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Preparation of lactic acid bacteria compound starter cultures based on pasting properties and its improvement of glutinous rice flour and dough

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ABSTRACT

The effects of 5 lactic acid bacteria (LAB) fermentation on the pasting properties of glutinous rice flour were compared, and suitable fermentation strains were selected based on the changes of viscosity, setback value, and breakdown value to prepare LAB compound starter cultures. The results revealed that *Latilactobacillus sakei* HSD004 and *Lactocaseibacillus rhamnosus* HSD005 had apparent advantages in increasing the viscosity and reducing the setback and breakdown values of glutinous rice flour. In particular, the compound starter created using the two abovementioned LAB in the ratio of 3:1 had better performance than that using a single LAB in improving the pasting properties and increasing the water and oil absorption capacity of glutinous rice flour. Moreover, the gelatinization enthalpy of the fermented samples increased significantly. For frozen glutinous rice dough stored for 28 days, the viscoelasticity of frozen dough prepared by compound starter was better than that of control dough, and the freezable water content was lower than that of control dough. These results indicate that compound LAB fermentation is a promising technology in the glutinous rice-based food processing industry, which has significance for its application.

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1. Introduction

Glutinous rice is one of the most important food crops in China with a long history of cultivation. Although it yields less than other rice varieties, it is an indispensable type of rice. The main difference between glutinous rice and other rice is that glutinous rice has extremely high amylopectin content, accounting for about 98% of the total starch^[1], while the amylose content only accounts for about 1.0%–2.3% of the total starch^[2]. Due to its advantages of high viscosity, easy gelatinization, slower rate of retrogradation, unique flavor, and nutrient richness, glutinous rice is often processed into traditional Chinese sticky snacks, such as glutinous rice cakes,

glutinous rice balls, glutinous rice crackers, glutinous rice dumplings, which are popular among consumers^[3–4]. The gel prepared from glutinous rice flour has excellent freeze-thaw stability, which enables frozen foods to better retain moisture during the freeze-thaw cycle. Thus, it is more suitable for the production of frozen foods compared with other rice flour^[5]. However, the drawbacks of glutinous rice flour cannot be ignored: its poor water absorption and water retention capacity cause glutinous rice dough to lack ductility and have a relatively loose structure. In addition, cooked glutinous rice products have a weak gel structure and low shear resistance^[6]. They are also prone to quality issues such as hardening, collapse, cracking, and short shelf life, problems which greatly affect the development and application of glutinous rice products. The main industrial use of glutinous rice flour in China is to produce quick-frozen glutinous rice balls. They generally have quality problems, such as cracking and poor taste, which seriously restrict the process of product industrialization^[7]. Therefore, to improve the processing

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properties and broaden the application range of glutinous rice flour, the modification of glutinous rice flour is imperative.

Microbial fermentation technology has been widely recognized as a processing technology for improving the quality of cereal foods. In particular, the application of lactic acid bacteria (LAB) in cereal fermentation has received extensive attention. Previous studies have shown that LAB fermentation promoted the physicochemical and processing properties of cereal, and imparted a special flavor to products^[8]. At the same time, the texture, shelf life and nutritional quality of products also became better^[9]. In addition, some LAB also produced exopolysaccharides (EPS), which improved the texture and taste of fermented cereal foods^[10-11], delayed the staling process^[12-13], and had a beneficial influence on dough stability during frozen storage^[14-15]. In the past years, most of the studies mainly focused on the non-glutinous rice (such as wheat, japonica rice, buckwheat and other grains), and numerous researches have been carried out to illustrate the advantages of LAB fermentation technology in these grain fermentation. However, there is little research on LAB fermentation of glutinous rice flour at present, and the physicochemical changes and aging mechanisms of glutinous rice flour after LAB fermentation are still unclear. In particular, we know sporadic about pasting and rheological properties of glutinous rice flour, which are closely related to viscosity, elasticity, hardness, texture and taste of glutinous rice-based foods^[16-19], leading to failure to provide valuable information for the glutinous rice processing industries. Up to now, no systematic study has been performed on the effects of LAB mixed-cultures on the glutinous rice flour. Therefore, it is necessary for us to carry out relevant research to provide theoretical reference for the wide application of glutinous rice flour.

The staple component of glutinous rice is starch. The gelatinized starch gel retrogrades during the storage process, causing the starch gel to gradually become hard and brittle and lose its water-holding capacity. In addition, the glutinous rice flour used in this experiment was prepared by the dry-milling method. A previous study was conducted to prove that the dry-milling method resulted in rice flour that had more damaged starch and lower peak viscosity (PV) and final viscosity (FV), drawbacks that restricted the development of rice products^[20]. Therefore, to solve these issues, 5 LAB strains (*Lactobacillus brevis* HSD001, *Lactiplantibacillus plantarum* HSD002, *Lactobacillus acidophilus* HSD003, *Latilactobacillus sakei* HSD004, and *Lacticaseibacillus rhamnosus* HSD005) were selected to ferment glutinous rice flour to reveal the variation of pasting properties. The suitable LAB strains were sought by determining the viscosity, setback value (SBV), and breakdown value (BDV) to prepare compound LAB starter cultures. The improvement effect of the compound starter cultures on glutinous rice flour was evaluated by measuring water/oil absorption capacity (WAC/OAC) and thermal characteristics. Furthermore, the freezable water (Fw) content and viscoelasticity of frozen glutinous rice dough were investigated to assess the effect of the LAB synergistic fermentation on frozen glutinous rice dough. These provided a theoretical basis and reference for the development and production of glutinous rice products.

2. Materials and methods

2.1 Materials

Glutinous rice and soybean oil were purchased from a local market in Harbin (China). Man Rogosa Sharp (MRS) agar and MRS broth were purchased from Beijing Aobo Star Biotechnology Co., Ltd. Sodium hydroxide and sodium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. *L. brevis* HSD001, *L. plantarum* HSD002, *L. acidophilus* HSD003, *L. sakei* HSD004, and *L. rhamnosus* HSD005 were isolated from glutinous rice fermented liquid obtained from Harbin Tianyi Ecological Agricultural and Sideline Products Co., Ltd.

2.2 Culture of LAB

According to the method of Hu et al.^[21], 5 LAB strains were stored in MRS broth containing 25% glycerol at -80°C . Prior to fermentation, each strain was streaked onto MRS agar and incubated for 24 h at 37°C . One colony LAB was cultivated in 5 mL of MRS broth at 37°C for 24 h. Finally, the above strains were inoculated into the MRS broth at a concentration of 1% (V/V) and cultured at 30°C for 18 h.

