free amino acid through enzymatic protein digestion in the early growth stage and used these amino acids to synthesize its bacteriophage protein. *T. versicolor* and *L. plantarum* increased the polysaccharide and GABA of the fermentation broth. Vitamin C was retained as much as possible, with a slight loss occurring mainly in the aerobic fermentation stage of *T. versicolor*. Its less loss in exchange was for a higher value of *T. versicolor* polysaccharide, protein enhancement, and bitterness reduction. This study provides a reference for the deep processing of Guizhou's unique agricultural products and edible mushroom fermented beverage.

1. Introduction

Rosa roxburghii Tratt (RRT) is a rose plant of Rosaceae, also known as Wenxian Fruit and Fargesia. The 100 g fresh fruit of RRT contains 2200–2500 mg of vitamin C, so RRT is "king of vitamin C" (Li et al., 2022). Due to the sour taste of RRT fruit, the public acceptance of fresh RRT fruit is not high. Hence, the deep processing of RRT fruit is essential. Reducing the bitterness from bio-fermentation transformation and introducing new active ingredients is possible. However, RRT lacks a readily biodegradable carbon source, which is not conducive to fermentation. This deficiency can be overcome by adding starch-rich grains such as coix seed (CS).

CS, a gramineous plant, has coix flavonoids, polysaccharides, and proteins. It has anti-tumor, improves immunity, removes hydroxyl free radicals and DPPH free radicals, anti-inflammatory and analgesic, and inhibits osteoporosis (Zhu, 2017). CS has problems such as not being easily gelatinized and must be soaked and boiled for a long time, a single

consumption model, and low prices. RRT and CS complement each other in nutrition. So processing them into a composite fermented beverage that is easy to consume and absorb is not only in line with modern beverage trends but also conducive to developing deep processed RRT and CS products.

The development of edible mushroom beverage products has been uninterrupted in recent years. Some drinks were directly prepared or fermented with edible mushroom fruiting bodies as raw materials, such as RRT *Hericium* beverage (Zhang et al., 2021) and *Ganoderma lucidum* fermented sea-buckthorn tea (Xin et al., 2021). Due to the low pH of the compound solution of CS and RRT, selecting an edible mushroom with strong adaptability to the acidic environment for fermentation is necessary. Due to the differences in the growth environment, wood rot fungi generally adapt strongly to acidic environments (Udhav et al., 2021). The current fermentation of RRT is mainly lactic acid strain fermentation or the joint fermentation of lactic acid strain and yeast. Still, it is limited in improving the bitterness of RRT, which may be

---

mainly because enzymes in lactic acid strain and yeasts are insufficient to convert the bitter taste substances in the RRT. Wood rot edible mushrooms such as *Agrocybe cylindracea*, and *Ganoderma lucidum* have rich enzymes, so their transformation effect on RRT may be better.

This study adopts staged fermentation, with the initial stage being edible mushroom fermentation and the later stage being co-fermentation with suitable probiotics and edible mushrooms. We explored the effects of factors such as fermentation time, fermentation temperature, inoculation amount, and sucrose addition on sensory, GABA, polysaccharide, and vitamin C content of the RRT and CS fermentation liquid. Finally, the fermentation parameters were optimized through a response surface test to obtain a high-quality compound fermented drink of RRT and CS.

2. Materials and methods

2.1. Materials

CS was purchased from Guizhou Renxin Agricultural Development Co., Ltd. RRT was purchased from Longli County, Guizhou Province. *T. versicolor*, *Shiitake*, *Hericium erinaceus*, *Agrocybe cylindracea* and *Grifola grifola* were purchased from Guizhou Xishui County Edible Mushroom Research Institute. *L. plantarum* NR1-7 (NR1-7), *L. plantarum* LB12 (LB12), *B. animalis* subsp. *lactis* BZ11 (BZ11), *L. pentosus* MT-4 (MT-4) and *B. animalis* subsp. *lactis* BZ25 (BZ25) were screened and conserved at our laboratory (Key Laboratory of Storage and Processing of Agricultural and Livestock Products, Guizhou University, Guiyang, China). *S. thermophilus* Q-1 (Q-1) was purchased from Tianyou Yogurt, China. *S. cerevisiae* (SC) and *Fragrant yeast* (FS) (*Wickerhamomyces anomalus*) were purchased from Angel Yeast Co., Ltd., China.

2.2. Fermentation of the compound liquid of RRT and CS

2.2.1. Preparation of starter culture

The edible mushroom was activated once on the potato bevel medium, and stored in a 4 °C refrigerator. The potato medium was prepared (25 g potato dextrose broth, 2 g peptone, 2 g potassium dihydrogen phosphate, 1 g magnesium sulfate heptahydrate, 1000 mL water, solid medium with 2 % agar). Edible mushroom seed liquid was prepared by the reference (Scarpari et al., 2017). Take uniformly sized strain blocks on edible mushroom bevel medium, mash them into a 250 mL erlenmeyer flask containing 100 mL of potato medium, incubated in a shaker for 4 days (27 °C, 160 × g), and homogenize the seed liquid under sterile conditions (3000 × g, 5 s) to obtain the seed suspension. *L. plantarum*, *L. pentose, S. thermophilus* and *B. animalis* subsp. *lactis* were cultured at 37 °C for 48 h by the MRS medium. *Yeast* was cultured in the potato medium at 28 °C and 120 rpm for 48 h. Then, the respective cell pellets were harvested by centrifugation (TGL20M high-speed refrigerated centrifuge, Changsha, China) at 8000 × g and 4 °C for 10 min and resuspended in 0.9 % (w/v) sterile physiological saline to obtain 1 × 10^8 CFU/mL as lactic acid strain or yeast seed liquid.

2.2.2. Pretreatment of sample RRT and CS

The preparation of hydrolysate of enzymatic CS was referred to the report (Chen, 2012) with slightly modified. The non-damaging CS was washed with clean water three times, soaked at 25 °C for 12 h, and then beaten at a feed-to-water ratio of 1:15. CS pulp was heated and gelatinized at 90 °C for 20 min. After complete gelatinization, the 200 U high-temperature α-amylase (Activity: 40,000 U/g, CAS: 9000-85-5, Jiangsu Ruiyang Biotechnology Co., Ltd) every mL of gelatinization solution was added for liquefaction at 90 °C for 45 min. Then, 300 U saccharification enzyme (Activity: 100,000 U/g, CAS: 9032-08-0, Jiangsu Ruiyang Biotechnology Co., Ltd) in every mL of liquefaction solution was added for saccharification at 65 °C for 80 min. Preparation of RRT juice: Thaw RRT was preserved at −20 °C at room temperature. Squeeze the juice with an original juicer to obtain the original RRT, and put it in a brown bottle for temporary storage. Hydrolysate of enzymatic CS (121 °C, 20 min) and RRT juice (90 °C, 20 min) were sterilized, respectively, and the sterilized hydrolysate of enzymatic CS and RRT juice were mixed in proportion to a total of 100 mL in a 250-mL erlenmeyer flask.

