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Research Article

# Fortification effect of mixed fermentation product of *Russula vinosa* Lindblad supplementation on physicochemical, sensory and antioxidant properties of wheat bread

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**Abstract:** *Russula vinosa* Lindblad is a traditional food and medicine resource with potential application in the field of functional foods, which is rich in polyphenolic compounds. In this study, the *R. vinosa* Lindblad was mixed fermented with *Saccharomyces boulardii* and *Lactobacillus lactis* and the antioxidant activity, composition and application in functional bread of the ethanol extract of the mixed bacterial fermentation product (EMFP) were investigated. The results showed that after fermentation, 186 new compounds appeared in EMFP, including organic acids and phenolic acids. The addition of EMFP significantly enhanced the quality and antioxidant activity of the bread, including reduction of hardness and chewiness, enhancement of resilience, and improvement of free radical scavenging activity and total reducing power. Furthermore, the EMFP addition affected color, odor, and texture indicators of the bread, and negatively affected at higher additions. 0.5% EMFP addition might be appropriate to positively affect the quality, sensory evaluation, and antioxidant activity of the bread. This study investigated the positive effects of mixed fermentation on the active ingredients of the *R. vinosa* Lindblad, providing a reference for the practical application of EMFP in the field of functional foods.

**Keywords:** *Russula vinosa* Lindblad; mixed bacterial fermentation; functional bread; physicochemical properties; antioxidant activity; sensory analysis

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## 1 Introduction

Edible mushrooms, as traditional Chinese food and medicine homology resources, have multiple beneficial health values, which are important resources in the development of functional foods[1-3]. Among them, the *Russula vinosa* Lindblad is gradually appreciated for its unique flavor and nutritional value, as well as its biological activities such as antioxidant, anti-inflammatory and activities anti-cancer[4,5]. It was revealed that the content of polyphenols in the *R. vinosa* Lindblad could reach up to (139.39 ± 2.41) mg/g, which might be an important part for its bioactivities[6]. However, the components of *R. vinosa* Lindblad polyphenols are still unclear and their practical application in food production has not been reported.

Microbial fermentation is a traditional and environment-friendly technology of food processing to optimize food properties[7]. In addition, the enzyme systems secreted by microorganisms during the fermentation process could promote cell lysis in the fermentation matrix and facilitate the release of active ingredients such as polyphenols and polysaccharides[8]. Notably, during fermentation, microorganisms utilize the nutrients in the substrate for growth and metabolism, which might facilitate the conversion of some active materials and enhance their bioactivity[9]. In particular, some studies have found that when suitable strains co-fermented the substrate, the enzyme systems and metabolic pathways of the different strains might interact and result in the synergistic fermentation effects[10,11]. For example, using *Saccharomycopsis fibuligera* and *Lacticaseibacillus paracasei* for mixed fermentation of *Dendrobium officinale* could significantly increase the total phenolic content of fermentation products and enhance the antioxidant activity *in vitro*[9]. In addition, the mixed bacterial fermentation on Chinese bayberry pomace could enhance the content of bioactive compounds such as gallic acid, protocatechuic acid, isoquercitrin in the



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fermentation product, with significantly better effect than that of pure strain fermentation[11]. Based on this, the present study was proposed to use *Saccharomyces boulardii* and *Lactobacillus lactis* for mixed bacterial fermentation treatment of the *R. vinosa* Lindblad, aiming to promote the release and conversion of polyphenolic components to enhance their bioactivities.

With the development of society and the enhancement of people's health awareness, some functional breads with natural bioactive ingredients have gradually become popular. These functional ingredients, such as polysaccharides and polyphenols, not only provide the bread with antioxidant, anti-inflammatory and other bioactivities, but also improve the quality of the bread[12]. Adding substances enriched with polyphenols can effectively improve the antioxidant activity of the bread, such as green coffee extracts, ginger powder, and grapeseed[13]. Additionally, owing to the strong reducing properties of some polyphenols, they could interact with proteins in gluten and result in changes in the secondary and tertiary structures of the proteins, affecting the ductility and elasticity of the gluten, and ultimately improving the quality of the bread[14].

The present study was carried out to co-ferment the *R. vinosa* Lindblad using *S. boulardii* and *L. lactis* and analyze the metabolite changes. Subsequently, the physicochemical quality, sensory evaluation and antioxidant activity of the mixed bacterial fermentation product (EMFP)-added wheat bread were evaluated. This study aims to provide a reference for mixed bacterial fermentation of edible mushrooms, as well as laying a foundation for the practical application of the *R. vinosa* Lindblad in the field of functional foods.

## 2 Materials and methods

### 2.1 Materials and chemicals

The *R. vinosa* Lindblad was purchased from Zhengzhou Xinji Flavor Food City and the producing area was Yunnan Province, China. The strains of *S. boulardii* and *L. lactis* were provided by Tianjin University of Science and Technology and kept in our laboratory (Zhengzhou University, China). 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). All other reagents and chemicals used in this study were of analytical grade.