2.3 Preparation of fermented glutinous rice flour

The glutinous rice was dry-milled and then passed through an 80-mesh sieve. The obtained glutinous rice flour and water were mixed at a ratio of 10:9 (m/m), and each of the 5 LAB strains was inoculated into the mixture at 1×10^8 CFU/g. Each mixture was sealed and placed in an incubator at 22°C for fermentation from 12 to 36 h. The fermented mixtures were first prefrozen at -80°C for 24 h, then dried in a lyophilizer (Alpha1-4LSC, Martin Christ, Germany), and finally ground into 80-mesh powder. F control, glutinous rice flour without fermentation; F I-12 h-F I-36 h, glutinous rice flour fermented by *L. brevis* HSD001 for 12-36 h; F II-12 h-F II-36 h, glutinous rice flour fermented by *L. plantarum* HSD002 for 12-36 h; F III-12 h-F III-36 h, glutinous rice flour fermented by *L. acidophilus* HSD003 for 12-36 h; F IV-12 h-F IV-36 h, glutinous rice flour fermented by *L. sakei* HSD004 for 12-36 h; F V-12 h-F V-36 h, glutinous rice flour fermented by *L. rhamnosus* HSD005 for 12-36 h.

After comparing the pasting properties of each fermented glutinous rice flour, *L. sakei* HSD004 and *L. rhamnosus* HSD005 were screened as compound strains, they were used to fermented glutinous rice flour in the ratios of 3:1, 2:1, 1:1, 1:2 and 1:3 for 24 h at 22°C , respectively. The total inoculation dose was 1×10^8 CFU/g, the other operations were the same as above. F VI-24 h, F VII-24 h, F VIII-24 h, F IX-24 h, F X-24 h represent glutinous rice flour fermented by *L. sakei* HSD004 and *L. rhamnosus* HSD005 in the ratios of 3:1, 2:1, 1:1, 1:2 and 1:3 for 24 h, respectively.

2.4 Determination of LAB fermentation properties

The pH value and total titratable acidity (TTA) of glutinous rice flour were investigated using the method reported by Tang et al.^[22] with minor modifications. Each sample (10.0 g) was added to distilled water (90.0 mL). The slurry was stirred in a magnetic stirrer for

30 min and then kept at ambient temperature for 10 min. The pH value was measured using a PHS-3C pH meter (Shanghai, China). Thereafter, the slurry was titrated with 0.1 mol/L of sodium hydroxide (NaOH) until the pH value rose to 8.5, recording the volume of NaOH solution consumed, which is the TTA.

2.5 Determination of pasting properties of glutinous rice flour

The pasting properties of glutinous rice flour were analyzed using a Rapid Visco Analyzer (RVA TecMaster, Perten, Sweden) according to the method proposed by Zhang et al.^[16]. Each sample and distilled water were mixed in a metal RVA canister to prepare a suspension (14.0% dry matter, *m/m*; total weight of 28.0 g). The suspension was heated at 50 °C for 1 min, after which the temperature was raised from 50 to 95 °C at a rate of 12 °C/min and maintained at 95 °C for 2.5 min. Thereafter, the paste was cooled to 50 °C at the same rate and maintained at 50 °C for 2 min. The stirring speed was 960 r/min for 10 s during testing, while it was 160 r/min for the rest time.

2.6 Determination of water/oil absorption capacity of glutinous rice flour

The WAC and OAC of glutinous rice flour were determined using the method reported by Teixeira et al.^[23] with minor modifications. Each sample (2.0 g) was mixed with 10 g of distilled water or soybean oil and kept at ambient temperature for 30 min. This was then centrifuged for 15 min at 4 000 × g, the supernatant was poured off, and its weight was recorded. WAC and OAC of glutinous rice flour were calculated as follows:

$$\text{WAC or OAC (g/g)} = \frac{10 - m_1}{m_2} \quad (1)$$

Where m_1 is the weight of the supernatant (g) and m_2 is the weight of the glutinous rice flour (g).

2.7 Determination of thermal properties of glutinous rice flour

The thermal properties of glutinous rice flour were measured using a differential scanning calorimeter (DSC, Q20, TA Instruments, USA) as described by Lin et al.^[24] with some modifications. Each sample (4 mg) and distilled water (8 µL) were added to an aluminum pan and hermetically sealed. The mixture, which was equilibrated at 4 °C for 12 h, was heated from 20 to 100 °C at a heating rate of 10 °C/min in a nitrogen atmosphere with an empty aluminum pan as a reference. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH) of each sample were determined.

2.8 Morphology of glutinous rice flour observation

A scanning electron microscopy (SEM, Regulus 8100, Hitachi, Japan) was used to explore the morphology of the glutinous rice flour according to the method previously reported by Wang et al.^[25]. Each sample was mounted on a metal stage with double-sided adhesive and then coated with gold. The morphology of each sample was observed at × 500 and × 1 000 magnifications at an acceleration voltage of 2.0 kV.

2.9 Fourier transform infrared spectroscopy analysis

The short-range ordered degree and double helix degree of starch and the secondary structure of protein in glutinous rice flour were determined by a Fourier transform infrared spectrometer (FTIR; PerkinElmer Spectrum 100, PerkinElmer, USA) according to the methods of Jia et al.^[26] and Zhang et al.^[27]. Each sample and KBr were mixed at a ratio of 1:100 and then pressed into a 0.5-mm flake. The flake was scanned 32 times with a spectrometer with a range from 4 000 to 400 cm^{-1} and a resolution of 4 cm^{-1} . The ratio of absorption peak intensities at 1 047 and 1 022 cm^{-1} ($R_{1\,047/1\,022}$) was used to characterize the short-range ordered degree, and the ratio of those at 995 and 1 022 cm^{-1} ($R_{995/1\,022}$) was used to quantify the double helix degree of starch in glutinous rice flour. The amide I peaks (1 600–1 700 cm^{-1}) of the secondary structure of protein were analyzed by PeakFit Software (Version 4.12, Systat Software, Inc., USA).

2.10 X-ray diffraction (XRD) analysis

The XRD patterns were determined with an X-ray diffractometer (D8 Advance, Bruker, Germany) at 40 kV and 40 mA with a scanning speed of 10.0°/min and a scanning range of 5°–40° (2θ)^[28]. The relative crystallinity (RC, %) of starch was calculated with Jade 7.0 software using the method of Zhan et al.^[29].

$$\text{RC (\%)} = \frac{A_c}{A_c + A_a} \times 100 \quad (2)$$

Where A_c is the area of the crystalline peak and A_a is the area of the amorphous peak.

2.11 Preparation of fresh and frozen glutinous rice dough

Glutinous rice flour (200 g) and distilled water (180 mL) were mixed and kneaded for about 15 min to achieve a smooth dough, and the dough was rested in a container for 30 min at 25 °C. Thereafter, the dough was divided into 30-g pieces that were wrapped with polyethylene film. Each dough sample was frozen in a –80 °C freezer (TSX40086A, Thermo Fisher, USA) for 6 h and then moved into a –20 °C freezer (BCD-321W, SIEMENS, Germany) for 7–28 days.

