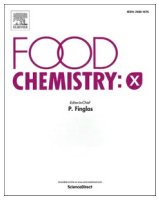
Food Chemistry: X 23 (2024) 101549

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Combined treatment of rice bran by solid-state fermentation and extrusion: Effect of processing sequence and microbial strains

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ARTICLE INFO

Keywords:
Aspergillus oryzae
Lactiplantibacillus plantarum
Neurospora sitophila
Treatment order
Bioactive components, arabinoxylan

ABSTRACT

Solid-state fermentation (SSF) and extrusion are effective methods to improve the nutritional and sensory quality of rice bran. The effect of the processing sequence of SSF and extrusion and microbial strains on the quality of rice bran was studied. The results showed that the first SSF followed by extrusion increased the contents of phenolic, flavonoid and γ-oryzanol, but the color changed to brown. The first extrusion followed by SSF caused damage to bioactive components and antioxidant activity, but significantly increased the content of arabinoxylans. The difference between the two processing sequences may be related to the process time and the effect of substrate on microbial induction. *Aspergillus oryzae* and *Neurospora sitophila* were suitable for increasing the bioactive components of rice bran, while *Lactiplantibacillus plantarum* was suitable for increasing water-extractable arabinoxylan content. Different processing sequences and microbial strains have their advantages, and these results can provide reference for rice bran processing.

1. Introduction

Rice bran is the main by-product of rice processing, which is a mixture of rice seed coat, aleurone layer, nucellus layer and a small amount of germ (Yu et al., 2023). Rice bran is rich in bioactive components, including vitamins, flavonoids, phenolic compounds, γ-oryzanol, dietary fiber, etc. (Yin et al., 2022). These bioactive ingredients have antioxidant properties and health benefits, including boosting immunity and reducing the risk of cardiovascular disease (Ritthibut et al., 2021). However, rice bran contains a large amount of insoluble dietary fiber, and the fat is easily oxidized, which makes the rice bran taste rough and smell bad, which limits the application of rice bran in the food field (Najamuddin et al., 2023). Currently, most rice bran is used as animal feed, resulting in a severe waste of food resources. As people pay more attention to natural nutrition and health, some food processing technologies have been used to improve the sensory and nutritional quality of rice bran to enhance its utilization value, such as fermentation, electronic irradiation, extrusion, etc.(Najamuddin et al., 2023; M. Wang et al., 2020).

In previous studies, solid-state fermentation (SSF) has been considered an attractive solution for rice bran modification due to its high production efficiency, low energy consumption and low environmental pollution (Chen et al., 2020). The selection of fermentation strain is the critical factor of SSF technology, which determines the production efficiency and product quality of SSF (J. Wang et al., 2023). Filamentous fungi such as *Aspergillus oryzae* and *Neurospora sitophila* are considered the most suitable organisms for SSF because their growth environment is similar to the conditions of SSF and they are classified as “generally recognized as safe” (GRAS), for food use, by the U.S. Food and Drug Administration (FDA) (D. Zhang et al., 2022). They can produce a large number of hydrolases, especially xylanase and cellulase, which promote the release of nutrients encapsulated in the plant cell wall and improve the taste and nutrition of rice bran. *Lactiplantibacillus plantarum* is one of the few bacteria adapted to SSF and is widely used in the production of fermented foods such as pickles, fruit vinegar and sourdough bread (M. Wang et al., 2020). It has been reported that using lactic acid bacteria to ferment grain bran produces a unique flavor and promotes the release of phenolic substances (Lin et al., 2024).

Extrusion is another widely studied rice bran processing method, which can destroy the fiber structure of rice bran and promote the

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https://doi.org/10.1016/j.fochx.2024.101549
Received 18 April 2024; Received in revised form 27 May 2024; Accepted 10 June 2024
Available online 20 June 2024

release of phenolic compounds through high temperature, high pressure and mechanical shear (Qiao et al., 2021; R. Zhang et al., 2018). Studies show that extrusion can promote the conversion of insoluble dietary fiber (IDF) to soluble dietary fiber (SDF), and improve the bioavailability and digestibility of nutrients in extrusion (Cao et al., 2021). Extrusion also affected the hydration properties of grain bran, showing that the water solubility was increased, but the water binding capacity was reduced (Roye et al., 2020). In addition, Wu et al. also reported that the extruded rice bran had a more substantial inhibitory effect on the deterioration of rice starch, which could expand the application of rice bran in the quality control of rice products Wu, Qiao, et al. (2021).

SSF and extrusion were used to modify rice bran through microbial enzymatic hydrolysis and thermomechanical treatment, respectively, and both of them could destroy the fiber structure of rice bran and promote the release of nutrients. At present, some researchers have used the two methods together and found that the combined treatment has a better modification effect than the separate treatment (Dang & Vasanthan, 2019; Rani et al., 2018). As two processing methods with different action principles, it can be speculated that the different processing sequences may lead to differences in the composition, sensory, and nutritional quality of rice bran. However, there are few studies on the effect of the processing sequence of the two methods on the composition and nutritional quality of rice bran.

Therefore, the purpose of this study was to investigate the effect of the processing sequence (first extrusion followed by SSF or first SSF followed by extrusion) on the quality of rice bran in the combined treatment of extrusion and SSF, and to compare the differences in the fermentation performance of three microorganisms (*A. oryzae*, *N. sitophila* and *L. plantarum*). The results of this study will provide helpful information for the joint application of the two technologies in rice bran processing.

2. Materials and methods

2.1. Materials

Fresh Rice bran milled from Nanjing 46 (brown) was provided by Shanghai Shengzhi Agricultural and Sideline Products Co., LTD (Shanghai, China). Active dry *A. oryzae* (CICC 41737), *N. sitophila* (CICC 40204), *and* L. *plantarum* (CICC 22696) were from the China Center of Industrial Culture Collection (CICC; Beijing, China). All other reagents were analytically pure and purchased from Sinopharm Shanghai Chemical Reagents Co., LTD (Shanghai, China).

Fresh rice bran was filtered through a 40-mesh screen (pore diameter 0.45 mm) to remove broken rice, husk and other impurities, and then spread in a metal plate, heated in an oven at 120 ± 5 °C for 2 h to stabilize, and stored in a refrigerator at 4 °C. The approximate composition of rice bran was water 8.22 ± 1.10%, starch 17.23 ± 0.25%, fat 12.53 ± 0.19%, protein 14.87 ± 0.16%, dietary fiber 32.96 ± 1.06%, ash 9.00 ± 0.10%.