2.2.3. Preparation of RRT and CS beverage (RCB)

After adding sucrose, hydrolysate of enzymatic CS and RRT juice were sterilized at 121 °C for 20 min and 90 °C for 20 min, respectively. *T. versicolor* seed liquid was added to the mixture of hydrolysate of enzymatic CS and RRT juice for fermentation, and finally, *L. plantarum* LB12 was added for fermentation.

2.2.4. Optimization of RCB fermentation technology

(1) One factor at a time (OFAT) experiment

The initial fermentation process of RCB was 30 % RRT juice, 70 % hydrolysate of enzymatic CS, 4 % *T. versicolor* fermentation for 2 days at 27 °C, and 3 % *L. plantarum* LB12 fermentation for 24 h at 37 °C. Using vitamin C, sensory, polysaccharide, and GABA content as screening indicators, the effects of fermentation time (12 h, 18 h, 24 h, 30 h, 36 h), fermentation temperature (29 °C, 33 °C, 37 °C, 41 °C, 45 °C), inoculation amount (1 %, 2 %, 3 %, 4 %, 5 %), and sucrose addition (1 %, 3 %, 5 %, 7 %, 9 %) on RCB were investigated.

(2) Based on the results of the OFAT experiment, four factors, including fermentation time, fermentation temperature, inoculation amount, and sucrose addition amount, were optimized using sensory, polysaccharide, and GABA content as response values. Each parameter is listed in Table S1.

2.3. Sensory evaluation

The sensory properties of RCB were evaluated by twenty panelists (ages 20–40) with experience. All testers were informed that they were conducting this experiment completely voluntarily and freely, and the Ethics Committee of Guizhou University has reviewed it. First, the 5-point system was used for scoring. Sensory evaluation was conducted from three aspects: color, aroma and taste. Rating values of 1–5 were utilized to assess these three sensory properties independently (5 = like very much, 4 = like more, 3 = average, 2 = dislike very much, 1 = dislike very much). Finally, the average values of color, aroma, and taste were multiplied by a factor of 5, 5, and 10, respectively, and added together to obtain a total score of 100. The higher the score, the better the sensory quality (Juliana et al., 2021; Yoshikawa et al., 2014).

2.4. Determination of pH, soluble solids, and color

The pH values were recorded using a digital pH meter (Shanghai Lichen Bangxi Instrument Technology Co., Ltd). A digital saccharometer (Shenzhen Ceyou Technology Co., Ltd) determined soluble solids. A hand-held colorimeter (X-Rite) counts the color, and the color change of the sample was evaluated from three aspects: L * (lightness), a * (red-green intensity), and b * (yellow-blue intensity).

2.5. Protein and free amino acid

The Coomassie Brilliant Blue method determines the protein content, regarding SN/T 3926-2014, for determining protein content in milk, eggs, and beans for export.

The content and composition of free amino acids were determined using an automatic amino acid analyzer (SYKAM, Germany). The fermentation sample was centrifuged (6000 × g, 10 min). Take 1 mL of supernatant, add 9 mL of 2 % sulfosalicylic acid (w/v), mix, and stand for 15 min. Centrifuge again and over 0.45 μm membrane. The membrane-passed liquid was injected into the amino acid analyzer to

determine free amino acids through qualitative and quantitative analysis of a single amino acid according to a standard amino acid.

2.6. Vitamin C and total phenol

The content of vitamin C was determined by the 2,6-dichloro-indophenol method. The specific experimental steps were referred to the standard GB/T 5009.86-2016 (National Food Safety Standard-Determination of Ascorbic Acid in Food).

The reference method (Yin, 2020) was used to determine the total phenol content with some modifications. Mix 2 mL of the diluted sample with 1 mL of Folin phenol reagent, add 13 mL of 10 % sodium carbonate solution, stand in the dark for 60 min, Measure the absorbance value of the sample reaction solution at 760 nm wavelength. With distilled water as the control, the total phenol content was calculated from the absorbance value and standard curve; the unit was mg/100 mL.

2.7. Reducing sugar and polysaccharide content

The reduced sugar content was determined by 3,5-dinitro salicylic acid method (Wang et al., 2021). Measure the absorbance of the reaction solution at 520 nm wavelength. The crude polysaccharide content was determined by the phenol sulfuric acid method. Measure the absorbance value of the sample solution at 490 nm wavelength.

2.8. γ- aminobutyric acid (GABA)

Take 0.2 mL of different concentrations of GABA standard solution (or sample supernatant) and add 0.2 mL of 0.5 mol/L sodium bicarbonate and 0.2 mL of 1 % FDNB solution dissolved in acetonitrile in a brown bottle at 60 °C for 1 h away from light for derivatization reaction. Then, add 1.4 mL of 0.12 % phosphoric acid solution by volume fraction, and pass through 0.22 μm film for HPLC (U3000, Thermo Fisher, USA). The fermentation sample was centrifuged ($8000 \times g$, 15 min) to collect the supernatant. The mobile phase comprised 50 % acetonitrile and 50 % of 0.12 % phosphoric acid solution. Other detection conditions: wavelength was 370 nm, column temperature 35 °C, flow rate 1.0 mL/min, injection volume 10 μL, and time 10 min.

2.9. Electronic tongue analysis

The Insent SA402B electronic tongue analysis system (Beijing Ying Sheng Heng Tai Technology Co., Ltd., Beijing, China) was used to collect the taste information of RCB. For sample testing, approximately 25 mL of RCB was added to the corresponding test measuring cup for taste value determination.

2.10. Statistical analysis

All data were obtained through three repeated experiments. SPSS 22, Excel 2019, and other software were used to process and analyze the data. The format of the analysis results was mean ± standard deviation. The significance of the difference ($P < 0.05$) was obtained through one-way ANOVA analysis and expressed in different letters. Design Expert 8.0 software was used for response surface design and analysis.

3. Results and discussion

3.1. Selection of edible mushrooms and mixed proportion of RCB

The edible mushrooms used in the study were found to have varying effects on sensory scores, as shown in Fig. S1. Among the five edible mushrooms tested, the composite liquid fermented with *T. versicolor* obtained the highest sensory score, reaching 84.2 points. That was followed by *Agrocybe cylindracea* and *Grifola* frondose, with scores of 79.7 and 75.9, respectively. On the other hand, the sensory values of the

composite liquid fermented with Shiitake and *Hericium erinaceus* were relatively low, with scores of 69.90 and 68.1, respectively. Additionally, the study investigated the impact of different CS and RRT compounding ratios on sensory scores. Among the five selected compounding ratios, the highest sensory score of 81.9 was achieved when the compounding ratio of CS and RRT was 7:3. The main reason why the compounding ratio of CS and RRT affected the flavor could be attributed to the heavy sourness and astringency of RRT juice (Xu et al., 2019). These characteristics likely influenced the overall sensory perception of the composite liquid.

