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Effect of enzymatic fermentation on the rheological properties of high-fiber incorporated bread dough

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Abstract: Wheat bran and barley bran were supplemented to produce high-fiber nutritional bread. The technology of sourdough was applied to improve the rheological properties, baking properties of high-fiber bread dough and reduce the phytic acid content of high-fiber breads. Here, we used enzymes to investigate the effects of fermented bran on the rheological- and rheo-fermentation properties of bread dough. It was observed that the rheological properties of bread dough were improved by adding proper amount of bran sourdough with proper fermentation time. CO2 production and gas retention capacity increased. At the same fermentation time, G′ and G″ declined with the increasing addition of bran sourdough, while Hm, Tx and R decreased to a certain extent. G′ and G″ of dough adding 10% barley bran sourdough fermented for 8h (10%-8 h) or 20% wheat bran sourdough fermented 8 h or 16 h were higher than that were unfermented. Hm, Tx and R values increased to maximum which were most close to the control by adding 20%-8 h barley bran sourdough or 20%-16 h wheat bran sourdough. In conclusion, the fermentation qualities were improved and further research to develop bread and check this impact on the characteristics of developed bread should be done.

Keywords: Lactobacillus plantarum; fermented bran; phytic acid; rheological properties

1. Introduction

Dietary fiber (DF) is a type of active ingredient and functional food ingredient, which is hailed as the “seventh nutrient” by scientists (Tian et al. 2022). As early as 1953, Hipsley first proposed the term "dietary fiber" to refer to the components of plant cell walls that cannot be digested. Nineteen years later, Trowell and his colleagues (Phillips 2011) adopted this term and defined it when measuring various nutrients in food. It was not until 2008 that an internationally standardized regulatory definition of “dietary fiber” was adopted at the 30th Session of the Codex Alimentarius Commission held in South Africa. Finally, in 2009, it was defined as a carbohydrate polymer with ten or more monomer units that cannot be hydrolyzed by endogenous enzymes in the human small intestine and belongs to the following categories: 1. Naturally edible carbohydrates in consumed foods compound polymers; 2. Food raw materials processed by physiological, enzymatic or chemical methods, carbohydrate polymers that are generally certified by experts to have positive physiological effects on health; 3. Carbohydrate polymers that are generally certified by experts to have positive physiological effects on health Synthetic carbohydrate polymers (Cummings et al. 2009).

Dietary fiber is divided into soluble fiber and insoluble fiber based on solubility in water. The former includes pectin, gum and viscose, and the latter includes cellulose and certain hemicelluloses. In addition, there are also lignin and resistant starch, among others (Ibrahim et al. 2024a). Although dietary fiber cannot be digested and absorbed by the human body, it has important physiological effects, especially in maintaining gastrointestinal health. Dietary fiber promotes intestinal peristalsis in the large intestine and has high water absorption properties, which can relieve constipation and prevent constipation and hemorrhoids. The intake of dietary fiber can increase the feeling of fullness, reduce sugar intake, and inhibit the rapid rise of blood sugar (Ibrahim et al. 2024b). It is high in fiber, improves symptoms of diabetes, and at the same time promotes fat consumption in the body, which is beneficial to weight loss; in addition, certain components in dietary fiber can bind

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cholesterol and cholic acid to prevent gallstones, lower blood lipids, and prevent coronary heart disease (Ibrahim and Wajahat 2024).

Dietary fiber has attracted widespread attention from researchers and consumers for its many physiological effects. Increasing the intake of dietary fiber in people's daily diet can reduce the risk of cardiovascular and cerebrovascular diseases and rectal cancer. These diseases have become urgent needs in every country (Sidhu et al. 1999). Bread has a long history, and the countries that use it as a staple food are concentrated in Europe, the United States, the Middle East and Australia. In our country, people's demand for bread is increasing day by day. Bread has gradually become people's staple food and daily necessities. Therefore, it is necessary to strengthen the content of dietary fiber in bread. The content is of great significance to people's daily intake of dietary fiber.

Cereal fiber is an important source of dietary fiber. Cereal bran is a very low-cost raw material with high dietary fiber content. Not only that, but wheat bran also enhances the flavor of the product and is rich in B vitamins and minerals; barley bran has higher total dietary fiber and β-glucan content and has functional properties such as reducing post-meal glycemic index and blood lipids. However, the addition of wheat bran and barley bran not only has a negative impact on the baking properties of bread dough, but also increases the content of the anti-nutritional factor phytic acid in bread. On the one hand, it results in poor bread processing properties and severely affects the sensory quality. On the other hand, the increase in phytic acid content reduces the utilization of minerals in the human body, which can cause nutritional deficiencies when mineral intake levels are at critical points.