2.2. Processing of rice bran

Rice bran samples were treated by first SSF followed by extrusion (FE) or first extrusion followed by SSF (EF) according to the following steps. In the SSF process, *A. oryzae* (A), *N. sitophila* (N) and L. *plantarum* (L) were inoculated respectively. In order to detect the enzyme activity in rice bran after SSF, the samples were taken in FE treatment group before extrusion treatment, namely only SSF with *A. oryzae* (OF-A), only SSF with *N. sitophila* (OF-N) and only SSF with L. *plantarum* (OF-L).

2.2.1. Extrusion

The water content of the sample was adjusted to 25% with distilled water, and a LEY33-II twin-screw extruder (Shandong LUERYA Machinery Manufacturing Co. LTD, Qingdao, China) was used for extrusion processing (the length of the extruder barrel was 90 cm, the diameter of the nozzle was 4 mm). The temperature of zone I, zone II and zone III in the extruder barrel was 70 °C, 98 °C and 134 °C, respectively, and the screw speed was 280 rpm. The extruded samples were dried in an oven at 60 °C for 3 h, then crushed using a grinder, passed through a 40-mesh screen, and stored in a refrigerator at 4 °C.

2.2.2. Solid-state fermentation

2.2.2.1. Sample pretreatment and strain activation. In order to avoid bacterial contamination and composition changes caused by high temperature, the samples were loaded into food-grade fermentation bags with unidirectional exhaust valves, and the samples were irradiated with electron beam at 8 kGy dose at room temperature by ESS-010-03 Linear Electron accelerator (IHI, Japan).

Strain activation was performed according to the method of Zhang et al. (D. Zhang et al., 2022) and the product manual. *L. plantarum* was activated in MRS Medium at 37 °C for 24 h. The second-generation bacterial solution was centrifuged at 3000 ×*g* and suspended in 0.85% sterile saline. *A. oryzae* and *N. sitophila* were cultured at 28 °C for 5 days on Cha's plate medium, and the spores were scraped and suspended in 0.85% sterile saline. All strain suspensions were stored in a refrigerator at 4 °C. The suspension of L. *plantarum* was diluted to 10^7 CFU/mL and the suspension of *A. oryzae* and *N. sitophila* were diluted to 10^7 spores/mL before inoculation.

2.2.2.2. Inoculation and SSF. Sterile water was used to adjust the water content of the samples to 50%, and the inoculation was carried out according to 1% (*v/w*) inoculum. All fermentation bags were incubated statically for 5 days in an incubator at 30 °C. After SSF, the culture was freeze-dried. The dried culture was crushed by a grinder, passed through a 40-mesh screen, and stored in cold storage at 4 °C.

2.3. Determination of composition and physicochemical properties of rice bran

To avoid the interference of fat, n-heptane was used to defat the rice bran sample, and the weight change of the sample after defatting was recorded, and the conversion coefficient was calculated. In the subsequent experiments, the content of dietary fiber, arabinoxylan, phenols, flavonoids and antioxidant activity were detected using defatted samples, and the results were corrected according to the conversion coefficient.

The moisture content was determined by the MA100 infrared moisture analyzer (Sartorius, Germany). Protein content was determined by the Dumas high-temperature combustion Dumas method using an Elementar rapid N cube (Hanau, Germany) and a 6.250 nitrogen-to-protein conversion factor. Fat content was determined by the Soxhlet extraction method using the SZC-101 fat measuring instrument (Hua-ye, Shanghai, China). Ash content was determined by carbonization in muffle furnace at 850 °C for 8 h. Starch content was determined using a starch content determination kit (BOXBIO, Beijing, China). The pH was determined using the DZS-706 multi-parameter analyzer (Lei-ci, Shanghai, China) after vortexing and mixing the sample with distilled water at a ratio of 1:10.

The color change of rice bran was measured by the CM-5 chromatic meter (Konica Minolta, China). The L^* represents brightness, the a^* represents redness/greenness, and the b^* represents yellowness/blueness. The color difference ($\triangle E$) was calculated according to Yao et al. to reflect the color change of rice bran (Yao et al., 2022).

2.4. Determination of dietary fiber content

Dietary fiber was determined according to the method of Sang et al., but with some modifications (Sang et al., 2022). In brief, starch and protein from defatted rice bran samples were removed using heat-stable

α-amylase, protease and starch glucosidase under different incubation conditions, and the precipitate obtained by centrifugation was dried as IDF. The supernatant was precipitated using a 4-fold volume of 95% ethyl alcohol preheated to 70 °C, and the precipitate obtained by centrifugation was dried as SDF. Residual ash and protein were determined according to the method of Zhang et al., and the results were corrected(D. Zhang et al., 2022).

2.5. Determination of total arabinoxylan (TAX) and water-extractable arabinoxylan (WEAX)

The contents of TAX and WEAX were determined according to the method of Zhang et al. (D. Zhang et al., 2022). 1.0 g of the defatted sample was suspended in 25 mL of distilled water, and 1 mL of the suspension was used for the determination of TAX. The mixture was continued to shake at room temperature for 30 min, centrifuged at 2500 ×*g* for 10 min, and 1 mL of the supernatant was used for the determination of WEAX. 1 mL of the test solution was mixed with 5 mL of reaction solution (glacial acetic acid: hydrochloric acid: 20% phloroglucinol: 1.75% glucose =110:2:5:1), and the liquid was bathed in boiling water for 25 min. The effect of hexose sugars was eliminated by calculating the difference between the absorbance at 558 nm and 505 nm. Xylose was used as a standard curve to calculate arabinoxylan content.

2.6. Determination of phenolic content

Free and bound phenols were extracted according to the methods of Chen et al. (Chen et al., 2019) and He et al.(He et al., 2023), respectively, but with some modifications. Briefly, 0.4 g of defatted rice bran sample was mixed with 40 mL of 80% acetone and shaken for 2 h at 4 °C. The precipitate was then centrifuged at 2500 ×*g* for 10 min at 4 °C and extracted again under the same conditions. The two supernatants were combined and concentrated by BUCHI Rotavapor (Switzerland) at 45 °C, followed by a constant volume of distilled water to 10 mL for the determination of free phenols. The precipitate after free phenol extraction was mixed with 20 mL of 2 M NaOH solution and shaken for 2 h at room temperature. The pH was adjusted to the range of 1 to 2 with hydrochloric acid, and then extracted twice with 20 mL ethyl acetate. The combined ethyl acetate was spin-steamed at 45 °C and reconstituted to 5 mL with distilled water for the determination of bound phenols.

Phenolic content was determined according to the method of Zhao et al. (Zhao et al., 2023). 0.3 mL of the diluted extract was mixed with 2.25 mL of 10% folinphenol reagent, and then 2.25 mL of 6% Na_2CO_3 solution was added. After incubation for 90 min at room temperature in the dark, the absorbance was measured at 760 nm. Gallic acid was used as a standard and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g sample dry weight (DW).