D control, glutinous rice dough prepared by F control; D IV, glutinous rice dough prepared by F IV-24 h; D V, glutinous rice dough prepared by F V-24 h; D VI, glutinous rice dough prepared by F VI-24 h; D control-7 d, D control-14 d, D control-21 d, D control-28 d, D control frozen for 7, 14, 21, and 28 days, respectively; D VI-7 d, D VI-14 d, D VI-21 d, D VI-28 d, D VI frozen for 7, 14, 21, and 28 days, respectively.

2.12 Determination of Fw content of frozen glutinous rice dough

The Fw content of glutinous rice dough with various frozen storage times was assayed using a DSC (Q20, TA Instruments, USA) based on a previous report^[30] with slight modifications. Each glutinous rice dough sample (12 mg) was placed into an aluminum pan and sealed, and the aluminum pan was frozen for 7–28 days. Before testing, the aluminum pan was taken out and thawed at room temperature for 1 h, cooled from 20 to –20 °C at a rate of

10 °C/min, equilibrated for 5 min, and finally heated to 20 °C at the same rate. The Fw content was calculated according to the following formula:

$$\text{Fw content (\%)} = \frac{\Delta H_1}{\Delta H_2 \times W} \times 100 \quad (3)$$

Where ΔH_1 is the enthalpy value of the dough sample, ΔH_2 is the water enthalpy value (335 J/g), and W is the moisture content of the dough sample (%).

2.13 Determination of dynamic rheological properties of glutinous rice dough

The dynamic rheological properties of glutinous rice dough were measured using a rheometer (MCR102, Anton Paar, Austria) equipped with a parallel plate (1 000- μm gap, 50-mm diameter) according to the method of Li et al.^[31] with minor modifications. Fresh dough and frozen dough thawed for 1 h were placed on the parallel plate, and the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan\delta = G''/G'$) were measured with 0.5% strain at 25 °C in the angular frequency sweep range from 0.5–126.0 rad/s.

2.14 Statistical analysis

The data were averaged by three parallel experiments. Origin 8.5 and SPSS 22.0 software were used in this research. Analysis of variance (ANOVA) and Duncan's test ($P < 0.05$) were used to assess significant differences.

3. Results and discussion

3.1 LAB fermentation properties

The pH value and TTA value are crucial indicators for evaluating acidification. Figs. 1A and B illustrate the changes in the pH value and TTA value of glutinous rice flour fermented by the 5 LAB strains. With the prolongation of fermentation time, the pH values of fermented glutinous rice flour gradually decreased, whereas the TTA values gradually increased. This was attributed to the metabolic activity of LAB, producing organic acids such as lactic and acetic acid^[32]. When the fermentation time was prolonged to 36 h, the pH values of samples fermented by *L. brevis* HSD001, *L. plantarum* HSD002, *L. acidophilus* HSD003, *L. sakei* HSD004, and *L. rhamnosus* HSD005 decreased to 4.15, 3.38, 3.24, 3.31, and

3.36, respectively. The corresponding TTA values increased to 7.14, 18.31, 17.61, 17.50, and 15.00 mL, respectively. During the whole fermentation process, the sample fermented by *L. brevis* HSD001 had the highest pH value and the lowest TTA value, indicating that the acid-producing performance of *L. brevis* HSD001 was the worst of all. In addition, the rapid increase in acidity can effectively inhibit the reproduction of spoilage microorganisms^[33]. Therefore, the other 4 LAB strains had apparent advantages over *L. brevis* HSD001 in ensuring food safety.

3.2 Pasting properties of glutinous rice flour fermented by single strain

3.2.1 Effects of single strain fermentation on viscosity of glutinous rice flour

The pasting viscosity represents extensive interaction between starch molecules during heating and cooling. The relatively higher PV and FV contributed to the elastic mouthfeel and smooth surface of the cooked rice-based foods^[26]. The PV reflects the ability of starch granules to absorb water and swell freely prior to physical breakdown^[34]. According to the information obtained from RVA curves (Figs. 2A-E), the PVs of glutinous rice flour fermented by the 5 LAB strains generally exhibited an increasing trend with the prolongation of fermentation time. When *L. brevis* HSD001 fermented for longer than 24 h, *L. plantarum* HSD002 and *L. rhamnosus* HSD005 fermented for at least 18 h, and *L. acidophilus* HSD003 and *L. sakei* HSD004 fermented for at least 24 h, the PVs of these samples were significantly higher than that of the control sample. In addition, the FV is another key indicator that reflects the association or retrogradation of starch after the cooling period^[34]. According to the RVA curves, the change trends of FV were basically the same as those of the PV.

To solve the problems of the insufficient viscosity of newly harvested glutinous rice and the viscosity reduction of glutinous rice flour caused by dry-milling, LAB fermentation can be used to increase the viscosity of glutinous rice flour. However, the viscosity of glutinous rice flour did not increase obviously after a short fermentation time, whereas the acidity of glutinous rice flour was too high after a long fermentation time, which affected the sensory quality of the products. After comprehensive consideration, 24 h was selected as the fermentation time for subsequent research. From Figs. 2F and 3A, apart from F I-24 h, the PVs, TVs, and FVs of other samples were

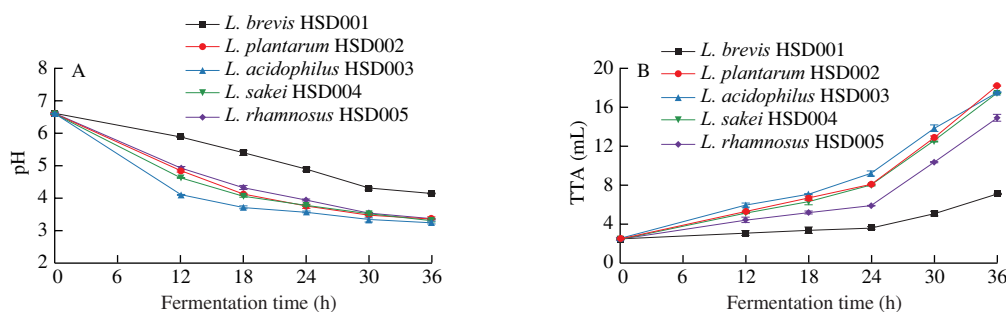


Fig. 1 Changes of (A) pH value and (B) TTA in glutinous rice flour fermented by LAB. Data represent the mean of three measurements \pm standard deviation.