### 2.2 Preparation of EMFP

The *R. vinosa* Lindblad was pretreated and the active components were extracted according to our previous method with some modifications[15]. The fresh *R. vinosa* Lindblad was washed, sliced, dried, crushed and passed through 40 mesh sieves to obtain *R. vinosa* Lindblad powders, which were then divided and sealed for storage. The glycerol-frozen strains of *S. boulardii* and *L. lactis* were secondarily activated respectively to obtain the strain inoculums. The activated *S. boulardii* and *L. lactis* were inoculated into the sterilized *R. vinosa* Lindblad medium (pre-sterilized at 121 °C for 15 min) at 0.5% inoculum, respectively, and then fermented at 180 r/min for 48 h at 30 °C. The fermentation product was mixed well, freeze-dried and preserved to obtain the mixed bacterial fermented product (MFP). The uninoculated *R. vinosa* Lindblad medium with the same treatment was collected and freeze-dried to obtain the unfermented product (UFP).

To investigate the effect of the mixed bacterial fermentation on the composition and biological activity of the MFP, the ethanol-soluble components of the MFP were extracted according to the previous study[16]. In brief, 50% (*v*/*v*) ethanol was used as an extraction solvent. The extraction conditions were as follows: material-liquid ratio of 1:30, extraction temperature of 70 °C, and extraction time of 50 min. Then the supernatant was separated by centrifugation at 2,250 g for 10 min. The precipitate was extracted again according to the above conditions. The two supernatants were combined, concentrated by a rotary evaporator and then lyophilized by a vacuum freeze dryer to obtain ethanol-soluble components in the MFP. The ethanol-soluble components extracted from MFP and UFP were named EMFP and EUFP, respectively.

### 2.3 Determination of EMFP antioxidant activity

The *in vitro* antioxidant activities of EMFP and EUFP was evaluated based on the previous method[17]. For detecting the DPPH radical scavenging activity, 2 mL of DPPH work solution (0.1 mol/L) was thoroughly mixed with 2 mL of sample solution with different concentrations. Then the mixture reacted at room temperature for 20 min in the dark condition. The absorbance of the reaction solution was measured at 517 nm.

The $ABTS^+$ radical scavenging experiment was performed as a previous study with some slight modifications[18]. In short, 0.1 mL of sample solution was mixed with 3.9 mL of ABTS work solution, shaken evenly and put in darkness at room temperature for 6 min and then its absorbance was measured at 734 nm.

Referring to the previous method, the total reducing power of EMFP and EUFP was detected[19]. 2 mL of phosphate-buffered saline (1×, pH 7.4), 2 mL of 1% potassium ferricyanide solution and 2 mL of sample solution with different concentrations were mixed and reacted at 50 °C for 20 min. Then 2 mL of 10% (*v*/*v*) trifluoroacetic acid was added to terminate the reaction. Then the supernatant was separated by centrifugation at 1,250 g for 10 min and its absorbance at 700 nm was detected. 2 mL of distilled water, 0.4 mL of ferric chloride, 2 mL of sample solution were mixed and used as blank control.

### 2.4 Composition analysis of EMFP by LC-MS

The main chemical components of EMFP and EUFP were detected by the liquid chromatography-mass spectrometry (LC-MS) method. In brief, 50 mg of EMFP or EUFP powder was mixed with 500 μL of methanol, ground for 5 min, and vortexed for 10 min. The homogenate was centrifuged at 20,000 *g* for 10 min at 4 °C. The supernatant was filtered and analyzed by the LC-MS system (UltiMate 3000 RS and Q Exactive of Thermo Fisher China). In the liquid chromatography detection stage, an AQ-$C_{18}$ column (150 mm × 2.1 mm, 1.5 μm) was employed, using 0.1% formic acid aqueous solution and methanol as mobile phases. The column temperature was set to 35 °C. The results of the LC-MS were matched with the compound library, and the Differential metabolic components in EMFP compared to EUFP were listed.

### 2.5 Preparation of wheat bread

The bread with different EMFP addition ratios was prepared according to the previous method with some modifications[20]. To prepare functional bread, 100 g of wheat flour, 10 g of sugar, 10 g of butter, 1 g of yeast, 60 g of distilled water, 1 g of edible salt and different masses of EMFP (0%, 0.025%, 0.05%, 0.1%, 0.5%, 1%, 2%, and 3%, respectively) were mixed thoroughly. The mixed dough was kneaded, fermented, divided and shaped, which was

then fermented again for another 60 min. Finally, the fermented dough was baked in a preheated oven at 180 °C for 40 min to obtain functional bread with different EMFP addition ratios.

### 2.6 Physicochemical properties of bread

#### 2.6.1 *Color detection*

The color of food is one of the important indicators of sensory evaluation, which can directly influence consumers' willingness. In this study, an NR 110+ colorimeter (Threenh Technology, China) was used to digitally analyze the color of the bread with different EMFP addition ratios. After the bread was cooled to room temperature, the crusts were removed and the lightness ($L^*$-value), redness ($a^*$-value), and yellowness ($b^*$-value) of the core portion of the bread were measured and recorded.

#### 2.6.2 *Textural evaluation*

Textural evaluation is commonly used to evaluate the physical properties of breads, including resilience, chewiness, and hardness. The effects of different EMFP addition ratios on the quality of the bread were examined using a texture analyzer (TA) TOUCH texture meter (BosinTech, China) according to the previous method[21]. After cooling to room temperature, the bread cores were cut into 2 cm thick slices for full texture detection. The experimental parameters were set as follows: the pre-test rate and in-test rate of 1 mm/s, the post-test rate of 2 mm/s, the deformation variable of 50%, the induction force of 5 gf, and the interval between two compressions of 5 s. The whole detection process was carried out with the TA/36R model probe.