3.2. The effect of T. versicolor fermentation on pH, soluble solids, and color

Table S2 shows that the pH of the fermentation broth decreased from 3.89 ± 0.009 on the first day to 3.54 ± 0.024 ($P < 0.05$). It could be that *T. versicolor* produces acids during the fermentation process. Studies show that the pH of soybean beverage fermented with edible mushrooms decreased from 6.5 to 4.0 due to the production of glucuronic acid and other organic acids during metabolism by edible mushrooms (Sun et al., 2022).

Soluble solids refer to substances soluble in water, including sugars, acids, vitamins, and minerals (Feng et al., 2022). The soluble solids in the fermentation broth showed a slow decline trend because the growth of *T. versicolor* requires the soluble solids as the energy source, which improved the conversion and utilization rate of the soluble solids. During the whole fermentation process, the variation range of the soluble solids in the mixed fermentation broth was 5.50 ± 0.082 and 4.43 ± 0.125. The soluble solids in the fermentation broth promoted the normal metabolism of *T. versicolor*.

Color is an essential feature of food and the first sensory attribute of consumers to food (Dimitra et al., 2020). The color change may be related to the complex enzyme system produced during the growth and metabolism of *T. versicolor* (Ana et al., 2020), such as laccase and manganese peroxidase, which were often used in decolorization, clarification, and lignin degradation (Kandhola et al., 2017).

3.3. The effect of T. versicolor fermentation on protein and free amino acid

During the early fermentation period of *T. versicolor*, the protein content in the compound solution of RRT and CS was continuously decreasing (Fig. 1). This may be due to the production of enzymolysis proteins during the early growth period, which was used as nutrients for the growth of *T. versicolor*. As a result, the protein content in the compound solution decreased. However, the protein content in the compound solution significantly increased during the late fermentation period. That may be because after the growth of *T. versicolor* was complete, the metabolism shifted towards protein synthesis. As a result, the fermentation liquid started to accumulate more protein.

Furthermore, throughout the fermentation process, the protein content in the fermentation liquid with strain (JFB) was higher than in the fermentation liquid (FB). That could be because the mycelia of *T. versicolor* utilized the nutrients in the fermentation liquid to synthesize intracellular proteins during growth. Then, these proteins were released into the fermentation liquid during processing, leading to increased protein content in JFB (Lv, 2020).

The composition and content of free amino acids were measured in 5 fermentation broth samples on the 0th, 2nd, and 4th days of fermentation (FB and JFB). Fifteen amino acids were detected in the samples, including seven essential amino acids such as threonine, histidine, isoleucine, leucine, lysine, valine, and phenylalanine (Table 1). Except for the decrease of amino acid types in the samples of fermentation 4 days, the amino acid composition of other samples did not change significantly, but the content changed significantly. The content of almost every amino acid increased first and then decreased. At the same
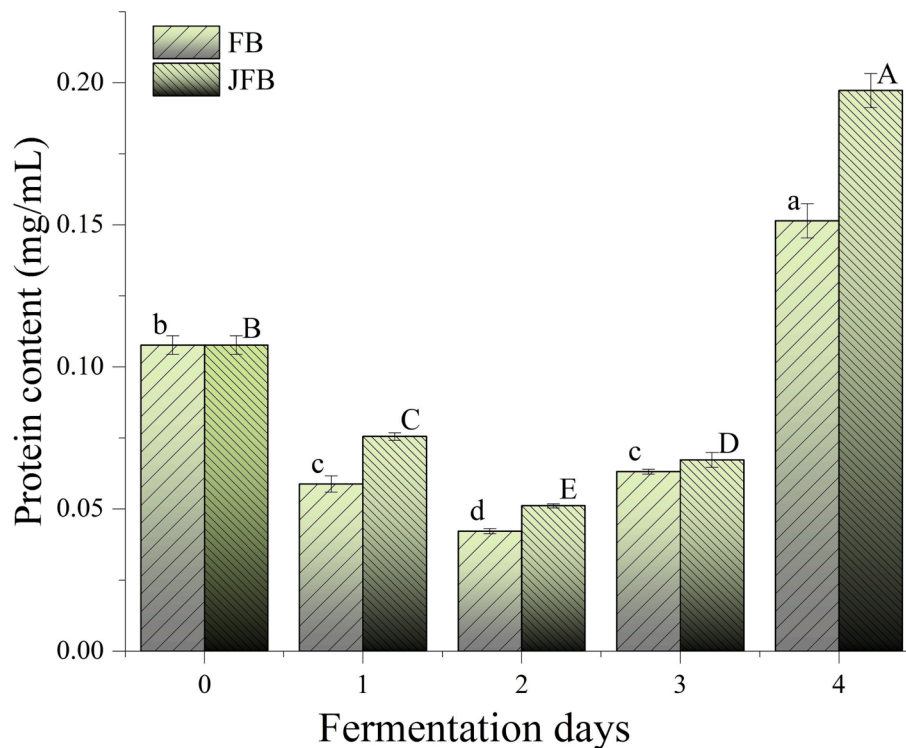
Fig. 1. Effect of *T. versicolor* fermentation time on the protein content in the RRT and CS mixture. Different letters on the same type of data in the figure indicate significant differences ($P < 0.05$), and the same letters indicate insignificant differences ($P > 0.05$). FB and JFB represent fermentation liquid samples and fermentation liquid samples with strain, respectively.

Table 1
Free amino acid composition and content comparison of the mixture of RRT and CS in different fermentation times of *T. versicolor* (mg/g).

Amino acid type	0 day	2 days (FB)	2 days (JFB)	4 days (FB)	4 days (JFB)
Threonine*	0.06 ± 0.002^c	0.1 ± 0.010^b	0.13 ± 0.010^a	0.01 ± 0.001^e	0.03 ± 0.002^d
Valine*	0.07 ± 0.001^c	0.12 ± 0.010^b	0.15 ± 0.001^a	ND	0.03 ± 0.002^d
Isoleucine*	ND	0.07 ± 0.001^b	0.09 ± 0.010^a	ND	ND
Leucine*	0.02 ± 0.002^d	0.12 ± 0.002^b	0.21 ± 0.01^a	ND	0.08 ± 0.010^c
Phenylalanine*	0.03 ± 0.002^d	0.11 ± 0.005^b	0.19 ± 0.001^a	ND	0.05 ± 0.010^c
Histidine*	0.1 ± 0.011^c	0.08 ± 0.001^d	0.12 ± 0.002^b	ND	0.16 ± 0.001^a
Lysine*	0.12 ± 0.010^c	0.21 ± 0.001^b	0.3 ± 0.005^a	ND	0.1 ± 0.002^d
aspartic acid	0.07 ± 0.005^c	0.1 ± 0.010^b	0.14 ± 0.001^a	0.02 ± 0.002^e	0.05 ± 0.001^d
Serine	0.09 ± 0.001^c	0.12 ± 0.004^b	0.18 ± 0.010^a	0.06 ± 0.020^d	0.04 ± 0.003^e
Glutamate	0.62 ± 0.006^c	0.71 ± 0.011^b	0.87 ± 0.001^a	ND	0.24 ± 0.002^d
Glycine	0.02 ± 0.003^d	0.11 ± 0.010^b	0.15 ± 0.004^a	ND	0.04 ± 0.005^c
Alanine	0.18 ± 0.001^c	0.21 ± 0.010^b	0.31 ± 0.005^a	ND	0.18 ± 0.007^c
cysteine	ND	ND	ND	ND	0.02 ± 0.001^a
tyrosine	0.04 ± 0.003^d	0.09 ± 0.012^b	0.15 ± 0.001^a	ND	0.05 ± 0.005^c
Arginine	0.87 ± 0.002^b	0.86 ± 0.010^b	1.1 ± 0.020^a	ND	0.2 ± 0.021^c
essential amino acids	0.4	0.81	1.19	0.01	0.45
total free amino acids	2.28	3.01	4.09	0.09	1.26