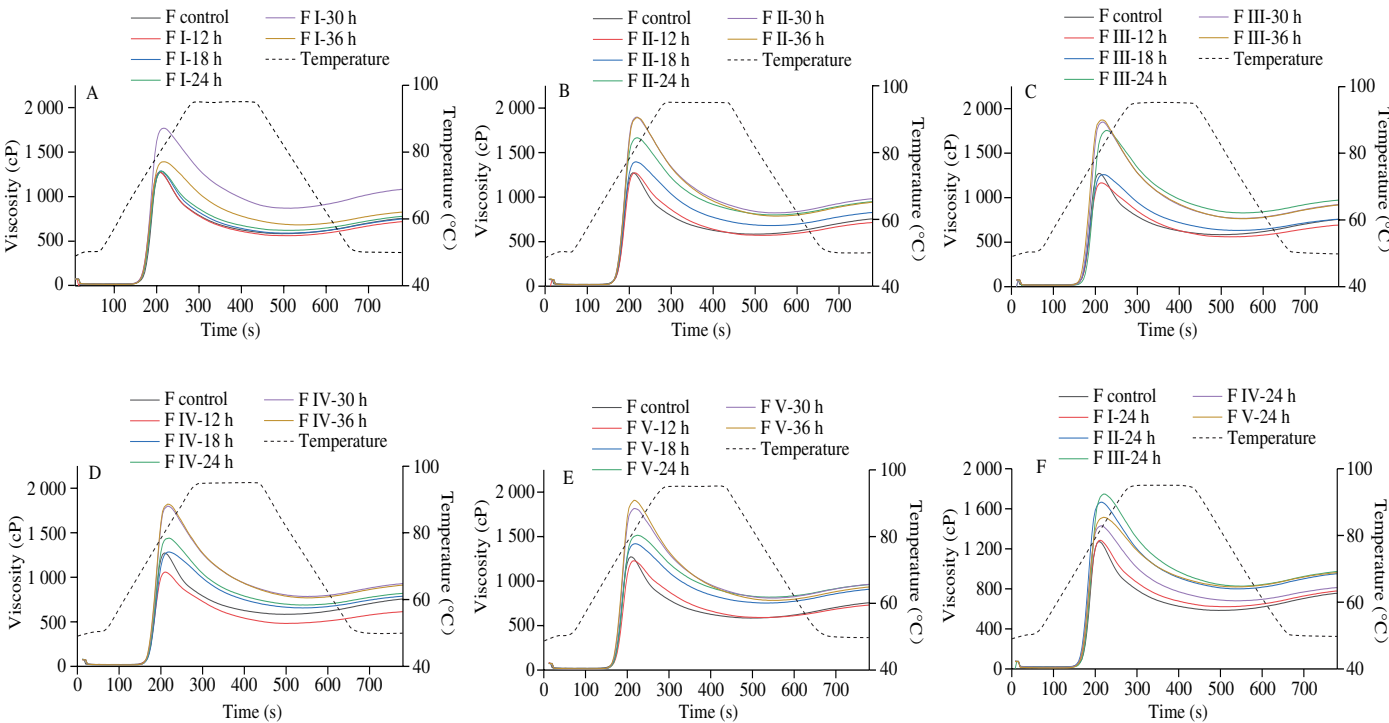


Fig. 2 RVA curve changes of glutinous rice flour fermented by LAB.

significantly higher than those of the control sample ($P < 0.05$), suggesting that the other 4 LAB had advantages in improving viscosity and that *L. brevis* HSD001 was not satisfactory in increasing the viscosity.

A possible reason for the increased viscosity of fermented glutinous rice flour is that the metabolites (organic acids or enzymes) produced by LAB caused the binding between protein and starch to become loose, and creating fissures and cavities on glutinous rice flour

granules (Fig. 4). This outcome facilitated water molecules to enter the granular interior and form intra- and inter-hydrogen bonds with stretched molecules and networked chains, thus increasing its $PV^{[35]}$. Generally, a higher WAC promotes starch to swell during pasting process, further resulting in a higher viscosity^[34]. According to the results displayed in Table 1, the WAC of each fermented glutinous rice flour was significantly higher than that of the control sample. Thus, this was considered another reason for the increase in PV, TV,

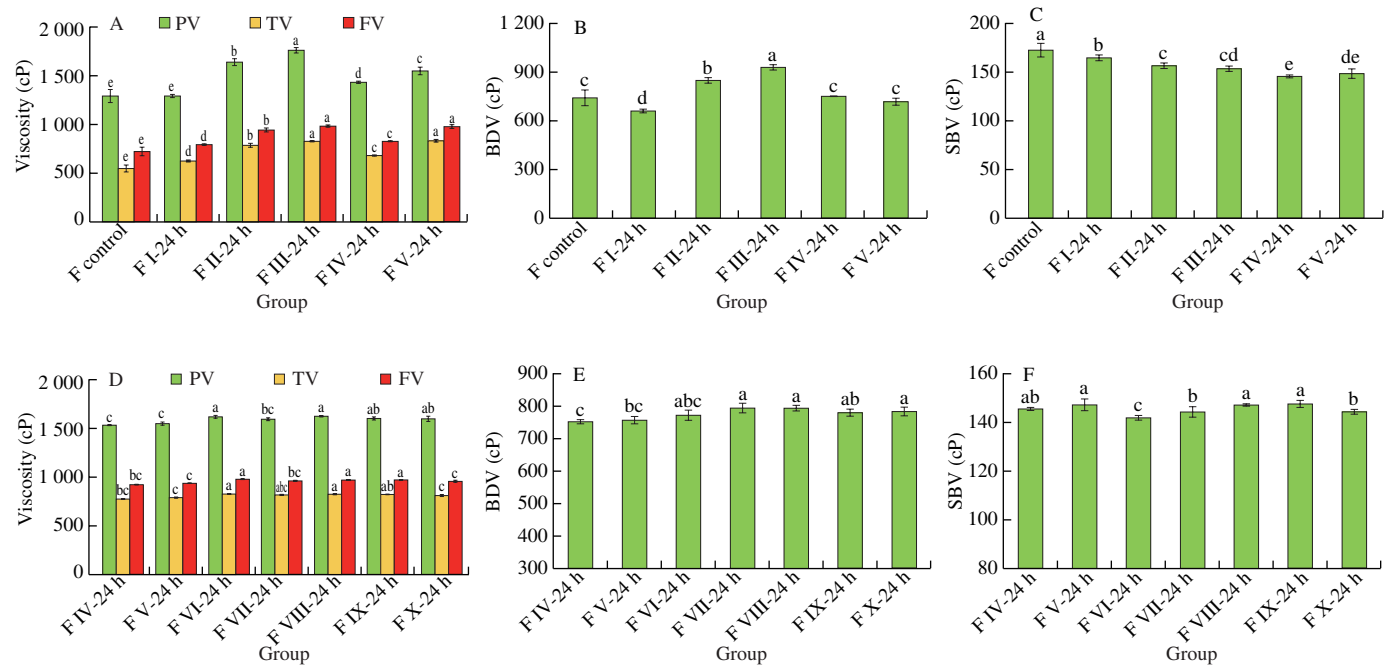


Fig. 3 Effects of LAB fermentation on viscosity, BDV and SBV of glutinous rice flour. Data represent the mean of three measurements \pm standard deviation, different letters in same index indicate significant differences ($P < 0.05$).