#### 2.6.3 *Specific volume detection*

In this study, the specific volume of the bread with different EMFP addition ratios was examined according to the previous study with some modifications[22]. The fresh bread was cooled to room temperature and weighed, and then its volume was determined by the rapeseed substitution method. The mass and volume of the bread were recorded and the specific volume was calculated according to the following equation.

$$P=V/m$$

where $P$ was the specific volume of the bread ($cm^3/g$). $V$ was the volume of the bread ($cm^3$). $m$ was the mass of the bread (g).

#### 2.6.4 *Acidity detection*

Acidity is one of the important indicators for evaluating the quality of breads, so the pH of the bread was examined concerning the previous method[23]. In brief, 15 g of bread crumbs and 100 mL of distilled water were put into a beaker and stirred magnetically at room temperature for 30 min. After standing for 10 min, the pH of the supernatant was measured with a pH meter.

### 2.7 Sensory evaluation of bread

To visually evaluate the effect of EMFP addition on the comprehensive quality of the bread, 10 trained panelists were selected for sensory evaluation of the prepared bread with different EMFP addition ratios according to the previous method[24]. During the sensory evaluation, panelists were allowed to clean their tastebuds with drinking water. The sensory evaluation mainly included the following indicators: appearance (0 = significant collapse and 15 = fullness without deformation), color (0 = dark brown and 15 = light yellow), odor (0 = sourness and 15 = baking aroma), palate (0= bitter & non-chewy and 15 = slightly sweet & chewy), texture (0 = no rebound after pressing and 15 = rebound to original shape after pressing), internal structure (0 = large & uneven holes and 15 = small & uniform holes) and overall acceptability (0 = very disliked and 15 = very liked).

### 2.8 Antioxidant activity of bread

The breads with different EMFP additions were extracted and detected for antioxidant activity with reference to the published studies[25,26]. In brief, the breads with different EMFP addition ratios were dried in an oven and ground to powder. Then 2 g of the bread powder was weighed in a beaker, mixed with 50 mL of 80% (*v/v*) ethanol, extracted by water bath (sonicated for 2 h at 37 °C). Finally, the supernatant and precipitate were separated by centrifugation at 2,250 g for 10 min. The supernatant was collected, named functional bread ethanol extract (FBEE), and its antioxidant activities were further determined as Section 2.3.

### 2.9 Statistical analysis

Data were presented as mean ± standard deviation (SD). All the experiments were performed in at least triplicate. The statistical significance was analyzed using a one-way analysis of variance (ANOVA) through GraphPad Prism software, and differences were considered significant at $P < 0.05$. The Pearson correlation coefficients were calculated to analyze the correlation between different indicators of the bread through Origin 2019 software.

## 3 Results and discussion

### 3.1 Antioxidant activity of EMFP

Threducing power and free radical scavenging abilities of EMFP and EUFP were detected and compared. As shown in **Fig. 1**, both EMFP and EUFP showed dose-dependent reducing power and scavenging ability on $ABTS^+$ and DPPH free radicals, exhibiting good antioxidant activity *in vitro*. It was worth

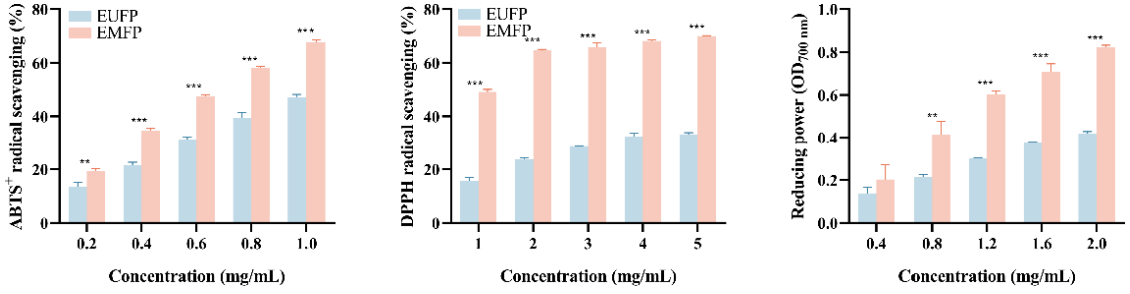


**Figure 1** Comparation of antioxidant activities between EMFP and EUFP. (A) $ABTS^+$ radical scavenging activity, (B) DPPH radical scavenging activity, (C) Reducing power. Values were expressed as means ± SD ($n = 3$). Significant difference between EUFP and EMFP groups was represented as $^{**}P < 0.01$ and $^{***}P < 0.001$.

emphasizing that EMFP possessed stronger antioxidant capacity ($P < 0.05$). During the fermentation process, cellulase, hemicellulase and other enzymes released by microorganisms could accelerate the cell lysis of the fermentation substrates and release bioactive compounds[14,27]. In addition, microorganisms could use the fermentation substrates for metabolic transformation during growth and produce bioactive ingredients such as flavonoids and polyphenols[28]. In the present study, the antioxidant activity of the *R. vinosa* Lindblad ethanol extract was significantly enhanced after fermentation, which might be related to the release and transformation of active components from the *R. vinosa* Lindblad by *S. boulardii* and *L. lactis*. In order to further investigate the changes of the small molecular compounds during the fermentation process, the compounds in EUFP and EMFP were subsequently analyzed.