The amino acid marked with * is an essential amino acid, and ND indicates that it is not detected; Different letters in the same column in the table indicate significant differences ($p < 0.05$), and the same letters indicate insignificant differences ($p > 0.05$). FB and JFB represent fermentation liquid samples and fermentation liquid samples with strain, respectively.

fermentation time, the content and composition of JFB after four days of fermentation were significantly higher than FB ($P < 0.05$).

At the early fermentation stage, protein content decreased, and free amino acid content increased. At the later fermentation stage, the content of free amino acids decreased, and the protein content grew. These changes may be related to a series of enzymes produced during the growth and metabolism of *T. versicolor*. *T. versicolor* is a fungus rich in protein, fat, polysaccharide peptide, polysaccharide, lignin, glucan, amino acid, and various inorganic salts, as well as protease, peroxidase, amylase, insect laccase, and leather enzyme (Xiong, 2021). It was reported that edible mushrooms would produce some extracellular enzymes during their growth, which either decompose the substrate for the

growth and development of strains or catalyze the synthesis of bioactive substances (Ning et al., 2020). A new immune regulatory protein TVC from *T. versicolor* purified by ammonium sulfate precipitation, ion exchange chromatography, and gel filtration chromatography can be used as an immune stimulator to enhance the proliferation of spleen cells (Li et al., 2011). Some scholars have studied that *Grifola frondosa* has a highly active proteolytic enzyme to digest soybean protein. *Grifola frondosa* protease releases free amino acids in soybean milk from soybean protein (Nishiwaki et al., 2009).

3.4. Reducing sugar and polysaccharide

The content of reduced sugar increases first and then decreases (Fig. 2a). The slow accumulation of reduced sugar content in the early stage was related to some enzymes produced during the growth and metabolism of *T. versicolor* (Zhang et al., 2015). In the later phase, *T. versicolor* fermentation required reducing sugar as the carbon source, so its content declined. The difference in reducing sugar content between JFB and FB was not apparent.

T. versicolor can produce extracellular polysaccharides during its growth (Scarpari et al., 2017). The polysaccharides extracted from mycelium and fermentation broth have vigorous anti-tumor activity (Jing et al., 2022). The polysaccharide content in FB and JFB increased first and then decreased (Fig. 2b). On the second day of fermentation, the polysaccharide content reached the highest, reaching 0.895 ± 0.007 mg/mL and 0.942 ± 0.014 mg/mL, respectively. The increase in polysaccharide content was due to the ability of *T. versicolor* to synthesize polysaccharides, including extracellular polysaccharides and intracellular polysaccharides. This decrease may be attributed to the requirement to metabolize polysaccharides for energy in the later stage as nutrients were depleted. At the early growth stage, *T. versicolor* will use the mixed liquid matrix to produce extracellular polysaccharides and synthesize intracellular polysaccharides to form a part of the mycelia (Hu et al., 2016; Zhang & Shi, 2010). So, the polysaccharide content in JFB was significantly higher than that in FB.

3.5. Vitamin C and total phenol

The growth of *T. versicolor* required a certain amount of ventilation and oxygen supply, so vitamin C content was affected by ventilation during the fermentation of *T. versicolor*. Vitamin C content in FB and JFB decreased with the progress of fermentation, especially in the late fermentation period (Fig. 3a). Compared with the unfermented mixed liquid, vitamin C content in the mixed liquid on the first day of fermentation decreased by about 20 %. The difference between vitamin C content in the mixed liquor on the second day of fermentation and the mixed liquor on the first day of fermentation was not noticeable. On the third day of fermentation, vitamin C content dropped sharply, reducing by about 70 %. On the fourth day, vitamin C had been exhausted, leaving only about 9 %. The rapid decrease in vitamin C content in the late stage of fermentation may be due to the utilization of carbon sources, nitrogen sources, and other nutrients by *T. versicolor* fermentation, leading to a continuous reduction of substances that can provide antioxidant protection for vitamin C (Liu et al., 2021). So, the vitamin C

content decreased slowly at first and then rapidly.

The total phenol content in each fermentation stage's fermentation liquid (FB and JFB) was similar (Fig. 3b). Still, the total phenol content in the two sample solutions decreased continuously with the extension of fermentation time and changed significantly. Compared with the unfermented mixed liquid of RRT and CS, the total phenol content in JFB and FB fermented for 4 days decreased by 79 % and 81 %, respectively. The degradation of phenols was the main reason for reducing total phenol content. The stress reaction process of microorganisms in the fermentation process and the enzymes generated in the metabolic process will promote the degradation of phenols, thus reducing the total phenol content. It was speculated that the decrease of total phenol content in this study has a significant correlation with the enzyme because the growth and metabolism of *T. versicolor* will produce laccase, a polyphenol oxidase that enables phenol substances to undergo oxidative polymerization. It was mainly used for decolorization, clarification, and lignin decomposition (Ana et al., 2020; Xu et al., 2020).

3.6. Effects of different strains on RCB

Lactic acid strain fermentation has no significant impact on vitamin C in the fermentation broth, and the preservation rate of vitamin C was more than 90 % (Table 2). Among them, NR1-7 fermentation has the smallest impact on vitamin C, and the preservation rate of vitamin C could reach more than 95 %. Although *Saccharomyces cerevisiae* (SC) and *Fragrant yeast* (FS) significantly affect vitamin C, their retention rates of vitamin C were also above 80 %.