Table 1

Effects of LAB fermentation on WAC/OAC and thermal properties of glutinous rice flour.

Samples	WAC (g/g)	OAC (g/g)	T_g (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
F control	1.024 ± 0.004 ^d	0.756 ± 0.006 ^c	58.08 ± 0.09 ^a	67.09 ± 0.04 ^a	85.59 ± 0.60 ^{ab}	3.81 ± 0.08 ^b
F IV-24 h	1.121 ± 0.004 ^c	1.005 ± 0.004 ^b	55.73 ± 0.36 ^b	66.45 ± 0.27 ^b	85.36 ± 0.67 ^b	4.26 ± 0.13 ^a
F V-24 h	1.142 ± 0.005 ^b	1.044 ± 0.002 ^a	50.79 ± 0.96 ^c	66.36 ± 0.22 ^b	83.09 ± 0.21 ^c	4.36 ± 0.05 ^a
F VI-24 h	1.149 ± 0.005 ^a	1.040 ± 0.004 ^a	55.33 ± 1.20 ^b	67.03 ± 0.11 ^a	86.50 ± 0.45 ^a	4.39 ± 0.13 ^a

Notes: Data represent the mean of three measurements ± standard deviation, different letters in each column indicate significant differences ($P < 0.05$).

and FV. Moreover, Zhu^[36] also have found that amylopectin with a higher proportion of long internal B-chains tends to exhibit higher viscosity during starch gelatinization. Therefore, it was speculated that LAB fermentation changed the ratio of long and short internal B-chains in amylopectin in glutinous rice flour, which eventually led to an increase in viscosity.

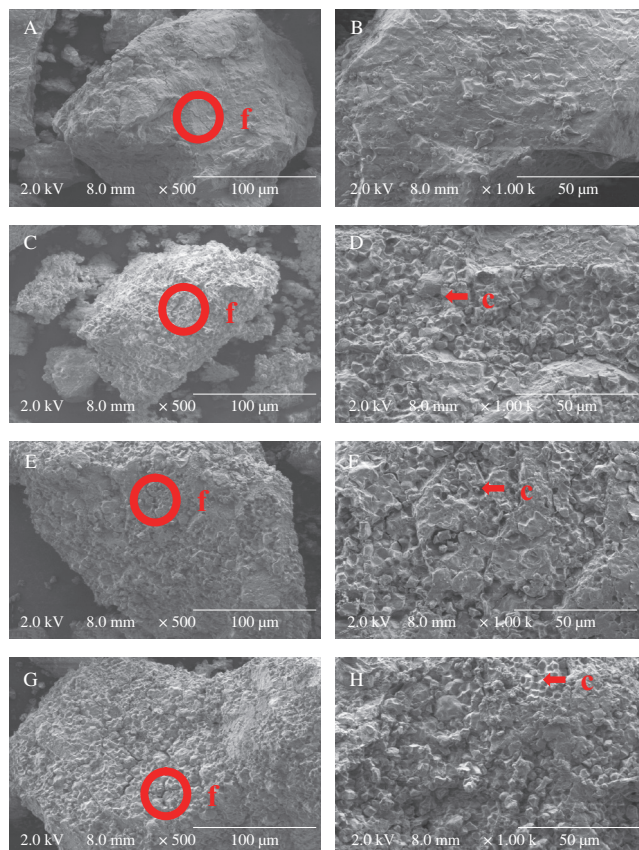


Fig. 4 Effects of LAB fermentation on the morphology of glutinous rice flour. (A and B) Glutinous rice flour without fermentation; (C and D) Glutinous rice flour fermented by *L. sakei* HSD004 for 24 h; (E and F) Glutinous rice flour fermented by *L. rhamnosus* HSD005 for 24 h; (G and H) Glutinous rice flour fermented by *L. sakei* HSD004 and *L. rhamnosus* HSD005 in the ratio of 3:1 for 24 h; f, fissure; c, cavity.

3.2.2 Effects of single strain fermentation on BDV and SBV of glutinous rice flour

The BDV reflects the shear resistance of the gelatinized sample at high temperature: the lower the BDV, the stronger the shear resistance^[16]. As depicted in Fig. 3B, the BDV of F I-24 h was lower than that of the control sample, the BDVs of F IV-24 h and F V-24 h were close to that of the control sample, while the BDVs of F II-24 h and F III-24 h were clearly higher than that of the control sample

($P < 0.05$). These results suggested that the shear resistance and thermal stability of the glutinous rice flour fermented by *L. plantarum* HSD002 and *L. acidophilus* HSD003 were not ideal. The SBV is an indicator of the short-term degree of rearrangement of the starch molecules and reflects the degree of retrogradation of a sample after cooling: the lower the SBV, the stronger the anti-retrogradation performance^[24]. As illustrated in Fig. 3C, the SBVs of the samples fermented by the 5 LAB strains were significantly lower than that of the control sample ($P < 0.05$), suggesting that these 5 LAB strains had outstanding anti-retrogradation properties, among which *L. sakei* HSD004 and *L. rhamnosus* HSD005 had the most prominent effect. Similar result was reported by Geng et al.^[8]. The retrogradation tendency of each fermented samples decreased significantly compared to the control sample, which is beneficial to the production and development of glutinous rice-based foods in the future.

3.3 Pasting properties of glutinous rice flour fermented by compound strains

Based on the previous research of this experiment, *L. sakei* HSD004 and *L. rhamnosus* HSD005 were selected to prepare LAB compound starter cultures, they were used to ferment glutinous rice flour in the ratios of 3:1, 2:1, 1:1, 1:2 and 1:3, respectively. Finally, the ratio of two LAB was determined by comparing the pasting properties of fermented glutinous rice flour.

3.3.1 Effects of compound strains fermentation on viscosity of glutinous rice flour

Adequate viscosity is related to better gelatinization and gel-forming ability of rice flour during cooking^[37]. The viscosity values of glutinous rice flour fermented by compound strains are depicted in Fig. 3D. The viscosity values (PV, TV, and FV) of F VI-24 h and F VIII-24 h were significantly higher than the corresponding viscosity values of samples fermented by a single strain. Thus, it was feasible to prepare LAB compound starter cultures to improve the viscosity of samples.

3.3.2 Effects of compound strains fermentation on BDV and SBV of glutinous rice flour

As presented in Figs. 3E and F, among all samples fermented by compound strains, only the BDV of F VI-24 h was close to that of F IV-24 h and F V-24 h, and F VI-24 h had the lowest SBV. For rice-based foods, the texture and properties depend on the degree of starch retrogradation during processing and storage. F VI-24 h had higher viscosity and lower SBV, exhibiting excellent pasting properties, so

after comprehensive consideration, the compound ratio of *L. sakei* HSD004 and *L. rhamnosus* HSD005 was determined to be 3:1.