### 3.2 Composition analysis of EMFP

The enhanced antioxidant activity might be related to the secondary metabolites produced by microorganisms during fermentation[10]. To investigate the factors responsible for the enhanced antioxidant activity of EMFP, the main chemical compositions of EMFP and EUFP were identified by LC-MS. The positive and negative ion spectrum of EMFP and EUFP were shown in **Fig. 2**. The differential metabolic components were further analyzed and presented in **Fig. 2A**. A total of 495 compounds were detected in EMFP, of which 186 compounds were differential metabolites. Compared to EUFP, the newly generated and significantly increased (area of EMFP/EUFP > 2) compounds in EMFP were listed in **Table 1**. Obviously, these compounds mostly belonged to phenolic acids, flavonoids and organic acids. Some of these compounds were good natural antioxidants, and their changes in EMFP might significantly contribute to the enhanced antioxidant activity of EMFP[9]. For instance, the 2-isopropylmalic acid and the 2-hydroxycinnamic acid were newly generated during the fermentation process, which have been reported to effectively scavenge free radicals *in vitro*[29,30]. In addition, the 2-hydroxycinnamic acid could also provide good protection against hydrogen peroxide-induced oxidative damage in mouse fibroblast cells[29]. Meanwhile, some compounds in EMFP with increased content compared to EUFP also exhibited good antioxidant activity, such as 3-phenyllactic acid, oleoyl ethanolamide, 4-hydroxybenzaldehyde[31-33]. The enhanced antioxidant activity of EMFP was mainly obtained through the fermentation of the *R. vinosa* Lindblad with strains used for food fermentation, providing possibility for the application of EMFP in the field of functional foods.

Besides *in vitro* antioxidant activity, these differential metabolic compounds of EMFP also exhibited other biological activities. For instance, the 4-hydroxybenzaldehyde could bind to xanthine oxidase active site and reduce uric acid level by inhibiting xanthine oxidase activity *in vitro*[34]. The advanced intervention of *Ferulago angulate* (FA), rich in the 2-hydroxycinnamic acid, could prevent *N*-nitrosodimethylamine-induced nephrotoxicity in rats[35]. These studies suggested that EMFP might also exhibit the above biological activities, which needed further investigation.

### 3.3 Effects of EMFP on the physicochemical properties of the bread

In this study, the effects of different EMFP addition ratios on

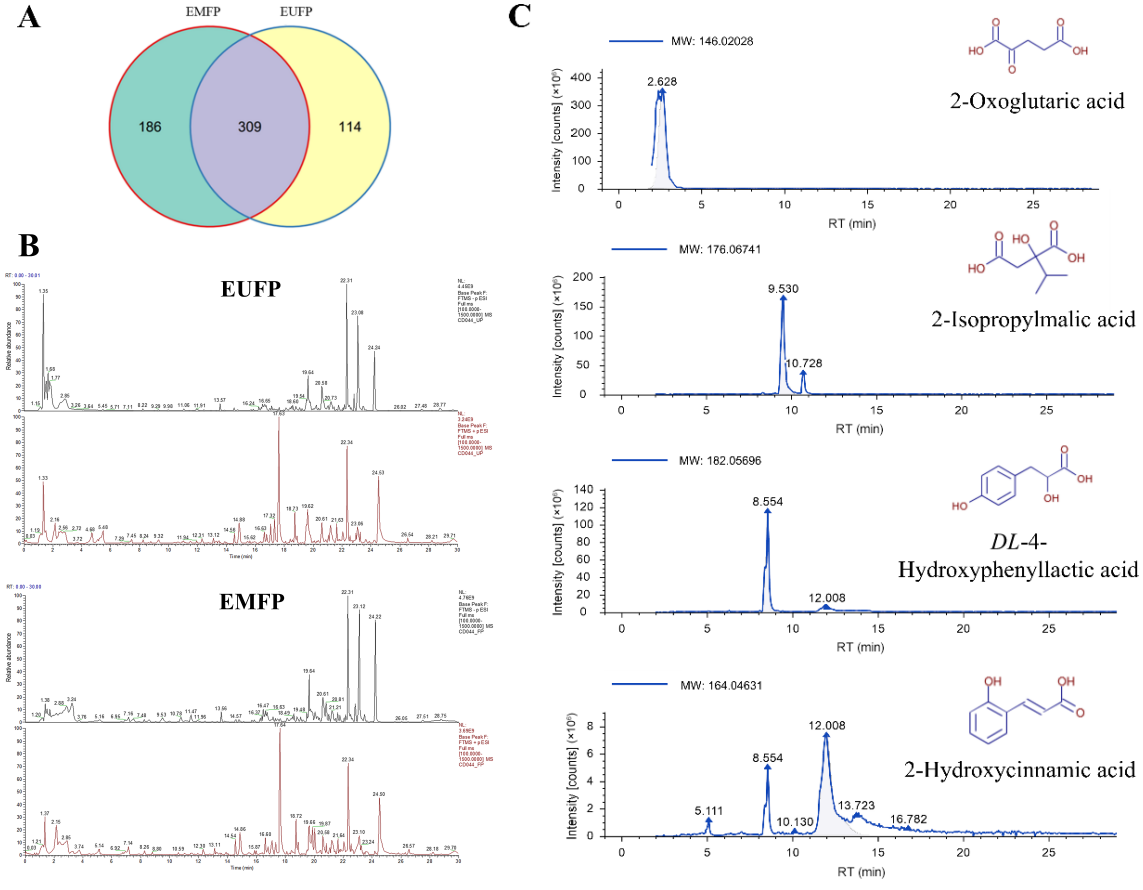


**Figure 2** Analysis of different components between EUFP and EMFP. (A) Venn diagram of different components, (B) The total ion flow diagram of EUFP and EMFP, (C) Mass spectrometry analysis of some major differential components of EMFP. MW means molecular weight and RT means retention time.