Some lactic acid strains and yeasts will produce extracellular polysaccharides when they grow and metabolize (Ana et al., 2021; Liu et al., 2021). These polysaccharides have many functions, such as hypoglycemic effect, anti-tumor effect, antioxidant effect, immunomodulatory effect, etc. (Ismail & Nampoothiri, 2010; Sasikumar et al., 2017; Zhai et al., 2021; Zhang et al., 2017). It can be seen from Table 2 that there were significant differences between the polysaccharide contents after fermentation by various strains. Compared with the single fermentation of *T. versicolor,* except for the substantial reduction of the polysaccharide content in the sample liquid fermented by two yeasts, FS and SC, the polysaccharide content in the sample liquid fermented by lactic acid strain has increased to varying degrees. Among them, the polysaccharide content in the fermentation liquid of LB12 was the highest, reaching 1.91 mg/mL. Compared with *T. versicolor* fermentation broth, the polysaccharide content increased by 35.6 %. The polysaccharide content in the fermentation broth of Q-1 and BZ11 was the second, reaching 1.89 mg/mL and 1.86 mg/mL, respectively. The capsular
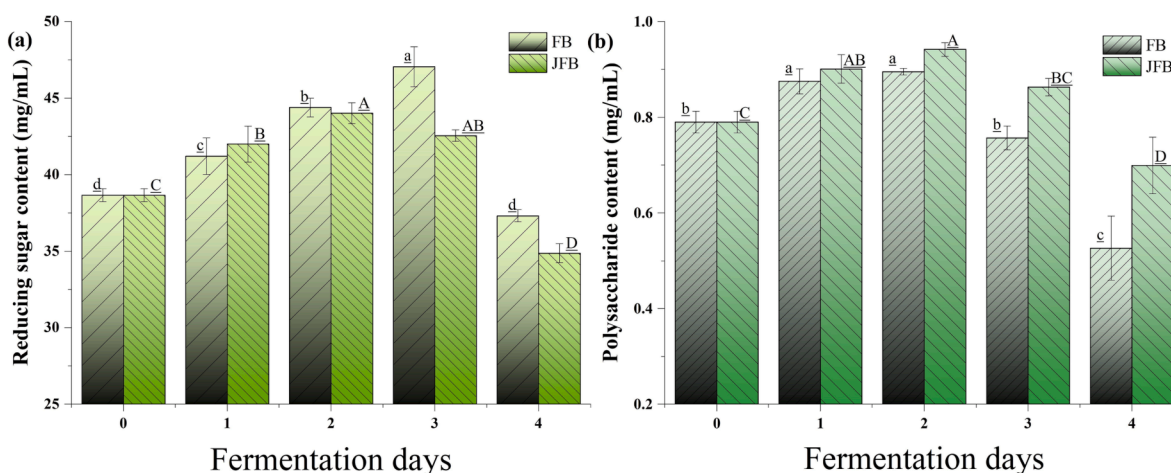

Fig. 2. Effect of *T. versicolor* fermentation time on reducing sugar (a) and polysaccharide content (b) in RRT and CS composite solution. Different letters on the same type of data in the figure indicate significant differences ($P < 0.05$), and the same letters indicate insignificant differences ($P > 0.05$). FB and JFB represent fermentation liquid samples and fermentation liquid samples with strain, respectively.
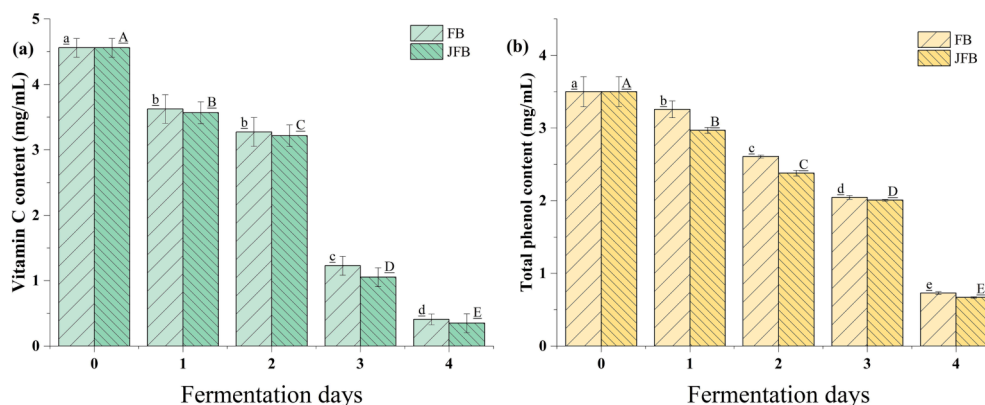
Fig. 3. Effect of fermentation time on vitamin C (a) and total phenols (b) in the complex liquid of RRT and CS. Different letters on the same type of data in the figure indicate significant differences ($P < 0.05$), and the same letters indicate insignificant differences ($P > 0.05$). FB and JFB represent fermentation liquid samples and fermentation liquid samples with strain, respectively.

Table 2
Effects of fermentation of different strains on RCB.

Strains	Vitamin C (mg/mL)	Polysaccharide (mg/mL)	Sensory value	GABA (mg/100 mL)
MT-4	3.31 ± 0.102[ab]	1.39 ± 0.021[c]	74.16 ± 0.770[ab]	3.65 ± 0.187[ab]
BZ25	3.39 ± 0.108[ab]	1.69 ± 0.017[b]	76.48 ± 0.610[ab]	3.39 ± 0.086[ab]
BZ11	3.28 ± 0.076[ab]	1.86 ± 0.164[a]	79.08 ± 0.572[a]	3.27 ± 0.4731[b]
NR1-7	3.41 ± 0.189[ab]	1.49 ± 0.011[c]	72.60 ± 0.427[ab]	3.85 ± 0.100[a]
LB12	3.31 ± 0.086[ab]	1.91 ± 0.019[a]	81.32 ± 0.627[a]	3.69 ± 0.081[ab]
SC	3.19 ± 0.066[b]	1.13 ± 0.013[d]	63.76 ± 0.720[b]	0.67 ± 0.023[c]
FY	3.17 ± 0.072[b]	1.03 ± 0.021[d]	63.68 ± 0.810[b]	0.65 ± 0.011[c]
Q-1	3.37 ± 0.150[ab]	1.89 ± 0.013[a]	80.80 ± 0.559[a]	3.62 ± 0.365[ab]
T. versicolor	3.56 ± 0.165[a]	1.40 ± 0.023[c]	78.80 ± 0.490[a]	3.30 ± 0.096[b]

Different letters in the same column in the table indicate significant differences ($p < 0.05$), and the same letters indicate insignificant differences ($p > 0.05$).

polysaccharide of *L. plantarum* can be used as a potential functional food component to fight against intestinal barrier dysfunction (Liu et al., 2022).

It can be seen from sensory that compared with the compound liquor fermented by *T. versicolor* alone, the sensory scores of the two kinds of yeast fermentation liquor, SC and FS, were significantly lower, which may be related to personal preferences. The difference in sensory scores of yeast fermentation liquor was relatively significant. Except for two kinds of yeasts, there was no significant difference between the sensory score of the sample liquid fermented by lactic acid strain and that of the single *T. versicolor* fermentation.

The glutamic acid content in the compound liquid of RRT and CS fermented by *T. versicolor* was high, accounting for about 20 % of the total free amino acid content. Most lactic acid bacteria can produce glutamate decarboxylase. Glutamate produces GABA under the action of glutamate decarboxylase, so some lactic acid bacteria fermentation can increase GABA content (Li et al., 2020; Shan et al., 2015). Table 2 showed that NR1-7 fermentation increases GABA, which has the strongest ability. Its content can reach 3.85 mg/100 mL, rising by about 17 %, LB12 fermentation increased by about 11 %, while MT-4, Q-1, and BZ25 have a decreasing ability to increase GABA through fermentation.