3.4 WAC and OAC of glutinous rice flour

The WAC and OAC play critical roles in the manufacturing of various rice-based foods. As listed in Table 1, the WAC of each fermented sample was significantly higher than that of the control sample ($P < 0.05$). The WAC of F VI-24 h was the best, followed by F V-24 h and F IV-24 h. The WAC represents the ability of a sample to associate with water. It is influenced by various factors, such as particle size, surface area, and damaged starch content, and it depends on the number of hydroxyl groups linked to the water and the number of hydroxyl groups that can actually interact with water molecules^[38]. Therefore, the number of hydrophilic hydroxyl groups might have changed after fermentation, contributing to the increase in WAC. Moreover, the surface of fermented glutinous rice flour granules became rougher than before, the surface area in contact with the water substantially increased, and deeper fissures and more cavities appeared (Fig. 4), promoting the capillary action of the water. This similar behavior was also observed by Teixeira et al.^[23], where fermentation resulted in more pores and holes appearing on the starch surface, significantly increasing the WAC of fermented sample.

The OAC is another important property, as it improves the mouthfeel and retains the flavor of rice-based foods. The OAC of each fermented sample was also significantly higher than that of the control sample ($P < 0.05$). The OACs of F V-24 h and F VI-24 h were close to each other and exceeded that of F IV-24 h. Kaur et al.^[39] indicated that exposed non-polar groups were closely related to OAC, so it is likely that more non-polar groups were exposed to bind the hydrocarbon side chains in oil during the LAB fermentation, promoting the increase of OAC.

3.5 Thermal properties of glutinous rice flour

Table 1 lists the values of T_o , T_p , T_c , and ΔH . The gelatinization temperatures reflect the crystalline heat stability^[37]. The gelatinization temperatures (T_o , T_p , and T_c) of fermented glutinous rice flour (F IV-24 h, F V-24 h, and F VI-24 h) generally decreased compared with control sample, implying that LAB fermentation was beneficial to gelatinization.

The ΔH value is the energy required to convert the crystalline structure to an amorphous structure, so it reflects the degree of starch crystallinity and the degree of molecular disorder^[40]. In this study, the ΔH values of F IV-24 h, F V-24 h, and F VI-24 h were significantly higher than that of the control sample ($P < 0.05$). Govindaraju et al.^[41] stated that a higher ΔH value indicated that a sample had greater crystallinity and more ordered double helices. The ΔH results in

this experiment were basically in agreement with the results of crystallinity in Table 2.

3.6 Morphology of glutinous rice flour

A SEM comparison revealed that F IV-24 h, F V-24 h, F VI-24 h, and the control sample had visible microstructural differences. The morphological characteristics of these samples at $\times 500$ and $\times 1\,000$ magnification are presented in Figs. 4A–H. Fissures (f in Figs. 4A, C, E, and G) appeared in all samples, possibly caused by damaged starch during dry-milling^[42]. The fissures of F IV-24 h, F V-24 h, and F VI-24 h were deeper than those of the control sample, so LAB fermentation aggravated the cracking of particles. The control sample exhibited irregular shapes and relatively smooth surfaces, whereas the glutinous rice flour granules were destroyed and eroded to different degrees after fermentation. The granules of F IV-24 h, F V-24 h, and F VI-24 h clearly had rougher surfaces than the control sample. In addition, the uneven surface of the fermented glutinous rice flour granules was even accompanied by the appearance of some small cavities (c in Figs. 4D, F, and H). These were all attributed to the synergistic action of organic acids and enzymes^[43–46]. In the process of LAB fermentation, LAB use carbohydrates to produce gases, acids, and enzymes. The glutinous rice flour granules were attacked by LAB and their metabolites (acids and enzymes), the starch and protein of the original component degraded, leading to changes in their contents and structures (such as crystal and amorphous regions of starch, secondary structure of protein). In addition, protein is primarily located in the periphery or subaleurone layer of the granule and gradually tapers off toward the interior of the granule. However, the protein body in this study could not be clearly observed in SEM images as in the experiment of Ibanez et al.^[42]. In particular, when the surface morphology of glutinous rice flour was severely damaged, it was more difficult to identify the protein body.

3.7 XRD patterns and FTIR spectra

The XRD can measure the repeating units of the long-range crystalline structure in starch granules. The XRD patterns are presented in Fig. 5A. F IV-24 h, F V-24 h, F VI-24 h, and the control sample displayed typical A-type XRD patterns with clear peaks at 2θ close to 15° , 17° , 18° , and 23° ^[40,47–48], suggesting that the crystalline structure of samples did not change after fermentation. Tu et al.^[45] and Zhang et al.^[46] also drew similar conclusions when they studied the effect of microbial fermentation on the crystal structure of grain starch. The RC results are demonstrated in Table 2. The RC values of F IV-24 h, F V-24 h, and F VI-24 h were remarkably higher than that of the control sample ($(21.32 \pm 0.19)\%$), at $(27.24 \pm 0.39)\%$, $(30.81 \pm 0.57)\%$, and $(32.83 \pm 0.12)\%$, respectively. These results

Table 2
Effects of LAB fermentation on the structures of starch and protein in glutinous rice flour.

Samples	RC (%)	$R_{1\,047/1\,022}$	$R_{995/1\,022}$	Relative content (%)			
				α -Helix	β -Sheet	β -Turn	Random coil
F control	21.32 ± 0.19^d	0.679 ± 0.008^c	1.015 ± 0.006^a	19.16 ± 0.00^a	32.31 ± 0.03^b	27.98 ± 0.00^b	20.55 ± 0.00^a
F IV-24 h	27.24 ± 0.39^c	0.747 ± 0.007^a	0.953 ± 0.009^b	14.50 ± 0.76^d	43.26 ± 0.16^c	24.34 ± 0.08^c	17.90 ± 0.54^b
F V-24 h	30.81 ± 0.57^b	0.730 ± 0.009^{ab}	0.928 ± 0.002^c	15.61 ± 0.39^c	41.62 ± 0.88^b	27.89 ± 1.93^b	14.88 ± 1.34^c
F VI-24 h	32.83 ± 0.12^a	0.729 ± 0.006^b	0.914 ± 0.007^d	17.18 ± 0.43^b	27.36 ± 0.48^c	38.17 ± 0.29^a	17.29 ± 0.30^b

Notes: Data represent the mean of three measurements \pm standard deviation, different letters in each column indicate significant differences ($P < 0.05$).

indicated that the starch in the fermented glutinous rice flour had a relatively higher-ordered crystal structure, which was in agreement with the previous results of thermal properties in Section 3.5. We speculated that LAB fermentation was insufficient to destroy the internal crystalline structure of starch and that the effect of LAB fermentation on starch primarily occurred in the amorphous region, thus leading to an increase in RC after fermentation. Similar result was found in the study of Lu et al.^[49], who suggested that the increase of starch crystallinity in fermented rice flour was due to the hydrolysis of the amorphous regions of starch granules. Furthermore, the reduction of amorphous regions in acid-modified wheat and corn starch has also been reported in previous study by Majzoobi et al.^[50] and Chen et al.^[51].