**Table 1** EMFP variance component analysis

| Differential metabolic compounds | Classes | Formula | Molecular weight | EMFP retention time (min) | Area (EMFP) | Area (EUFP) | EMFP/EUFP (area) |
|---|---|---|---|---|---|---|---|
| 3-Phenyllactic acid | Organic acid | $C_9H_{10}O_3$ | 166.06 | 11.46 | 39,297,886.22 | 915,110.91 | 42.94 |
| Oleoyl ethanolamide | Lipid | $C_{20}H_{39}NO_2$ | 371.30 | 21.87 | 11,016,089.35 | 391,435.46 | 28.14 |
| 10-Nitrolinoleate | Fatty acid | $C_{18}H_{31}NO_4$ | 307.21 | 13.11 | 20,872,299.71 | 1,390,464.05 | 15.01 |
| Kynurenic acid | Organic acid | $C_{10}H_7NO_3$ | 189.04 | 10.92 | 3,382,798.77 | 366,704.56 | 9.22 |
| Dodecyl sulfate | Organic acid | $C_{12}H_{26}O_4S$ | 266.16 | 19.50 | 8,265,796.11 | 1,465,581.36 | 5.64 |
| 4-Hydroxybenzaldehyde | Phenolic acid | $C_7H_6O_2$ | 122.04 | 9.91 | 2,669,077.32 | 499,343.43 | 5.35 |
| *N*-Acetyl-5-aminosalicylic acid | Organic acid | $C_9H_9NO_4$ | 195.05 | 2.83 | 21,165,143.85 | 5,279,941.81 | 4.01 |
| 16-Hydroxyhexadecanoic acid | Organic acid | $C_{16}H_{32}O_3$ | 272.24 | 22.33 | 30,368,632.36 | 2,323,341.59 | 3.97 |
| 2,3-Dihydroxybenzoic acid | Phenolic acid | $C_7H_6O_4$ | 154.03 | 10.46 | 652,757.82 | 167,106.46 | 3.91 |
| Arjungenin | Flavonoid | $C_{30}H_{48}O_6$ | 504.34 | 18.04 | 4,338,728.20 | 1,897,297.81 | 2.29 |
| Azelaic acid | Organic acid | $C_9H_{16}O_4$ | 188.10 | 13.55 | 34,146,545.09 | 15,397,201.54 | 2.22 |
| 3-Hydroxydecanoic acid | Fatty acid | $C_{10}H_{20}O_3$ | 188.14 | 16.64 | 2,582,630.10 | 1,189,031.46 | 2.17 |
| Salicylic acid | Phenolic acid | $C_7H_6O_3$ | 138.03 | 9.26 | 21,165,143.85 | 685,589.40 | 2.16 |
| Indole-3-acetic acid | Organic acid | $C_{10}H_9NO_2$ | 175.06 | 12.42 | 580,627.84 | 278,618.24 | 2.08 |
| Citraconic acid | Organic acid | $C_5H_6O_4$ | 130.03 | 4.62 | 3,619,037.97 | 1,752,613.34 | 2.06 |
| 2-Oxoglutaric acid | Organic acid | $C_5H_6O_5$ | 146.02 | 2.64 | 190,018,656.80 | | — |
| 2-Isopropylmalic acid | Organic acid | $C_7H_{12}O_5$ | 176.07 | 9.50 | 43,815,506.80 | | — |
| *DL*-4-Hydroxyphenyllactic acid | Phenolic acid | $C_9H_{10}O_4$ | 182.06 | 8.56 | 24,357,579.86 | | — |
| 2-Hydroxycinnamic acid | Phenolic acid | $C_9H_8O_3$ | 164.05 | 11.97 | 5,945,985.14 | | — |
| 6-Hydroxynicotinic acid | Alkaloid | $C_6H_5NO_3$ | 139.03 | 2.72 | 2,211,692.12 | | — |
| α-Pyrrolidinobutiophenone | Flavonoid | $C_{14}H_{19}NO$ | 217.15 | 16.98 | 1,639,323.99 | | — |
| *DL*-Mandelic acid | Phenolic acid | $C_8H_8O_3$ | 152.05 | 8.063 | 1,400,584.55 | | — |
| 4'-Methoxyacetophenone | Flavonoid | $C_9H_{10}O_2$ | 150.07 | 22.09 | 882,188.52 | | — |
| 4-Methylphenol | Phenolic acid | $C_7H_8O$ | 108.06 | 8.27 | 642,712.92 | | — |
| 3-Methylsalicylic acid | Phenolic acid | $C_8H_8O_3$ | 152.05 | 10.31 | 166,277.38 | | — |

Note: — represented the new substances of EMFP compared to EUFP.