According to sensory score, polysaccharide content, vitamin C content, and analysis of four indicators, such as GABA content, the fermentation effect of LB12 was relatively good.

3.7. Effect of process parameters on RCB

The effect of fermentation time on the quality of RCB is shown in Fig. S2(a) and (b). When the fermentation time was 18 h, the polysaccharide content was the highest, reaching 2.84 mg/mL ($P < 0.05$); The effect of fermentation time on sensory scores was not significant ($P > 0.05$). Therefore, 18 h was chosen as the optimal fermentation time.

The effect of fermentation temperature on the quality of RCB is shown in Fig. S2(c) and (d). As the fermentation temperature increased, the polysaccharide content increased and then decreased. At a fermentation temperature of 33 °C, the polysaccharide content increases significantly, reaching 2.58 mg/mL ($P < 0.05$). The sensory score was also the highest at this fermentation temperature, reaching 84.95 points ($P < 0.05$). The fermentation temperature did not significantly affect GABA and vitamin C content ($P > 0.05$). The GABA content was the highest at a fermentation temperature of 33 °C, reaching 4.59 mg/100 mL ($P < 0.05$). Considering various indicators, 33 °C was selected as the optimal fermentation temperature.

The effect of the inoculation amount on the quality of RCB is shown in Fig. S2(e) and (f). When the inoculation amount was 2 %, the polysaccharide content in the fermentation broth was the highest, reaching 2.75 mg/mL ($P < 0.05$). The sensory score was also the highest at this inoculation amount, with 80.9 points. The effect of inoculation amount on the vitamin C content in the fermentation broth was insignificant, and the vitamin C content was maintained at around 3 mg/mL. However, it significantly impacted the GABA content in the fermentation broth ($P < 0.05$). When the inoculation amount was 3 % to 4 %, the GABA content increased significantly, reaching 4.61 mg/ 100 mL to 4.68 mg/100 mL. After comprehensive consideration, 2 % was chosen as the optimal inoculation amount.

The effect of sucrose addition on the quality of RCB was shown in Fig. S2(g) and (h). As the sucrose addition increased, the polysaccharide content gradually increased. When the sucrose addition was 7 % −9%, the polysaccharide content in the fermentation broth was the highest, reaching 2.34 mg/mL to 2.55 mg/mL, and there was no significant difference between the two concentrations ($P > 0.05$). When sucrose was added at 7 %, the sensory score was the highest, reaching 89.95 points. The effect of sucrose addition on vitamin C and GABA content was not significant ($P > 0.05$), therefore. Choose 7 % as the optimal amount of sucrose added.

3.8. Optimization of fermentation conditions using response surface methodology

The response surface results are shown in Table S3. The test results were analyzed by the ANOVA program in the DesignExpert software. The second-order polynomial equations of GABA (Y1), polysaccharide (Y2) and sensory value (Y3) for the independent variables such as fermentation time (A), fermentation temperature (B), strain ratio (C), and inoculation amount (D) were obtained as follows:

$Y1 = +5.09 + 0.058 \times A - 0.032 \times B - 0.11 \times C - 0.21 \times D - 0.096 \times AB - 0.13 \times BC + 0.058 \times A^2 - 0.070 \times B^2 - 0.16 \times C^2 + 0.023 \times D^2$.

$Y2 = +2.89 + 0.057 \times A - 0.015 \times B + 0.029 \times C - 0.20 \times D - 0.072 \times AB + 0.072 \times AD - 0.15 \times BC - 0.11 \times A^2 - 0.25 \times B^2 - 0.16C^2 - 0.020 \times D^2$.

$Y3 = +82.79 - 0.43 \times A + 2.29 \times B + 0.70 \times C + 3.25 \times D - 1.30 \times AB + 0.41 \times AC - 0.54 \times AD - 1.28 \times A^2 + 1.18 \times C^2 - 2.41 \times D^2$.

GABA ($P < 0.0001$), polysaccharide ($P < 0.0001$), and sensory value ($P < 0.0001$) of the model were significant, but the missing item was not significant. The response value of GABA was $R^2 = 90.83$ %, polysaccharide was $R^2 = 89.95$ %, and sensory value was $R^2 = 84.33$ % (Tables S4–S6), indicating that the correlation between actual and predicted values of GABA, polysaccharide and sensory value was high (Sriwiang et al., 2022). There were interactions among various factors to varying degrees, $P = 0.0033 < 0.01$ for BC and $P = 0.0228 < 0.05$ for AB, so the interaction of BC has a more significant impact on the content of GABA (Fig. S3(a) and (b)). With the increase of fermentation temperature and time, the content of GABA increased slowly at first and tended to decline. That may be due to fermentation time and temperature, which affect the growth and fermentation activity of microorganisms, influencing the content of GABA. It can be seen that the interaction of fermentation temperature and fermentation time has a very significant impact on the polysaccharide content (Fig. S3(c) and (d)). In contrast, the interaction of inoculation amount and fermentation temperature and inoculation amount and sucrose addition amount have no significant impact on the polysaccharide content. With the increase in fermentation temperature and fermentation time, the polysaccharide content first increased and then decreased. That was due to the influence of fermentation time and temperature on the growth and fermentation activity of microorganisms, thus affecting their ability to produce extracellular polysaccharides. Each factor has different degrees of interaction, and the interaction *P* values between factors AB, AC, and AD was greater than 0.05, indicating that the interaction between the factors has no significant impact on the sensory value and the order of the interaction between the factors on the sensory value was AB > AD > AC (Fig. S3(e) and (f)).

According to the Design-Expert analysis, the optimal fermentation parameters were: the inoculation amount was 1.7 %, the fermentation temperature was 35 °C, the fermentation time was 15 h, and the sucrose addition was 6 %. Under the modified optimization conditions, the GABA content of RCB was 5.123 mg/100 mL, the polysaccharide content was 2.825 mg/mL, and the sensory value was 82.75. The result was close to the predicted value of the response surface.

3.9. Electronic tongue analysis

The electronic tongue can convert electrical signals into taste signals to distinguish the taste of fermented beverages, and its sensory threshold is small, which can exclude the subjectivity of sensory evaluation (Jiang et al., 2018). The taste change of RCB in three stages: unfermented, *T. versicolor* fermentation, and LB12 tandem fermentation (Fig. 4). It can be seen that with the fermentation, the sourness and richness increased, the umami decreased, the fermentation of *T. versicolor* reduced the bitterness and astringency of the fermentation broth. The tandem fermentation of LB12 increased the astringency of the fermentation broth. The bitterness decreased due to the formation of acid masking other flavors (Sun et al., 2022).
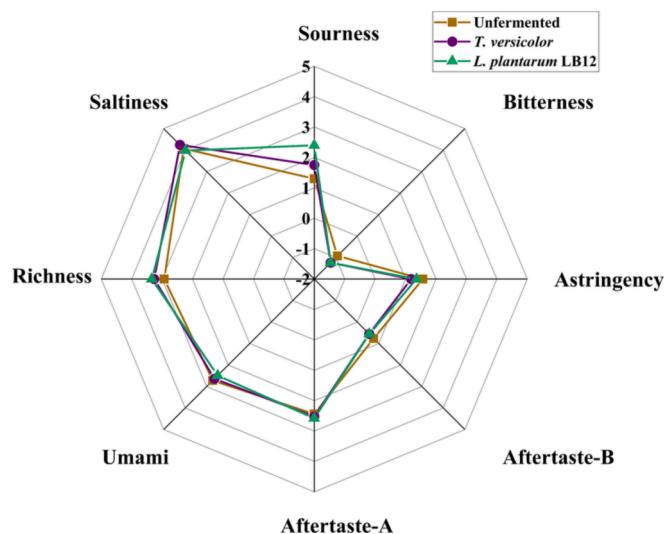

Fig. 4. Electronic tongue analysis at different stages of fermentation.