2.7. Determination of flavonoid content

Follow the method of Liu et al. (Liu et al., 2015) with some modifications. 0.5 mL of diluted free phenol extract was mixed with 1.35 mL of distilled water, and then 0.15 mL of 5% sodium nitrite solution was added. After 5 min of reaction in the dark, 0.15 mL of 10% aluminum chloride solution was added, and the reaction was continued for 6 min before 1 mL of 1 M NaOH solution was added. The absorbance was measured at 415 nm after another 15 min of reaction. Results were expressed as milligrams of rutin equivalent (RE) per 100 g sample dry weight, using rutin as a standard.

2.8. Determination of antioxidant activity

The antioxidant activity was determined by Ferric ion reducing antioxidant power (FRAP) and DPPH radical scavenging rate (DPPH-RSR) according to the methods of Wu et al. Wu, Liu, et al. (2021), respectively, but with some modifications.

2.8.1. FRAP

280 μL of FRAP working solution preheated to 37 °C (10 mM TPTZ solution:0.02 M $FeCl_3$ solution:0.3 M acetate buffer =1:1:10) was mixed with 20 μL of diluted free phenol extract and kept at 37 °C for 10 min. Absorbance was immediately measured at 593 nm.

2.8.2. DPPH-RSR

100 μL of diluted free phenol extract was mixed with 3.9 mL 0.2 mM DPPH/ethanol solution and reacted for 30 min at room temperature in the dark before absorbance was measured at 517 nm.

The L-ascorbic acid solution was used as the standard curve according to the methods of Sdiri et al. (Sdiri et al., 2020). Antioxidant activity results were expressed as milligrams of ascorbic acid equivalent (AAE) per 100 g sample dry weight.

2.9. Determination of γ-oryzanol content

The method of Renata et al. was used for the extraction and determination of γ-glutamyl alcohol, with some modifications (Heidtmann-Bemvenuti et al., 2012). 2.0 g of rice bran sample was mixed with 10 mL of 40 g/L ascorbic acid solution, shaken and extracted at 40 °C for 40 min, then added 15 mL of extraction reagent (hexane: isopropanol =1:3), vortexed for 30 s, centrifuged at 1320 ×*g* for 15 min, and the organic phase was separated. An additional 8 mL of extraction reagent was added to the precipitate and the extraction was repeated once. The organic phase was combined, concentrated by spin evaporation at 70 °C, and reconstituted with 8 mL isopropanol, and the absorbance was measured at 326 nm. the γ-oryzanol standard was used as the standard curve.

2.10. Determination of hydration properties

The water binding capacity (WBC), water solubility index (WSI) and oil binding capacity (OBC) of rice bran samples were determined according to the method of Yin et al. (Yin et al., 2022).

In brief, the sample was mixed with distilled water at a ratio of 1:20 and left at room temperature for 12 h before centrifugation. WBC was calculated according to the precipitate weight. Another sample was mixed with distilled water at a ratio of 1:20 and kept at 80 °C for 30 min. Then, the supernatant was centrifuged and concentrated at 85 °C, and dried in an oven at 80 °C for 30 min. The weight of the rotary steam bottle was measured before rotary steam and after drying, and the WSI was calculated. Another sample was thoroughly mixed with vegetable oil at a ratio of 1:10, left at room temperature for 12 h before centrifugation, and OBC was calculated based on the precipitate weight.

2.11. Microstructure analysis

The rice bran samples were evenly scattered on the copper posts. After plating a thin layer of gold by sputtering under vacuum conditions, scanning electron microscopy (TM 4000 plus, Hitachi) was performed under high vacuum conditions at an accelerating voltage of 5.0 kV. The images of midpoints in the cross-section were captured using ×500 magnification.

2.12. Statistical analysis

SPSS version 19.0 statistical software package was used to analyze the data, and OriginPro 2021 was used for principal component analysis and drawing data graphs. Each sample was tested in triplicate and data are reported as mean ± standard deviation. Significant differences among rice bran samples were evaluated by one-way ANOVA. The significance threshold was set at $p < 0.05$.

3. Results and discussion

3.1. Protein, fat, and pH

Table 1 shows the protein, fat content and pH values of untreated rice bran and processed rice bran samples. Compared with the control group (14.87% and 12.53%), the protein and fat contents of the processed rice bran samples were significantly increased, ranging from 7.1 to 11.4% and 3.9–19.2%, respectively. It can be found that the processing sequence of extrusion and SSF will not cause a huge difference in protein content. At the same time, the influence of fermentation bacteria is significant, and the samples treated with *A. oryzae* and *N. sitophila* are significantly higher than those treated with L. *plantarum* under the same processing sequence (Table 1). FE-N sample obtained the highest fat content (14.93%), while the difference between other treatment groups was not significant. The pH value of rice bran samples was affected by the processing sequence and fermentation strain. The pH value of all treatments was lower than that of the control group, but the difference between different treatment groups was significant. According to Table 1, the pH value of samples in the FE treatment group was 0.47–0.54 lower than that in the EF treatment group when the fermentation strain was the same. When the processing sequence was the same, the pH values of different fermentation bacteria samples were ranked as *A. oryzae* > *N. sitophila* > *L. plantarum*.

The increase of protein and fat content in processed rice bran was mainly due to the effect of microorganisms during SSF. Microbial SSF agricultural by-products (soybean meal, rice bran, straw, etc.) are considered to be effective ways to obtain microbial protein resources (J. Wang et al., 2023). The bacteria or mycelium of microorganisms are called single-cell proteins. They not only have high protein content, but also help to enrich the quality of protein and improve the nutritional value of products (Ranjan et al., 2019). The increase of microbial biomass during SSF caused an increase of the protein content of the product (Oduguwa et al., 2008). In this study, the protein content of the fungal (*A. oryzae* and *N. sitophila*) treatment group was higher than that of the bacterial (*L. plantarum*) treatment group, which was since the fungi had more adaptive growth conditions and stronger enzyme production ability under the studied fermentation conditions, so they had higher efficiency of bacterial protein production (Table S1). SSF also caused an increase in fat content, which is consistent with the study of Oduguwa et al. (Oduguwa et al., 2008).