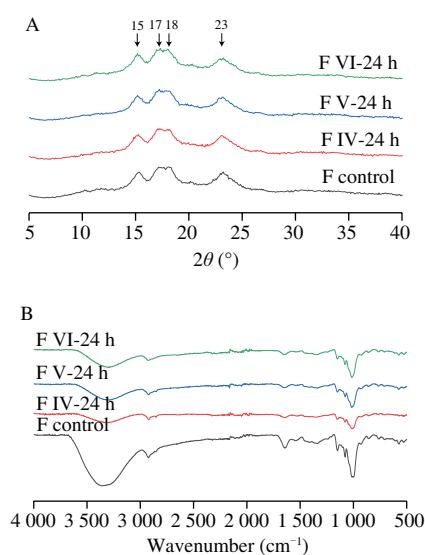


Fig. 5 Effects of LAB fermentation on (A) XRD patterns and (B) FTIR spectra.

The FTIR can provide insights into the molecular structure of starch granules on the scale of short-range order, while it can also be used to characterize the secondary structure of protein^[52]. The FTIR spectra are presented in Fig. 5B. All samples had similar peak positions at the region of 4 000 to 500 cm^{-1} , and no new absorption peaks appeared, only the absorbance intensity of each peak varied, indicating that LAB fermentation did not cause any new chemical group formation or molecular dissociation. The absorption peaks at 995 and 1 047 cm^{-1} are linked to an ordered and crystalline structure. The absorption peak at 1 022 cm^{-1} is related to the amorphous structure of starch^[53–54]. $R_{1\,047/1\,022}$ and $R_{995/1\,022}$ are frequently used to quantify the short-range ordered degree and double helix degree of starch granules^[55–56], which can be obtained from the deconvoluted spectra of starch that range from 1 200 to 800 cm^{-1} ^[57]. As shown in Table 2, the short-range ordered degree of F IV-24 h, F V-24 h, and F VI-24 h was generally higher than that of the control sample, whereas their double helix degree trended to decrease. Tu et al.^[45] reported a similar result, the $R_{1\,047/1\,022}$ of starch fermented by 7% starter significantly increased, which corresponded to the increase in the short-range ordered degree. In addition, a previous study mentioned that the $R_{995/1\,022}$ of fermented wheat starch decreased^[56], similar to the research of the double helix in the current experiment. These structural changes might be largely attributed to the degradation of the amorphous structure of starch and the reassembly of amylopectin.

The amide I band (1 600–1 700 cm^{-1}) is used to characterize the secondary structure of protein. The β -sheets, random coils, α -helices, and β -turns correspond to the 1 610–1 640, 1 640–1 650, 1 650–1 660, and 1 660–1 670 cm^{-1} peaks, respectively^[58]. The content of these four secondary structures is summarized in Table 2. The β -sheets are considered the predominant secondary structure in grain protein^[59]. From Table 2, the relative content of β -sheets in the control sample was indeed the highest, followed by β -turns, random coils, and α -helices. LAB fermentation greatly influenced the secondary structure of protein in glutinous rice flour. F IV-24 h and F V-24 h had common features: the content of α -helices, β -turns, and random coils decreased, while the content of β -sheets increased significantly. However, the secondary structure of protein in F VI-24 h exhibited particular changes: the content of α -helices, β -sheets, and random coils decreased, while the content of β -turns increased greatly. The changes in disordered structures (β -turns, random coils) and ordered structures (α -helices, β -sheets) have a certain influence on the viscoelasticity of dough^[60], which might also be one of the reasons for the difference in dough viscoelasticity in Section 3.8.

3.8 Fw content of frozen glutinous rice dough

Water in dough can be divided into freezable and unfreezable water. The Fw content reflects the number of ice crystals during frozen storage, which is closely related to the microstructure and quality of cereal foods^[61]. The Fw content was determined by DSC, and the results are presented in Fig. 6. The Fw content in all frozen dough exhibited a gradually increasing trend with the extension of frozen storage time, similar behavior appeared in the study conducted by Li et al.^[31]. This increase could be ascribed to the migration and redistribution of water molecules that occurred inside the glutinous rice dough during the long-term frozen storage, accelerating the formation and enlargement of ice crystals and thus increasing the proportion of Fw^[30]. Meanwhile, the enlargement of ice crystals damaged the network structure of dough, possibly weakening the binding of water and macromolecules (such as starch and protein).

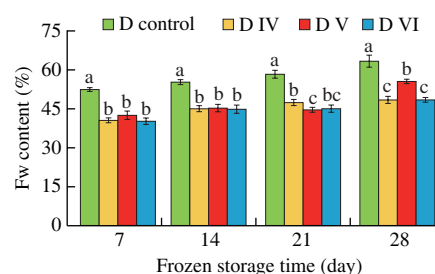


Fig. 6 Effects of LAB fermentation on Fw content of frozen glutinous rice dough. Data represent the mean of three measurements \pm standard deviation, different letters in same day indicate significant differences ($P < 0.05$).

In each frozen storage period, the content of Fw in D IV, D V, and D VI was lower than that of D control, indicating that LAB fermentation delayed the generation of Fw and had a positive impact on the dough, which might be related to the increase of the WAC in glutinous rice flour (Section 3.4). A comparison of the Fw content of D IV, D V, and D VI revealed that the Fw content of D VI was the lowest, implying that compound LAB starter cultures delayed the separation of bound water in frozen dough and that the effect of

increasing the water-holding capacity and stability of the dough was better than that of a single strain.

3.9 Rheological properties of fresh and frozen glutinous rice dough

The viscoelasticity of rice-based foods is usually assessed by the determination of rheological properties. The variations of G' , G'' , and $\tan\delta$ of the glutinous rice dough with the angular frequency at 25 °C are depicted in Fig. 7. G' was always greater than G'' for all samples, and $\tan\delta$ was also less than 1, indicating that samples exhibited solid-like behavior and possessed a typical gel network structure. In addition, the lower the value of $\tan\delta$, the higher the rigidity of starch gel. As shown in Figs. 7A–C, D VI had the highest G' and G'' and the lowest $\tan\delta$, while the G' , G'' , and $\tan\delta$ of D IV and D V were lower than those of D control, suggesting that D VI had the best viscoelasticity and that its total structure became the strongest. The

above results clearly indicated that the positive effect of compound strain fermentation on viscoelasticity surpassed that of single strain fermentation.