4. Conclusions

This study investigated the effect of *T. versicolor* and *L. plantarum* LB12 on RCB by phased fermentation. It has been proven that *T. versicolor* fermentation improved the physicochemical quality of RCB, significantly increased polysaccharides and free amino acids, and reduced the bitter taste. On this basis, *L. plantarum LB12* fermentation elevated 35.6 % polysaccharides and 11 % GABA, and the retention rate of vitamin C reached more than 90 %. Response surface methodology optimized RCB fermentation conditions and produced the best sensory experience, polysaccharides, and GABA. This study provides a reference for extending the processing chain of industries of unique agricultural products (such as RRT and CS) in Guizhou and developing new high-value fermented beverages.

CRediT authorship contribution statement

Mengqi Liu: Data curation, Formal analysis, Writing – original draft. **Xueyi Tian:** Data curation, Software. **Laping He:** Writing – review & editing, Resources, Supervision. **Cuiqin Li:** Supervision, Validation, Software. **Han Tao:** Funding acquisition, Resources. **Xiao Wang:** Software, Supervision. **Shunbin Qiao:** Funding acquisition, Supervision. **Xuefeng Zeng:** Supervision, Resources.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

Consent

All participants stated that they agreed to participate in the study and use their information. All authors stated that this study protects the rights and privacy of all participants.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101041.

References

Ana, B.-K., Fátima, F., Tània, S., Mirela, P., Marina, T., Gordana, Š., … B, A. P. (2020). Enhancement of the anti-inflammatory properties of grape pomace treated by *Trametes versicolor*. *Food & function, 11*(1). https://doi.org/10.1039/c9fo02296a

Ana, H., Panagiota, S., Sreeram, U., Ana, R., Maria, E., Francesca, B., … Ken, N. (2021). Bifidobacterium breve exopolysaccharide blocks dendritic cell maturation and activation of CD4+ T Cells. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.653587

Chen, L. (2012). *Study on the processing technology of coix seed solid beverage.* Fujian Agricultural and Forestry University. Unpublished Master thesis.

Dimitra, D., Nikoletta, S., Evangelos, K., & Panagiotis, K. (2020). Yogurts supplemented with juices from grapes and berries. *Foods, 9*(9). https://doi.org/10.3390/foods9091158

Feng, Y., Li, Z., Zhu, L., Liu, Y., Yang, X., Zhang, Y., … zhu, W. (2022). Research progress of soluble solid content in tomato. *Molecular Plant Breeding, 20*(15), 5158–5163. https://doi.org/10.13271/j.mpb.020.005158

Hu, J., Ren, Y., & Lu, Z. (2016). Optimization of liquid fermentation conditions of polysaccharide from *Coriolus versicolor*. *Edible Fungi, 38*(01), 17–18.

Ismail, B., & Nampoothiri, K. M. (2010). Production, purification and structural characterization of an exopolysaccharide produced by a probiotic *Lactobacillus plantarum* MTCC 9510. *Archives of Microbiology, 192*(12), 1049–1057. https://doi.org/10.1007/s00203-010-0636-y

Jiang, H., Zhang, M., Bhandari, B., & Adhikari, B. (2018). Application of electronic tongue for fresh foods quality evaluation: A review. *Food Reviews International, 34*(8), 746–769. https://doi.org/10.1080/87559129.2018.1424184

Jing, Y., Zhang, S., Li, M., Ma, Y., Zheng, Y., Zhang, D., & Wu, L. (2022). Research progress on the extraction, structure, and bioactivities of polysaccharides from *Coriolus versicolor*. *Foods, 11*(14). https://doi.org/10.3390/foods11142126

Juliana, M., Habtu, S., Jolien, D., J., S. J., Xavier, G., Ann, d. W., O., M. A., & Katleen, R. (2021). Effect of lactic acid fermentation of watermelon juice on its sensory acceptability and volatile compounds. *Food Chemistry, 358*. https://doi.org/10.1016/j.foodchem.2021.129809.

Kandhola, G., Rajan, K., Labbé, N., Chmely, S., Heringer, N., Kim, J.-W., … Carrier, D. J. (2017). Beneficial effects of *Trametes versicolor* pretreatment on saccharification and lignin enrichment of organosolv-pretreated pinewood. *RSC Advances, 7*(72), 45652–45661. https://doi.org/10.1039/c7ra09188e

Li, F., Wen, H., Zhang, Y., Aa, M., & Liu, X. (2011). Purification and characterization of a novel immunomodulatory protein from the medicinal mushroom *Trametes versicolor*. *Science China Life Sciences, 54*(4), 379–385. https://doi.org/10.1007/s11427-011-4153-2

Li, H., Fang, W., Wang, Z., & Chen, Y. (2022). Physicochemical, biological properties, and flavour profile of *Rosa roxburghii* Tratt, *Pyracantha fortuneana*, and *Rosa laevigata* Michx fruits: A comprehensive review. *Food Chemistry, 366*, Article 130509. https://doi.org/10.1016/j.foodchem.2021.130509

Li, H., Pan, D., Wu, Z., Zeng, X., & Wu, A. (2020). Screening and identification of GABA-producing lactic acid bacteria and its fermentation conditions. *Science and Technology of Food Industry, 41*(23), 78–84. https://doi.org/10.13386/j.issn1002-0306.2019030272

Liu, K., Chen, Y.-Y., Zha, X.-Q., Li, Q.-M., Pan, L.-H., & Luo, J.-P. (2021). Research progress on polysaccharide/protein hydrogels: Preparation method, functional property and application as delivery systems for bioactive ingredients. *Food Research International, 147*. https://doi.org/10.1016/j.foodres.2021.110542

Liu, L., Xu, J., Du, R., Ping, W., Ge, J., & Zhao, D. (2021). The response surface optimization of exopolysaccharide produced by *Saccharomyces cerevisiae* Y3 and its partial characterization. *Preparative Biochemistry & Biotechnology, 52*(5). https://doi.org/10.1080/10826068.2021.1972428