The pH value will affect the storage form and bioavailability of some active ingredients in the sample. During SSF, microorganisms produce organic acids as they decompose the substrate, causing a significant decrease in the pH of the sample (Dessie et al., 2018; J. Wang et al., 2023). Among them, lactic acid bacteria can significantly reduce the pH of the medium by secreting lactic acid, thus affecting the bioavailability of active components such as phenolic acid (Lin et al., 2024). In this study, *L. plantarum* treated samples had the lowest pH (4.01 or 4.48). In addition, the processing sequence also significantly affected the pH of the sample. The extrusion treatment changed the physical structure of rice bran and produced more small molecular components (Cao et al., 2021). This may reduce the difficulty for microorganisms to decompose and utilize the matrix in the subsequent SSF process, thereby reducing the secretion of organic acids and increasing the pH value of the sample (J. Wang et al., 2023). For the FE treatment group, the organic acids produced by microbial fermentation may promote the release of phenolic acids and other acidic components from the rice bran from the fiber structure during the high temperature and high-pressure extrusion treatment, thus further reducing the pH value.

3.2. Color

Processing changed the color of the rice bran samples, especially in the FE treatment group (Fig. S1). The color data of rice bran samples are shown in Table 1. It can be found that the processing sequence is the critical factor affecting the color of rice bran. Compared with the EF treatment group, the L^* value of samples in the FE treatment group was significantly reduced, and the a^* and b^* values were significantly increased, indicating that the rice bran color was deeper and closer to brown. While the EF treatment group was also significantly different from the control group, the $\triangle E$ value was relatively low (< 6.00), and the color change was relatively small.

The color change of rice bran during processing is mainly due to the production of Maillard reaction products during extrusion (Liao et al., 2020). In this study, all treatment groups were extruded, so the color of rice bran samples changed to some extent. However, the rice bran color in the FE treatment group was darker than in the EF treatment group, indicating that fermentation followed by extrusion produced more Maillard reaction products. This is because during SSF, enzymes produced by microorganisms destroy the rice bran fiber structure, exposing more proteins on the one hand, and degrading the fiber to small molecular sugars on the other hand, thus providing more Maillard reaction substrates for the subsequent extrusion process (Jia et al., 2019). As a food processing raw material, the color of rice bran will affect the sensory quality of subsequent processed products, so the processing sequence of rice bran may affect the application range of rice bran (Yin et al., 2022).

3.3. Dietary fiber and arabinoxylan

The dietary fiber and arabinoxylan contents of processed rice bran are shown in Table 2. Compared with the control group, the total dietary fiber content of processed rice bran samples did not change significantly, but the IDF content was significantly reduced (6.3–15.3%). The processing sequence of extrusion and SSF had a significant effect on SDF content. FE treatment generally increased the SDF content (16.2–28.4%), and the SDF content of FE-N treatment was the highest (11.89 g/100 g DW). EF-N and EF-L treatments were improved by 7.9% and 19.7%, respectively, but EF-A group had no significant change. On

Table 1
Effect of processing on composition and color of rice bran.

Sample	Protein/ (g/100 g)	Fat/ (g/100 g)	pH	Color			
				L^*	a^*	b^*	$\triangle E$
CK	14.87 ± 0.16e	12.53 ± 0.19d	6.65 ± 0.03a	70.45 ± 0.69a	3.89 ± 0.16e	20.73 ± 0.52f	–
FE-A	16.23 ± 0.08b	14.93 ± 0.04a	6.02 ± 0.03d	57.68 ± 0.17 cd	9.02 ± 0.08a	32.55 ± 0.19a	18.14 ± 0.21a
FE-N	16.56 ± 0.08a	13.81 ± 0.29b	5.95 ± 0.06d	58.19 ± 0.20c	9.01 ± 0.06a	32.70 ± 0.53a	17.89 ± 0.34ab
FE-L	15.93 ± 0.08 cd	13.02 ± 0.24bcd	4.01 ± 0.05f	57.29 ± 0.06d	8.79 ± 0.08b	31.25 ± 0.15b	17.55 ± 0.10b
EF-A	16.25 ± 0.05b	13.83 ± 0.11b	6.56 ± 0.01b	70.81 ± 0.38a	3.83 ± 0.12e	22.91 ± 0.46e	2.25 ± 0.40e
EF-N	16.01 ± 0.01bc	13.62 ± 0.13bc	6.45 ± 0.02c	69.33 ± 0.30b	4.23 ± 0.09d	23.70 ± 0.11d	3.20 ± 0.22d
EF-L	15.92 ± 0.06d	13.54 ± 0.07 cd	4.48 ± 0.01e	69.52 ± 0.11b	4.54 ± 0.02c	26.01 ± 0.10c	5.40 ± 0.08c

Note: There is a significant difference between the values with different letters ($p < 0.05$).
CK: the control group; FE-A: first SSF with *A. oryzae* followed by extrusion; FE-N: first SSF with *N. sitophila* followed by extrusion; FE-L: first SSF with L. *plantarum* followed by extrusion; EF-A: first extrusion followed by SSF with *A. oryzae*; EF-N: first extrusion followed by SSF with *N. sitophila*; EF-L: first extrusion followed by SSF with L. *plantarum*.

Table 2
Effects of processing on the contents of dietary fiber and arabinoxylan in rice bran (g/100 g DW).

Sample	Dietary fiber			Arabinoxylans	
	IDF	SDF	Total	TAX	WEAX
CK	26.65 ± 0.35a	9.26 ± 0.68d	35.91 ± 1.16a	6.65 ± 0.02b	3.44 ± 0.02de
FE-A	22.58 ± 0.55d	10.76 ± 0.20b	33.34 ± 0.42a	5.59 ± 0.24d	3.55 ± 0.11d
FE-N	23.13 ± 0.33 cd	11.89 ± 0.19a	35.02 ± 0.54a	5.85 ± 0.35d	3.82 ± 0.06bc
FE-L	23.77 ± 1.21bcd	11.74 ± 0.17a	35.51 ± 1.38a	6.28 ± 0.04c	3.32 ± 0.04e
EF-A	24.17 ± 0.24bc	9.05 ± 0.15d	33.22 ± 1.42a	6.45 ± 0.13bc	3.95 ± 0.08b
EF-N	24.96 ± 0.10b	9.99 ± 0.15c	34.95 ± 1.02a	7.74 ± 0.16a	3.76 ± 0.08c
EF-L	23.85 ± 0.53bcd	11.08 ± 0.48b	34.93 ± 1.08a	6.53 ± 0.24bc	5.01 ± 0.08a

Note: There is a significant difference between the values with different letters ($p < 0.05$).
DW: dry weight; SDF: soluble dietary fiber; IDF: insoluble dietary fiber; TAX: total arabinoxylans; WEAX: water-extractable arabinoxylans.
CK: the control group; FE-A: first SSF with *A. oryzae* followed by extrusion; FE-N: first SSF with *N. sitophila* followed by extrusion; FE-L: first SSF with *L. plantarum* followed by extrusion; EF-A: first extrusion followed by SSF with *A. oryzae*; EF-N: first extrusion followed by SSF with *N. sitophila*; EF-L: first extrusion followed by SSF with *L. plantarum*.

the other hand, the variation of TAX and WEAX content showed a correlation with the processing sequence. FE treatment generally reduced TAX content (5.6–15.9%), while EF treatment had no significant effect. In FE treatment group, only FE-N treatment significantly increased the content of WEAX by 11.0%, while EF treatment generally increased the content of WEAX by 9.3–45.6%, and EF-L treatment had the highest content of WEAX (5.01 g/100 g DW).