the color and texture of the bread were evaluated. As shown in **Table 2**, when the EMFP addition ratio excessed 0.5%, both *a**-value and *b**-value of the bread increased significantly, and the bread color turned dark. This phenomenon might be attributed to the pigments present in EMFP. Meanwhile, the appropriate amount of EMFP could improve the texture of the bread to some extent. For example, at 0.5% EMFP addition, the hardness of the bread decreased from (525.74 ± 45.60) gf to (233.60 ± 19.67) gf ($P < 0.05$), the gumminess decreased from (378.30 ± 27.34) gf to (199.51 ± 31.92) gf ($P < 0.05$), while the resilience increased from 0.13 ± 0.01 to 0.44 ± 0.02 ($P < 0.05$), resulting in softer and more resilient bread. As also evident from **Fig. 3A**, the addition of EMFP resulted in the increase of pore space in the cross-section of the bread, causing an increase in the softness of the bread[36]. The specific volume of the bread gradually decreased with the increase of EMFP addition ratio, which significantly decreased from (3.35 ± 0.39) $cm^3/g$ to (2.76 ± 0.30) $cm^3/g$ ($P < 0.05$) at the addition ratio of 0.5%. And the specific volume decreasing trend of the bread tended to flatten out when the EMFP addition ratio exceeded 0.5%. Similarly, previous studies have found that the addition of polyphenolic compounds could significantly change the texture of the bread, such as tannic acid, tea polyphenols, and caffeic acid[37-39]. The texture of the bread was related to the number and distribution of disulfide bonds in gluten proteins, and these highly reducing compounds might react with the disulfide bonds leading to changes in the protein structure, which consequently affected the hardness, resilience and other qualities of the bread[12]. In addition, some phenolic acids might interact with sulfhydryl groups in gluten, weakening the gluten network and reducing the specific volume of the bread[12,39]. In this study, EMFP was enriched with phenolic acids, including 4-hydroxybenza ldehyde, salicylic acid, and others, which might react with proteins in the dough and result in higher resilience and lower hardness and specific volume of the bread.

In summary, although the addition of EMFP decreased the specific volume of the bread to some extent, it improved the bread in terms of texture, including reduction of the hardness, improvement of the resilience of the bread. The general quality of the bread was better with 0.5% EMFP addition ratio.

### 3.4 Effects of EMFP on the sensory evaluation of the bread

Sensory evaluation was used to evaluate the effect of EMFP addition on the acceptability of the bread[24]. As shown in **Fig. 3A**, the color of the bread core gradually deepened with the increase of EMFP addition, which was consistent with the results of the bread physicochemical property detection (**Table 2**). As shown in **Fig. 3B**, the addition ratio of EMFP affected relatively more on the color, odor and texture, and relatively less on the appearance, internal structure and palate. For sensory evaluation of color, odor and texture, the bread with 0.025%, 0.05% and 0.5% EMFP addition were the most acceptable, respectively. In terms of overall acceptability, the bread with 0.5% EMFP addition scored the highest. Comprehensively, at EMFP additions above 0.5%, there was negative effects on the overall sensory evaluation results of the bread, while lower additions caused positive effects. Similar trend

**Table 2** Effect of different EMFP addition ratios on bread quality

| Indicators | | EMFP addition ratio (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.025 | 0.05 | 0.1 | 0.5 | 1.0 | 2.0 | 3.0 |
| Color | *L** | 77.77 ± 2.34$^{bc}$ | 80.06 ± 2.52$^{ab}$ | 80.89 ± 0.99$^{a}$ | 80.91 ± 1.85$^{a}$ | 76.49 ± 1.00$^{cd}$ | 74.04 ± 1.55$^{d}$ | 74.09 ± 0.49$^{d}$ | 71.04 ± 1.48$^{e}$ |
| | *a** | 1.62 ± 0.29$^{d}$ | 1.45 ± 0.31$^{d}$ | 1.74 ± 0.31$^{d}$ | 1.50 ± 0.22$^{d}$ | 2.93 ± 0.47$^{c}$ | 3.21 ± 0.09$^{c}$ | 3.98 ± 0.21$^{b}$ | 5.20 ± 0.61$^{a}$ |
| | *b** | 15.70 ± 0.81$^{de}$ | 15.88 ± 1.73$^{de}$ | 16.87 ± 0.88$^{d}$ | 14.84 ± 0.87$^{e}$ | 18.76 ± 0.69$^{c}$ | 19.50 ± 0.52$^{bc}$ | 20.65 ± 0.91$^{ab}$ | 21.91 ± 0.67$^{a}$ |
| Texture | Hardness (gf) | 525.74 ± 45.60$^{a}$ | 386.24 ± 23.54$^{bc}$ | 545.39 ± 14.38$^{a}$ | 538.24 ± 5.81$^{a}$ | 233.60 ± 19.67$^{d}$ | 332.20 ± 30.87$^{c}$ | 413.24 ± 37.72$^{b}$ | 449.76 ± 38.30$^{b}$ |
| | Chewiness (gf) | 343.05 ± 32.52$^{ab}$ | 265.19 ± 16.55$^{cd}$ | 374.51 ± 12.25$^{a}$ | 349.09 ± 8.21$^{a}$ | 198.09 ± 23.75$^{e}$ | 232.76 ± 17.76$^{de}$ | 277.00 ± 22.37$^{cd}$ | 300.91 ± 19.46$^{bc}$ |
| | Gumminess (gf) | 378.30 ± 27.34$^{a}$ | 293.48 ± 20.14$^{bc}$ | 409.18 ± 8.37$^{a}$ | 392.83 ± 6.14$^{a}$ | 199.51 ± 31.92$^{d}$ | 253.84 ± 27.74$^{c}$ | 302.61 ± 22.65$^{bc}$ | 327.39 ± 28.42$^{b}$ |
| | Resilience | 0.13 ± 0.01$^{c}$ | 0.15 ± 0.00$^{c}$ | 0.15 ± 0.00$^{c}$ | 0.14 ± 0.01$^{c}$ | 0.44 ± 0.02$^{a}$ | 0.43 ± 0.02$^{a}$ | 0.41 ± 0.03$^{ab}$ | 0.39 ± 0.02$^{b}$ |
| Specific volume ($cm^3$/g) | | 3.35 ± 0.39$^{a}$ | 3.27 ± 0.22$^{ab}$ | 3.03 ± 0.01$^{abc}$ | 2.86 ± 0.08$^{abc}$ | 2.76 ± 0.30$^{bcd}$ | 2.68 ± 0.17$^{bc}$ | 2.41 ± 0.30$^{d}$ | 2.3 ± 0.18$^{d}$ |
| pH | | 5.45 ± 0.10$^{a}$ | 5.53 ± 0.10$^{a}$ | 5.55 ± 0.10$^{a}$ | 5.59 ± 0.09$^{a}$ | 5.66 ± 0.01$^{a}$ | 5.62 ± 0.01$^{a}$ | 5.73 ± 0.02$^{a}$ | 5.82 ± 0.02$^{a}$ |