Liu, Y., Liu, Q., Zhao, J., Zhang, H., Zhai, Q., & Chen, W. (2022). Strain-specific regulative effects of *Lactobacillus plantarum* on intestinal barrier dysfunction are associated with their capsular polysaccharides. *International Journal of Biological Macromolecules, 222*(Pt A), 1343–1352. https://doi.org/10.1016/j.ijbiomac.2022.09.136

Lv, H. (2020). Effect of chemical components of mycelium of *Coriolus Hirsutus* in sports health care. *Edible Fungi of China, 39*(03), 222–225. https://doi.org/10.13629/j.cnki.53-1054.2020.03.069

Ning, Y., Zhu, C., Pan, J., Zheng, Y., Feng, Y., & Zhang, D. (2020). Advances in studies on edible fungi enrichment for trace elements and their effects on enzyme activity. *Modern, Food(13)*, 109–112. https://doi.org/10.16736/j.cnki.cn41-1434/ts.2020.13.036

Nishiwaki, T., Asano, S., & Ohyama, T. (2009). Properties and substrate specificities of proteolytic enzymes from the edible basidiomycete *Grifola frondosa*. *Journal of Bioscience and Bioengineering, 107*(6). https://doi.org/10.1016/j.jbiosc.2009.01.008

Sasikumar, K., Vaikkath, D. K., Devendra, L., & Nampoothiri, K. M. (2017). An exopolysaccharide (EPS) from a Lactobacillus plantarum BR2 with potential benefits for making functional foods. *Bioresource Technol, 241*. https://doi.org/10.1016/j.biortech.2017.05.075

Scarpari, M., Reverberi, M., Parroni, A., Scala, V., Fanelli, C., Pietricola, C., … Rizzo, R. (2017). Tramesan, a novel polysaccharide from *Trametes versicolor*. Structural characterization and biological effects. *PLoS ONE, 12*(8). https://doi.org/10.1371/journal.pone.0171412

Shan, Y., Man, C. X., Han, X., Li, L., Guo, Y., Deng, Y., … Jiang, Y. J. (2015). Evaluation of improved γ-aminobutyric acid production in yogurt using *Lactobacillus plantarum* NDC75017. *Journal of Dairy Science, 98*(4), 2138–2149. https://doi.org/10.3168/jds.2014-8698

Sriwiang, R., Ratchanee, C., Natthaya, C., Wanticha, S., & Wiboon, R. (2022). Response surface optimization for antioxidant extraction and attributes liking from roasted rice germ flavored herbal tea. *Processes, 10*(1). https://doi.org/10.3390/pr10010125

Sun, H., Chen, X., Xiang, Y., Hu, Q., & Zhao, L. (2022). Fermentation characteristics and flavor properties of Hericium erinaceus and Tremella fuciformis fermented beverage. *Food Bioscience*. https://doi.org/10.1016/j.fbio.2022.102017

Sun, T., Jiang, H., Yang, K., Li, X., Wang, S., Yao, H., … Sun, D. (2022). Nutritional Function and Flavor Evaluation of a New Soybean Beverage Based on Naematelia aurantialba Fermentation. *Foods, 11*(3). https://doi.org/10.3390/foods11030272

Udhav, G. V., Pandit, M. V., R.L., G., L., R., M, N., L., R., A, R., R.E., T., T.E., O. D., R.G., T., B, V., B, W., B.K., C., Jiao, H., HJ, L., P., D., & B.S., J. (2021). Some wood-rotting fungi from Gautala Autram ghat Sanctuary, Maharashtra(India). *BIOINFOLET - A Quarterly Journal of Life Sciences, 18*(3).

Wang, D., Wang, Y., Zhang, Y., Zuo, Z., Liu, S., Zhang, Y., & Li, Z. (2021). Study on the components and antioxidant function of kidney bean enzyme during fermentation. *Science and Technology of Food Industry, 42*(18), 18–24. https://doi.org/10.13386/j.issn1002-0306.2020120042

Xin, Y.-H., Zhao, S.-H., Yang, J., Zhang, T.-D., Zhang, J.-H., & Wang, Y. (2021). Effect of microwave-drying on the quality and antioxidant properties of *Ganoderma lucidum* fermented sea-buckthorn tea. *International Journal of Food Engineering, 17*(1), 65–74. https://doi.org/10.1515/ijfe-2019-0271

Xiong, Y. (2021). *Study on variety selection and primary cultivation techniques of Trametes versicolor.* Hunan Agricultural University. Unpublished Master thesis.

Xu, J., Vidyarthi, S. K., Bai, W., & Pan, Z. (2019). Nutritional constituents, health benefits and processing of *Rosa Roxburghii*: A review. *Journal of Functional Foods, 60*(C). https://doi.org/10.1016/j.jff.2019.103456

Xu, L., Sun, K., Wang, F., Zhao, L., Hu, J., Ma, H., & Ding, Z. (2020). Laccase production by *Trametes versicolor* in solid-state fermentation using tea residues as substrate and its application in dye decolorization. *Journal of Environmental Management, 270*. https://doi.org/10.1016/j.jenvman.2020.110904

Yin, X. (2020). *Study on the technology and quality analysis of fermented pomegranate juice.* Xihua University. Unpublished Master thesis.

Yoshikawa, Y., Chen, P., Zhang, B., Scaboo, A., & Orazaly, M. (2014). Evaluation of seed chemical quality traits and sensory properties of natto soybean. *Food Chemistry, 153*. https://doi.org/10.1016/j.foodchem.2013.12.027

Zhai, Z., Xie, S., Zhang, H., Yi, H., & Hao, Y. (2021). Homologous over-expression of chain length determination protein EpsC Increases the molecular weight of exopolysaccharide in *Streptococcus thermophilus*. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.696222

Zhang, J., Zhao, X., Jiang, Y., Zhao, W., Guo, T., Cao, Y., … Yang, Z. (2017). Antioxidant status and gut microbiota change in an aging mouse model as influenced by exopolysaccharide produced by *Lactobacillus plantarum* YW11 isolated from Tibetan kefir. *Journal of Dairy Science, 100*(8). https://doi.org/10.3168/jds.2016-12480

Zhang, Y., Fraatz, M. A., Müller, J., Schmitz, H.-J., Birk, F., Schrenk, D., & Zorn, H. (2015). Aroma characterization and safety assessment of a beverage fermented by *Trametes versicolor*. *Journal of Agricultural and Food Chemistry, 63*(31). https://doi.org/10.1021/acs.jafc.5b02167

Zhang, Y., Wang, D., Chen, Y., Liu, T., Zhang, S., Fan, H., … Li, Y. (2021). Healthy function and high valued utilization of edible fungi. *Food Science and Human Wellness, 10*(4), 408–420. https://doi.org/10.1016/j.fshw.2021.04.003

Zhang, Z., & Shi, J. (2010). Liquid cultivation of edible mushroom in oat emulsion and development of beverage from the culture. *Food Science, 31*(05), 169–174.

Zhu, F. (2017). Coix: Chemical composition and health effects. *Trends in Food Science and Technology, 61*, 160–175. https://doi.org/10.1016/j.tifs.2016.12.003