Although IDF cannot be digested by the human body, it has the effect of promoting intestinal peristalsis and maintaining intestinal flora, which can reduce the risk of colon cancer (Manzoor et al., 2023). However, high IDF content in rice bran can lead to poor eating quality and consumption experience, and may cause flatulence (D. Zhang et al., 2022). SDF is mainly composed of hemicellulose, sodium alginate, guar gum, pectin, glucan and other oligosaccharides, and has physiological activities such as reducing postprandial blood glucose and cholesterol levels (Jia et al., 2019). It has been shown that both extrusion and SSF have the effect of promoting the conversion of IDF to SDF (Jia et al., 2019; Roye et al., 2020). In this study, IDF content decreased and SDF content increased in processed rice bran. Among them, FE treatment obtained higher SDF content. Although both extrusion and SSF promote the conversion of IDF to SDF, the SSF process relies on plant cell wall degrading enzymes actively produced by microorganisms (J. Wang et al., 2023). In this study, higher cellulase and cellobiose activities were observed in the samples fermented only than those fermented after extrusion, among which there were significant differences in cellulase activities in the samples fermented by *A. oryzae* and L. *plantarum*, and significantly differences in cellulobiase activities in the samples fermented by *N. sitophila* (Table S1). Compared with extruded rice bran, unextruded rice bran has higher IDF content and less small molecule nutrients, which may be conducive to inducing microorganisms to produce more enzymes (Roye et al., 2020).

Arabinoxylan is the main component of grain bran dietary fiber, which has good gelability and emulsification (Li et al., 2023; X. Zhang et al., 2019). In recent years, arabinoxylan has received extensive attention for its functional properties in controlling diabetes, cardiovascular diseases, weight management and prevention of colon cancer (Yan et al., 2019). In this study, TAX was observed to be significantly reduced after FE treatment, which may be related to the production of more cell wall degrading enzymes during SSF (Table S1). On the other hand, the EF treatment group obtained more WEAX. The increase in WEAX content is thought to be related to matrix acidification with xylanase activity(D. Zhang et al., 2022). This may be due to the different numbers and types of degradation enzymes produced by microorganisms during SSF induced by different treatment sequences. According to Table S1, the xylanase activity in the samples only SSF with *N. sitophila.* or L. *plantarum* was significantly higher than that of the samples only SSF with *N. sitophila* or L. *plantarum*, which may be due to the different amounts and types of degrading enzymes produced by microorganisms in the SSF process induced by different treatment sequences (D. Zhang et al., 2022). In addition, the EF-L treatment obtained the most WEAX possibly due to the lower substrate pH.

3.4. Phenols and flavonoids

Table 3 shows the phenols and flavonoid content of processed rice bran. The total phenolic content of untreated rice bran samples was 492.86 mg GAE/100 g DW, and FE treatment significantly increased the total phenolic content of rice bran samples (33.9–61.7%). The total phenolic content of samples fermented by *A. oryzae* and *N. sitophila* increased by 17.7% and 16.2%, respectively, while the total phenolic content of samples fermented by L. *plantarum* decreased by 4.1%. The change of free phenol was consistent with that of total phenol, and the FE-N sample had the highest free phenol content (514.45 mg GAE/100 g DW), which was 120.5% higher than that of the control group. The content of bound phenol was significantly different among the treatment groups, and the content of bound phenol in processed rice bran increased by 8.6–30.0%. Similarly, FE treatment also significantly increased the flavonoid content of rice bran (9.7–53.8%), while EF treatment had no significant difference in most cases.

The abundance of phenolic compounds and flavonoids in rice bran is an essential source of health promoting functions such as anti-inflammatory, anti-cancer, and anti-cardiovascular diseases (Zhao et al., 2023). Compared with fruits and vegetables, most phenolic compounds in rice bran exist in the cell wall in the bound state, covalently binding with cell wall components (such as cellulose, hemicellulose, lignin, structural proteins) (Shin et al., 2019). Studies have shown that extrusion and fermentation can destroy covalent bonds, promote the conversion of bound phenolic compounds into free forms, and improve the accessibility of phenolic substances in rice bran (R. Zhang et al., 2018). Chen et al. also showed that extrusion and *Rhizopus oryzae* SSF alone could increase free, bound and total phenolic content in rice bran (Chen et al., 2019). In this study, the processing sequence of extrusion and SSF had a significant effect on the phenolic content of rice bran. The content of phenolic in the samples after FE treatment increased significantly, while the increase in the samples after EF treatment was small and even decreased in some samples (Table 3). The increase in phenolic in processed rice bran was mainly attributed to the release of bound phenols (Zhao et al., 2023). It should be noted that although both extrusion and SSF promoted the release of bound phenols, the processing time of extrusion treatment was shorter (3–5 min), while that of the SSF process was longer (5 d). Shin et al. reported that the highest content of major phenolic compounds in rice bran was obtained on the third day of SSF, while it was greatly reduced after the fourth day. They hypothesized that this was due to the breakdown of phenolic acids or their incorporation into insoluble polysaccharides on the fourth day (Shin et al., 2019). Zhang et al. also found that the total phenol content of brown rice was significantly reduced during 30–48 h of SSF, and believed that this was related to microbial metabolism reducing its concentration (D. Zhang et al., 2022). Therefore, it can be speculated that in the EF process, the phenolic compounds released in the extrusion process may be decomposed or absorbed and utilized by microorganisms during SSF, resulting in a large loss of phenolic compounds. In the process of FE process, more phenolic compounds released were retained in the short extrusion process. On the other hand, the contents of

Table 3
Effects of processing on bioactive components and antioxidant activity of rice bran.