Note: Values were expressed as means ± SD ($n$ = 3). Different letters between the same line implied significant differences among the EMFP addition ratios ($P$ < 0.05).

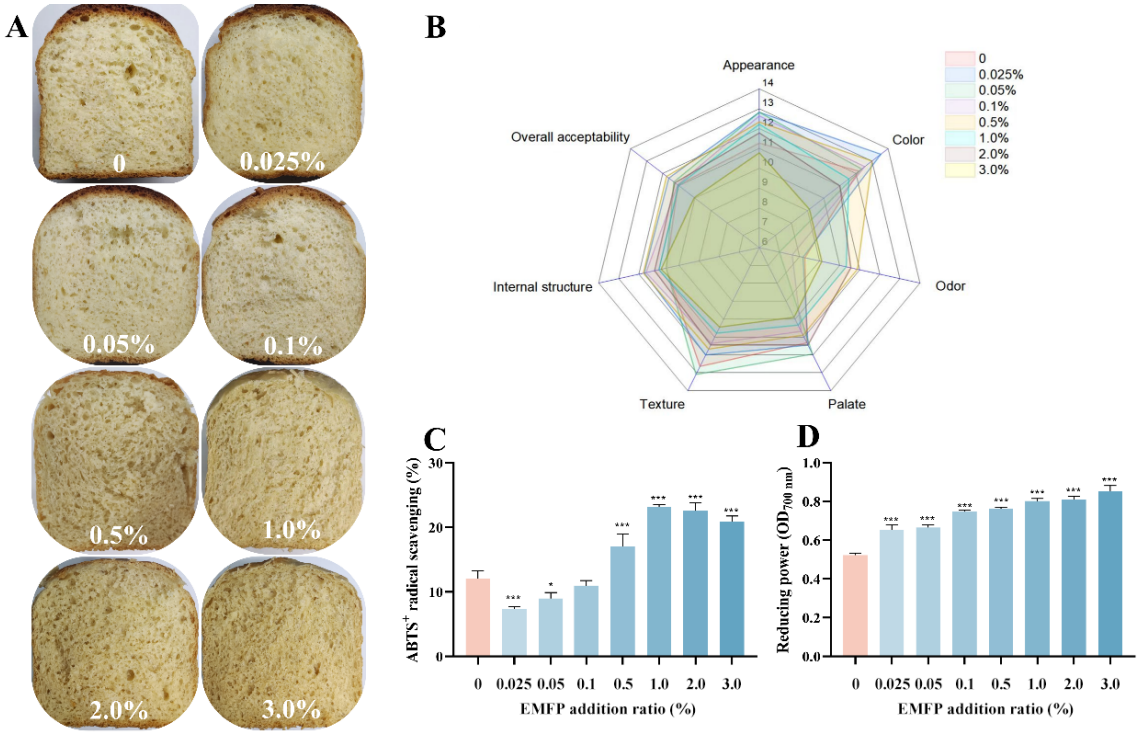


**Figure 3** Effects of EMFP addition ratio on the display, sensory evaluation and antioxidant activities of the bread. (A) Cross-sectional display of the bread, (B) Sensory evaluation results of the bread, (C) ABTS$^+$ radical scavenging activity of the bread, (D) Reducing power of the bread. Data were expressed as means ± SD ($n$ = 3). Significant difference from no EMFP addition group were represented as *$P$ < 0.05 and ***$P$ < 0.001.

was observed in the previous study where 2% *Agaricus bisporus* mushroom powder addition positively affected the overall acceptability of the bread, while 4% addition decreased the acceptability of the bread[40]. Overall, the influence of functional ingredients on the acceptability of the bread was complex, which was influenced by the color, odor, taste and other characteristics of the ingredients[12]. Preparation of antioxidant bread needed balancing the effect of exogenous antioxidants on the antioxidant activity and acceptability of the bread, and in the present study 0.5% EMFP addition might be more appropriate.