A subsequent study was conducted to investigate the effect of frozen storage on the viscoelasticity of D control and D VI. With the prolongation of the frozen storage time, both D control and D VI exhibited a trend of decreasing and then increasing viscoelasticity, and the viscoelasticity of D VI was always better than that of D control for each frozen storage time (Figs. 7D–I). This result was quite interesting, as it was obviously different from that of Li et al.^[31], who found that the viscoelasticity of the control dough and dough to which short-clustered maltodextrin, trehalose, and guar gum were added decreased consistently with the extension of storage time. However, in the study of Wang et al.^[57], the G' and G'' of starch gel displayed a gradually increasing trend, while $\tan\delta$ exhibited a gradually decreasing trend after the freeze-thaw cycle, indicating that repeated freeze-thaw enhanced the viscoelasticity and rigidity of starch gels.

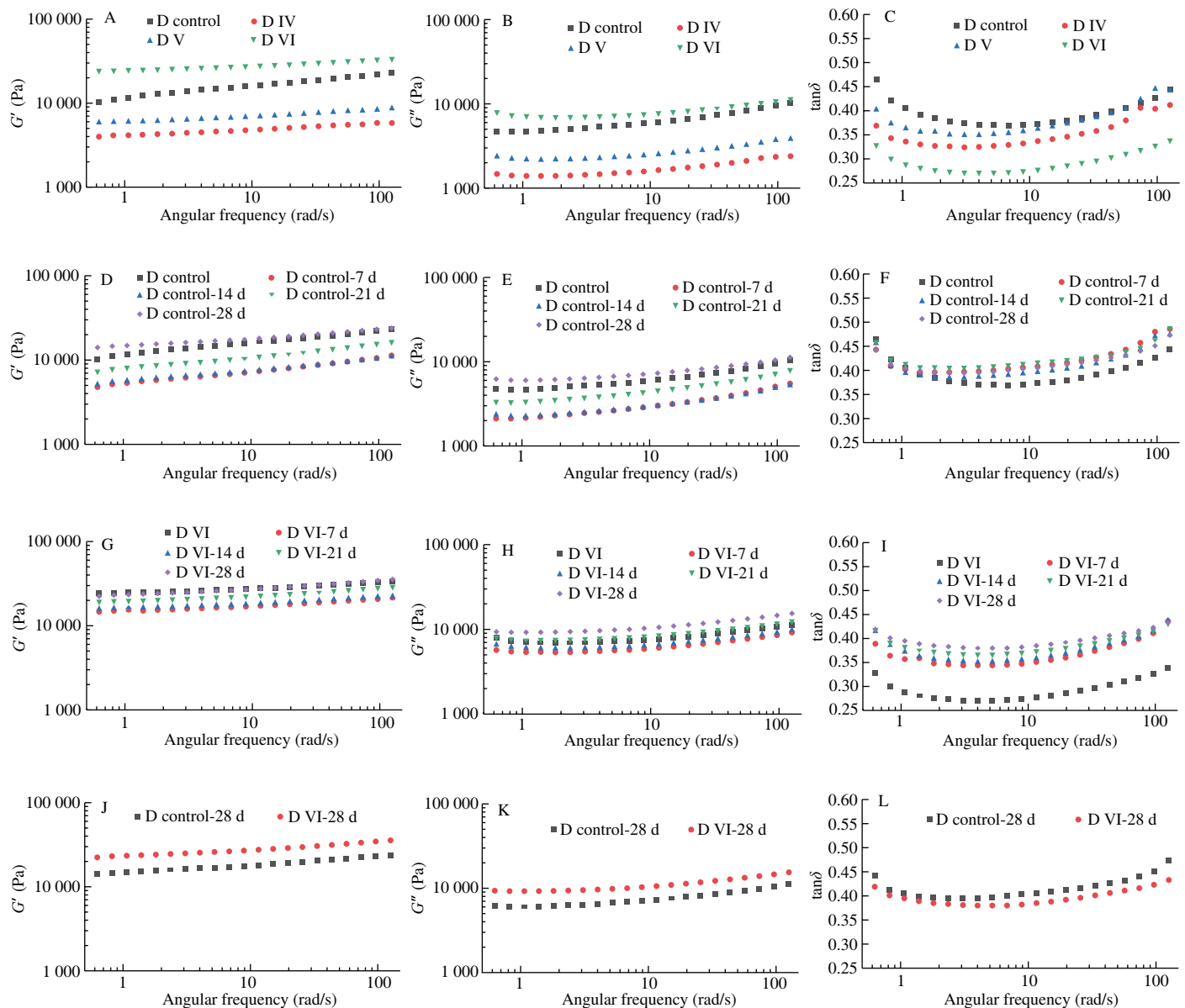


Fig. 7 Effects of LAB fermentation on G' , G'' and $\tan\delta$ of fresh and frozen glutinous rice dough.

This outcome was attributed to the reordering and rearrangement of starch molecules, which was related to the effect of ice crystals on the morphological features, semi-crystalline lamella structure, crystalline structure, short-range ordered structure, and helical structures of starch granules. The results of previous studies were worthy of reference, but there are many other factors to consider in this study, as fermented glutinous rice flour is a mixed system, besides the main ingredient, starch, there are other components, such as protein. The content of free thiol groups and secondary structure changes in protein also affects the dough viscoelasticity^[60,62]. Therefore, it is necessary to conduct detailed and in-depth research on the changes of starch and protein structure at each frozen storage stage, which will be the focus of our later research. Finally, Figs. 7J–L reveals that D VI-28 d presented stronger viscoelasticity and rigidity compared with D control-28 d after freezing and storage for 28 days. Thus, the use of compound LAB is a feasible and ideal method of improving the viscoelasticity of glutinous rice dough.

4. Conclusion

This study investigated the effects of 5 LAB fermentation on the pasting properties of glutinous rice flour. The suitable LAB strains for the fermentation of glutinous rice flour was screened by comparing the viscosity, BDV, and SBV. Among the 5 LAB strains, *L. sakei* HSD004 and *L. rhamnosus* HSD005 had obvious advantages in increasing the viscosity and reducing the SBV and BDV of glutinous rice flour. The LAB compound starter cultures prepared by the above two strains in a ratio of 3:1 improved the pasting properties and increased the WAC, OAC, and ΔH of glutinous rice flour. The viscoelasticity and stability of the dough (D VI) prepared with the abovementioned compound starter cultures were obviously better than those of other samples and the control sample. After 28 days of frozen storage, D VI-28 d had the lowest Fw content and the best viscoelasticity and stability. This study offered some guidance for improving the quality of glutinous rice flour and glutinous rice dough and provided a theoretical basis for the application of glutinous rice.

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.

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