Sample	Phenolic content (mg GAE/100 g DW)			Flavonoid content (mg RE/100 g DW)	Antioxidant activity (mg AAE/100 g DW)		γ-oryzanol content (mg/100 g DW)
	Free	Bound	Total		FRAP	DPPH-RSR	
CK	233.33 ± 6.39f	259.54 ± 1.64e	492.86 ± 6.00e	403.19 ± 13.03d	2742.21 ± 43.22d	198.90 ± 4.61b	357.01 ± 7.17b
FE-A	478.17 ± 12.06b	300.24 ± 1.68c	785.38 ± 1.68b	549.60 ± 22.81b	3765.01 ± 51.13a	256.17 ± 2.47a	362.05 ± 8.26b
FE-N	514.45 ± 3.38a	281.98 ± 1.54e	796.99 ± 5.07a	620.25 ± 35.47a	3568.47 ± 60.91b	254.58 ± 2.80a	390.79 ± 8.50a
FE-L	351.98 ± 5.30c	307.67 ± 1.50b	659.97 ± 7.28c	442.25 ± 11.96c	3083.35 ± 60.05c	167.05 ± 1.70d	327.36 ± 9.10 cd
EF-A	273.21 ± 2.76d	307.04 ± 2.89b	580.24 ± 1.73d	423.19 ± 3.41 cd	2695.30 ± 35.64d	193.11 ± 1.41bc	357.32 ± 5.15b
EF-N	253.68 ± 1.723	319.17 ± 2.74a	572.86 ± 1.04d	429.81 ± 12.08 cd	2505.11 ± 95.33e	185.85 ± 4.77c	335.98 ± 8.18c
EF-L	170.23 ± 2.51 g	302.25 ± 3.63c	472.47 ± 6.13f	348.41 ± 13.56e	2405.57 ± 2.64f	138.26 ± 7.08e	317.40 ± 9.77d

Note: There is a significant difference between the values with different letters ($p < 0.05$).
DW: dry weight; GAE: gallic acid equivalent; RE: rutin equivalents; AAE: ascorbic acid equivalent; FRAP: ferric ion reducing antioxidant power; DPPH-RSR: DPPH radical scavenging rate.
CK: the control group; FE-A: first SSF with *A. oryzae* followed by extrusion; FE-N: first SSF with *N. sitophila* followed by extrusion; FE-L: first SSF with L. *plantarum* followed by extrusion; EF-A: first extrusion followed by SSF with *A. oryzae*; EF-N: first extrusion followed by SSF with *N. sitophila*; EF-L: first extrusion followed by SSF with L. *plantarum*.

phenols and flavonoids in the samples fermented by *A. oryzae* and *N. sitophila* were higher than those in the samples fermented by L. *plantarum*. This is because filamentous fungi (*A. oryzae* and *N. sitophila*) are more capable than L. *plantarum* in producing plant cell wall-degrading enzymes, such as cellulase (D. Zhang et al., 2022). In addition, the content of phenolic compounds in rice bran in this study was lower than that reported by Lin et al.(Lin et al., 2024), which may be related to the variety of rice bran and the pretreatment method.

3.5. Antioxidant activity and γ-oryzanol content

In this study, FRAP and DPPH-RSR were measured to reflect the changes in rice bran antioxidant activity (Table 3). Compared with the control group, FE treatment increased the FRAP and DPPH-RSR of rice bran by 12.4–37.3% and 28.0–28.8%, respectively, and EF treatment reduced the antioxidant activity of rice bran. Among them, the FRAP of FE-L was not consistent with the trend of antioxidant changes reflected by DPPH-RSR, because the oxidative potential of DPPH radical scavenging activity was relatively low, and some antioxidant substances may not be detected (Zhuang et al., 1999). On the other hand, the samples fermented by *A. oryzae* under FE treatment showed stronger antioxidant activity, with FRAP and DPPH-RSR of 3765.01 and 256.17 mg AAE/100 g DW, respectively. The content of γ-oryzanol in rice bran was also determined (Table 3). The content of γ-oryzanol in FE-N treated rice bran increased from 357.01 mg/100 g DW to 390.79 mg/100 g DW. FE-A and EF-A treatments did not significantly affect the content of γ-oryzanol, but the content of γ-oryzanol decreased by 5.9–11.1% after FE-L, EF-N and EF-L treatments.

The antioxidant activity of rice bran are derived from phenols, phytosterols, γ-oryzanol and various phytosterol conjugates. The role of antioxidant activity of rice bran in coping with oxidative damage and metabolic diseases has been widely studied, in which γ-oryzanol plays a key role (Behl et al., 2021).γ-oryzanol can reduce serum cholesterol and triglyceride concentrations and is more effective than tocopherol and tocotrienol in preventing oxidation of cholesterol and linoleic acid (Sahini & Mutegoa, 2023). In this study, the antioxidant activity of rice bran showed similar changes with the content of γ-oryzanol. FE-A and FE-N treatment groups significantly improved the antioxidant activity of rice bran, and FE-N treatment also significantly increased the content of γ-oryzanol. On the contrary, EF treatment reduced the antioxidant activity and γ-oryzanol content of rice bran to different degrees. γ-oryzanol (39–63%) and phenols (33–43%) are considered to be the most prevalent antioxidants in rice bran (Sahini & Mutegoa, 2023). The significant effect of the processing sequence may be due to the longer processing time of SSF and the loss of antioxidants. On the other hand, the rice bran fermented by *A. oryzae* and *N. sitophila* showed higher antioxidant activity and γ-oryzanol. Zhang et al. showed that the increase in γ-oryzanol content caused by SSF can be attributed to the degradation of esterified ferulate by microbial feruloyl esterase (D. Zhang et al., 2022). Filamentous fungi (*A. oryzae* and *N. sitophila*) have stronger enzyme production capacity in SSF, which may be the reason for the difference in antioxidant activity and γ-oryzanol content of rice bran.

3.6. Hydration properties

Hydration and adsorption properties of rice bran were analyzed by WBC, WSI, and OBC (Fig. 1). The WBC of processed rice bran was 38.6–57.0% lower than that of the control group. In the FE treatment groups, the WBC of the samples treated with the three SSF strains from high to low was as follows: *A. oryzae*, *N. sitophila* and L. *plantarum*. In EF treatment groups, the WBC of samples fermented by *A. oryzae* was the lowest (0.81 g/g), and there was no significant difference between *N. sitophila* and *L. plantarum*. The WSI of rice bran after processing was significantly increased by 22.9–60.8% compared with the control group. The rice bran treated with FE-L had the highest WSI (35.87 g/100 g). The OBC of rice bran showed significant differences under different processing sequences. FE treatment significantly reduced the OBC of rice bran. In contrast, EF treatment only reduced the OBC of *the A. oryzae* treatment group, and there was no significant difference between *the N. sitophila* and *L. plantarum* treatment groups and the control group.