### 3.5 Effects of EMFP on the antioxidant activities of the bread

From the results of bread quality tests, it was observed that the appropriate addition of EMFP could improve the quality of the bread. To further evaluate the effect of EMFP addition on the bread antioxidant activity, the bread was extracted by 80% (*v*/*v*) ethanol and the extract was assayed for its antioxidant activity[25,26]. As shown in **Fig. 3C**, compared to the control group, EMFP addition ratios ranging from 0.5% to 3.0% significantly enhanced the scavenging activity of the bread against ABTS$^+$ radicals ($P$ < 0.05). Moreover, **Fig. 3D** showed that the reducing power of the bread with EMFP addition ratios ranging from 0.5% to 3.0% was also significantly enhanced ($P$ < 0.05). The bread without EMFP addition also showed antioxidant activity, which might be related to the polyphenolic compounds contained in the wheat flour[41]. Polyphenolic compounds were natural antioxidants that could effectively scavenge excessive free radicals in the body and alleviate oxidative stress, thus preventing some chronic diseases[42]. Exogenous addition of natural antioxidants could effectively enhance the antioxidant ability of the bread[12]. For example, adding green caffeic extracts, tea polyphenols, and *A. bisporus* could effectively enhance the antioxidant function of breads[38,40,43]. Consumption of these exogenous antioxidants could prevent and

alleviate oxidative stress-induced chronic diseases and positively affect the health of the body[44].

### 3.6 Correlation analysis

In this study, Pearson correlation coefficients were calculated to analyze the correlation between different indicators of the bread. As shown in Fig. 4, acceptability of bread odor was significantly and negatively correlated with bread hardness, chewiness and gumminess, suggesting that the addition of EMFP negatively affected the odor of bread while improving its texture. According to the previous report, excessive addition of exogenous substances in bakery products might affect the aroma of the bread and decrease consumer acceptability[45]. Furthermore, in the present study, there was a significant positive correlation between bread resilience and antioxidant activity while negative correlation between resilience and hardness, chewiness and gumminess (Fig. 4). These results indicated that the addition of EMFP significantly improved the antioxidant activity of the bread, but also decreased the hardness, chewiness and gumminess of the bread, which might be related to that the phenolic acids could change the structure of the gluten proteins[38]. Additionally, the overall acceptability of the bread showed a significant positive correlation with appearance and color, while the other indicators did not significantly affect it, suggesting that the appearance and color of the bread might be the most important factors contributing to the evaluation of consumers[40]. In the present study, 0.5% EMFP addition was considered appropriate in a comprehensive manner, which improved the antioxidant activity of the bread while improving the texture and acceptability.

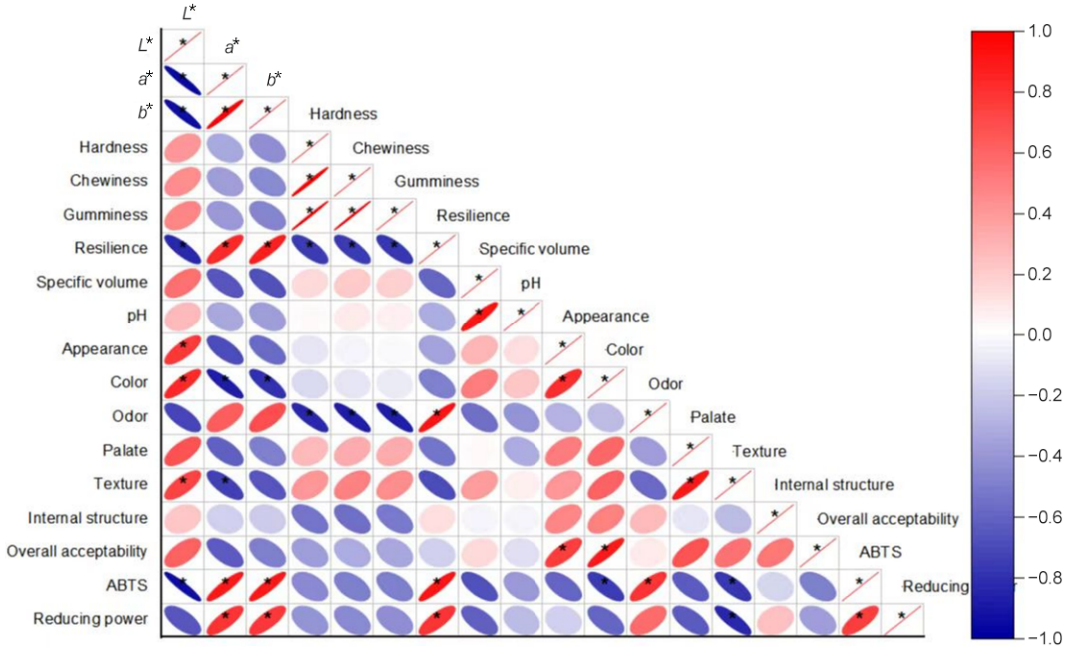


**Figure 4** Correlation analysis between bread quality indicators and antioxidant properties. *$P \leq 0.05$.

## 4 Conclusion

In the present study, the fermentation treatment of the *R. vinosa* Lindblad was carried out using *S. boulardii* and *L. lactis* to promote the release and conversion of bioactive components and improve the antioxidant activity of *R. vinosa* Lindblad polyphenols. Additionally, EMFP significantly reduced the hardness and enhanced the resilience of the bread. 0.5% EMFP addition provided stronger antioxidant activity to the bread while improving the quality and enhancing the sensory acceptability of the bread. This study initially demonstrated the positive effect of mixed bacterial fermentation on polyphenols of edible mushrooms, and provided a reference for the application of the *R. vinosa* Lindblad in the field of functional foods.

## Conflict of interest

The authors declare that there are no conflicts of interest in this work. Jike Lu is an associate editor of Food & Medicine Homology, but was not involved in the processing, peer review or decision making of this article.

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