Fiber void is considered to be an essential factor affecting WBC in rice bran (Yin et al., 2022). In the process of extrusion and SSF treatment, the fiber structure of rice bran was destroyed, and the original fiber space collapsed, resulting in the decrease of WBC in rice bran. In addition, Roye et al. also found that samples with lower pH during extrusion would have lower WBC and pointed out that this could be due to acid hydrolysis of the fiber structure during extrusion (Roye et al., 2020). In the FE treatment groups, the pH values from high to low were: *A. oryzae*, *N. sitophila* and *L. plantarum*., and the order was consistent with the WBC of rice bran. The WSI of rice bran reflects the dissolution efficiency of water-soluble components, and the destruction of fiber structure and the improvement of solubility will increase WSI (Speroni et al., 2020). In this study, all treatments were able to enhance the WSI of rice bran. Among them, the lifting amount of FE treatment is higher than that of EF treatment, which is consistent with the analysis results of water-soluble components such as SDF and free phenol. OBC is related to the surface properties of the sample, total pore volume, hydrophilicity and the number of lipophilic sites (Naumann et al., 2021). In this study, FE treatment significantly reduced OBC, which may be related to the reduction of void volume due to structural changes caused by extrusion (Naumann et al., 2021). EF did not have a significant effect on OBC, probably because the SSF process increased the lipophilic sites of the sample.

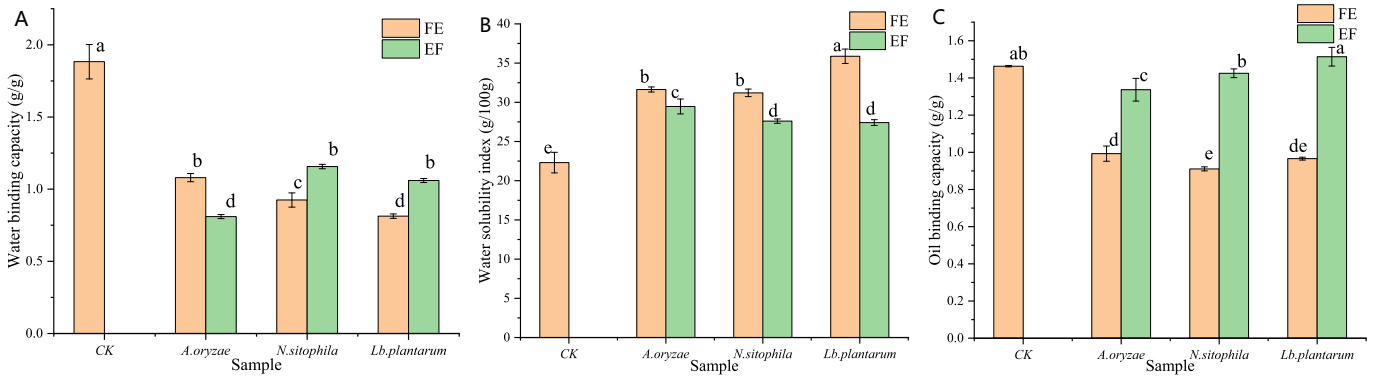

Fig. 1. Effects of processing on the hydration and adsorption properties of rice bran. CK: the control group; FE: first SSF followed by extrusion; EF: first extrusion followed by SSF. There is a significant difference between the values with different letters ($p < 0.05$).

3.7. Microstructure

The microstructure of processed rice bran particles was observed by scanning electron microscopy (Fig. 2). Unprocessed rice bran particles had regular edges and blocky shapes, and a large number of starch particles could be observed on the surface (Fig. 3-CK). After FE treatment, the edge of rice bran particles became smooth, the structure became loose, the surface starch particles disappeared and large pores appeared. It indicates that extrusion and SSF damage the fiber structure of rice bran and decompose the edge part, which is beneficial to improve the bioavailability of bioactive compounds and improve the functionality of rice bran (D. Zhang et al., 2022). Compared with the FE treatment group, more fine particles can be observed in the rice bran after EF treatment, and the rice bran surface is more fragmented. This may be due to the destruction of fibers caused by extrusion, resulting in more gaps and promoting starch gelatinization, which is conducive to the invasion and rapid growth of microorganisms in the rice bran structure during the subsequent SSF process, resulting in more bacterial debris and small and broken pores. On the other hand, under the same treatment sequence, it can be found that there are more pores on the surface of the samples treated with *A. oryzae* and *N. sitophila*, and the number of starch particles is less. This is because both have a more strong ability to produce degradation enzymes, resulting in more damage to the rice bran fiber structure (Table S1).

3.8. Principal component analysis (PCA)

The PCA of the correlation between processing treatment and rice bran quality parameters is shown in Fig. 3. PC1 is mainly related to nutrition and bioactive components of rice bran, such as protein, fat, total phenols, total flavonoids, etc. PC2 is mainly related to fiber structure, such as pH, WBC, SDF, etc.

The PCA results showed that the contents of IDF, TAX and WEAX tended to decrease, and pH, WBC and OBC tended to decrease in the processed rice bran. Nutrients, bioactive components, antioxidant activity, $\triangle E$ and WSI tended to increase. PC1 (55.3%) can reflect the variation trend of most quality parameters. It can be found that although all treatment groups are moving toward the X-axis relative to the control group, the FE treatment group is in the positive part of the X-axis and the EF treatment group is in the negative part. This indicates that FE treatment is more beneficial in improving the nutritional quality of rice bran than EF treatment, but it is also accompanied by more color changes. PC2 (22.2%) mainly reflects the related parameters of rice bran fiber structure, and the negative direction of the Y-axis tends to the degradation of fiber, such as the increase of SDF, WEAX content, and the increase of WSI. Fig. 3 shows that all treatment groups moved in the negative direction of the Y-axis compared with the control group, indicating that the processing treatment promoted the degradation of rice bran fiber. Among them, the samples treated with L. *plantarum* were in the negative value part of the Y-axis, while the samples treated with

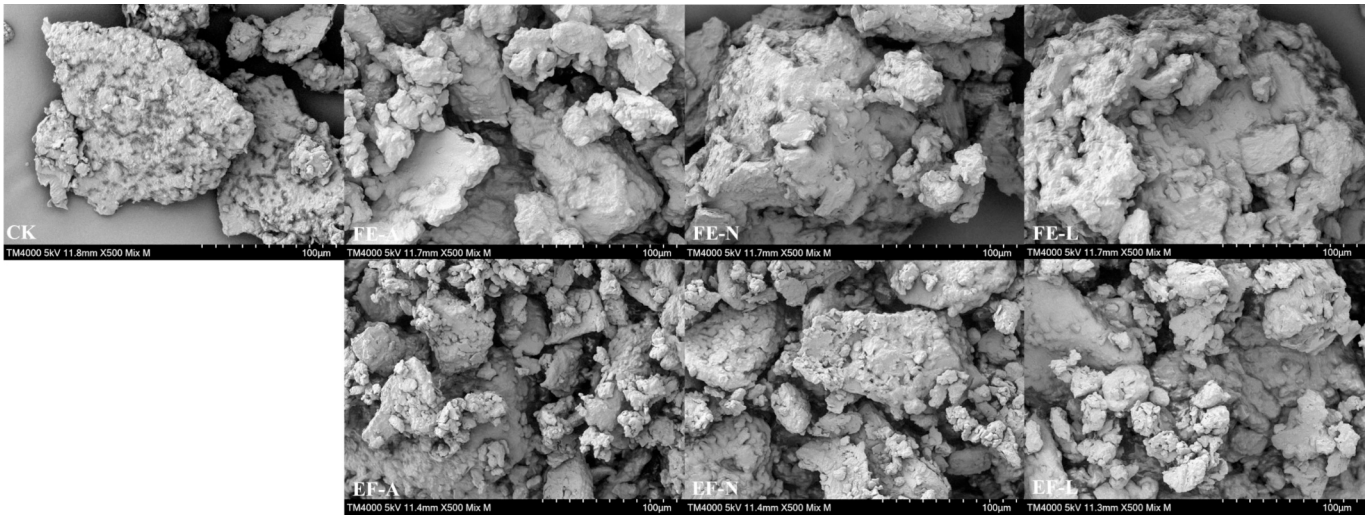

Fig. 2. Sem observation of rice bran (500×). CK: the control group; FE-A: first SSF with *A. oryzae* followed by extrusion; FE-N: first SSF with *N. sitophila* followed by extrusion; FE-L: first SSF with L. *plantarum* followed by extrusion; EF-A: first extrusion followed by SSF with *A. oryzae*; EF-N: first extrusion followed by SSF with *N. sitophila*; EF-L: first extrusion followed by SSF with L. *plantarum*.

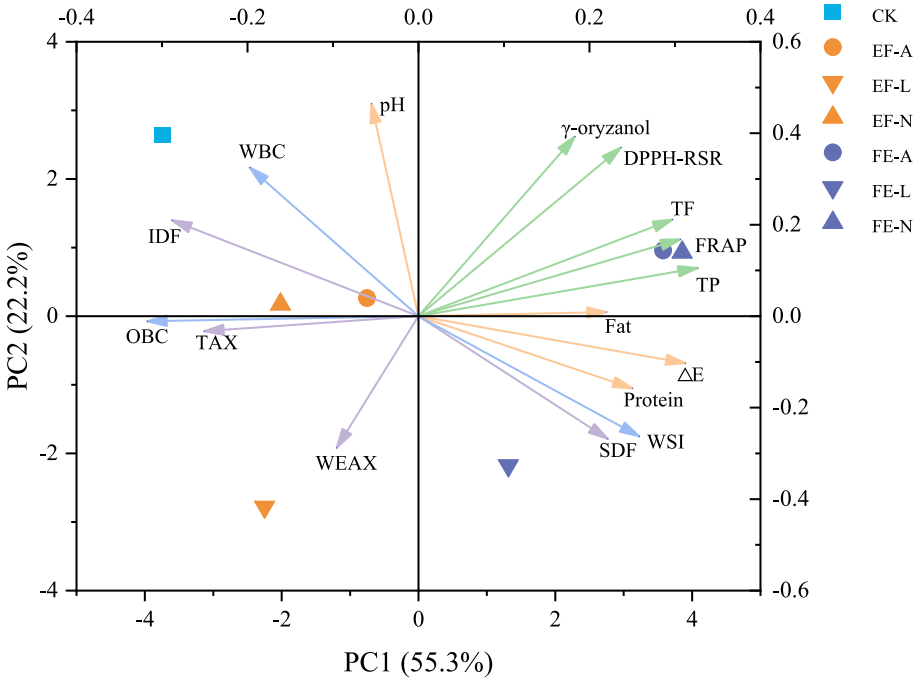

Fig. 3. Principal component analysis of processed rice bran. CK: the control group; FE-A: first SSF with *A. oryzae* followed by extrusion; FE-N: first SSF with *N. sitophila* followed by extrusion; FE-L: first SSF with *L. plantarum* followed by extrusion; EF-A: first extrusion followed by SSF with *A. oryzae*; EF-N: first extrusion followed by SSF with *N. sitophila*; EF-L: first extrusion followed by SSF with *L. plantarum*; SDF: soluble dietary fiber; IDF: insoluble dietary fiber; TAX: total arabinoxylans; WEAX: water-extractable arabinoxylans; FRAP: ferric ion reducing antioxidant power; DPPH-RSR: DPPH radical scavenging rate; TP: total phenol; TF: total flavonoid; WBC: water binding capacity; WSI: water solubility index: OBC: oil binding capacity.

A. oryzae and *N. sitophila* were in the positive value part of the Y-axis. This suggests that fermentation of rice bran with L. *plantarum* may be beneficial to destroy fiber structure and increase SDF and WEAX content.

4. Conclusion

In this study, the effects of extrusion and SSF processing sequences and fermentation strains on appearance, bioactive components and functional properties of rice bran were compared. The results showed that the combined treatment of extrusion and SSF could increase the protein and fat content of rice bran, and promote the conversion of IDF to SDF. However, the different processing sequences of the two treatments would bring different results to the changes in color, bioactive components, and antioxidant activity. The FE treatment increased the contents of SDF, phenols, flavonoids and γ-oryzanol, and significantly reduced the WBC and OBC of rice bran, but the color of rice bran also changed to brown obviously. The EF treatment caused damage to the bioactive components and antioxidant activity of rice bran, but significantly increased the content of TAX and WEAX, and the color change was small. There are two main reasons for the difference in results caused by the processing sequence: 1) the significant difference in processing time; 2) The induction effect of matrix components on SSF microorganisms. Under the same processing sequence, *A. oryzae* and *N. sitophila* showed better effects in enhancing the bioactive components of rice bran due to their strong production capacity of degrading enzymes. *L. plantarum* can reduce the pH of the substrate and obtain the best effect in increasing the content of WEAX in rice bran. Different processing sequences and fermentation strains have their advantages on the nutritional results of rice bran, so the appropriate processing sequences and fermentation strains should be selected according to the production needs.

Acknowledgment/Funding

This study was financially supported by the the Shanghai Agricultural Science and Technology Innovation Project, grant number I2023007, and the Shanghai agricultural products preservation and processing professional technical service platform, grant number 21 DZ2292200.

CRediT authorship contribution statement

Songheng Wu: Writing – original draft, Methodology, Investigation, Conceptualization. **Yi Zhang:** Writing – review & editing, Methodology. **Bingjie Chen:** Methodology. **Xiao Wang:** Software. **Yongjin Qiao:** Project administration, Formal analysis. **Jianyu Chen:** Resources.

Declaration of competing interest

All authors approve the authorship; the financial supports have also been thanked. There is no conflict of interests in this work.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101549.

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