Sourdough fermentation is a process in which grains and water produce many metabolites under the action of active microorganisms. By selecting appropriate bacterial strains, fermentation substrates and fermentation conditions, their application in the bread making process can improve the baking characteristics and nutritional properties of the dough. The combined application of sourdough technology and enzyme preparations has profound implications for the biotechnology of modern bakery products, especially on bread preservation properties such as aging and shelf life.

This paper adds dietary fiber-rich wheat bran and barley bran to bread to make high-fiber nutritious bread, applies sourdough technology to ferment the bran to improve bread nutrition, rheology, and baking characteristics, and uses sourdough technology to combine starch Enzyme or xylanase combination was used to explore the aging characteristics of high-fiber bread. This provides a certain basic theoretical basis for the development of high-fiber bread with high nutritional value, good quality characteristics and anti-aging properties.

2. Materials And Methods

2.1 Experimental materials and equipment

2.1.1 Experimental materials and agents

High-gluten flour: Pengtai (COFCO Noodles) Co., Ltd.; barley bran: Dafeng Dade Wheat Kernel Factory; wheat bran: Xuzhou Yuanda Grain Co., Ltd.; Lactobacillus plantarum Biogreen300: Danisco (China) Co., Ltd. ; α-amylase, xylanase: Novozymes (China) Investment Co., Ltd.; instant active dry yeast: Panyu Meishan-Marley Yeast Co., Ltd.; shortening: Jiangmen Baixian Food Co., Ltd.; white sugar, Salt: commercially available food grade; other reagents are of analytical grade or above.

2.2 Experimental methods

2.2.1 Determination of components of high-gluten flour, barley bran, and wheat bran

The contents of moisture, protein, ash and total dietary fiber in high-gluten flour, barley bran, and wheat bran were determined according to AACC methods (2000) 44-15A, 46-12, 08-01, and 32-07 respectively.

2.2.2 Preparation of *Lactobacillus plantarum* fermented bran sourdough

In the MRS culture medium, *Lactobacillus plantarum* was activated twice and cultured to the late logarithmic phase. Take 20 mL of bacterial liquid (bacterial concentration >108 CFU/mL), centrifuge at 4500 r/min for 15 min, and rinse with sterile saline. After two times, add 300 g of water, mix evenly with 200 g of bran, and put it into a 28°C constant temperature and humidity incubator for culture (Wang et al. 2021).

2.3 Determination of total bacterial colonies in bran sourdough

Ten grams (10 g) of fermented bran was weighed and 90 g of sterile physiological saline was added for crushing, Afterwards, 0.2 mL of each solution was poured into a sterile petri dish along with MRS agar culture medium mixed immediately. After the plates were solidified, inverted it and incubated at 28 °C in incubator for 48 h before counting. Each gradient was repeated three times and the average value was taken.

2.4 Preparation of bread dough and bread

The recipe for making bread dough was high-gluten flour, salt (1.5%, based on the quality of high-gluten flour), yeast (1.5%), white sugar (6%), shortening (4%), water (60%) and fermented bran skin. Bread dough without bran sourdough was used as the blank group. Barley and wheat bran sourdough were added at 10%, 20% and 30% of the high-gluten flour mass, respectively. All ingredients were stirred except bran sourdough until gluten was completely formed. Afterwards, bran sourdough was added, stirred slowly for 5 minutes. Later, it was taken out and covered with film and rested at room temperature for 10 minutes. Divide the relaxed dough into 90 g/piece, rolled it into a ball and left for proofing in a proofing box (38°C, relative humidity 85%) for 1.5 hours, baked for 22 minutes, and set the upper/lower heat temperature to 170/210° C.

2.5 Determination of pH value and total titrated acidity (TTA) of bran sourdough and bread dough

According to AACC method (2000) 02-52, 10 g of the sample was weighed, 90 mL of CO2-free distilled water was added, and let it stand for 10 min before measuring the pH. Then it was titrated with 0.1 mol/L NaOH solution to the titration endpoint pH=8.6. The number of milliliters of 0.1 mol/L NaOH solution consumed was the total acidity.

2.6 Analysis of dynamic rheological properties of bread dough

2.5 g of dough (without yeast) was sampled, and dynamic rheology experiment was conducted using a dynamic rheometer. The experimental temperature was 25 °C, a flat plate with a diameter of 40 mm, a scanning frequency of 0.1~40 Hz, and a stress of 0.5% was used.

2.7 Analysis of rheological and fermentation characteristics of bread dough

The F3 rheological fermentation instrument was used to measure the rheological fermentation characteristics of bread dough. 150 g of bread dough was taken and put into the fermentation basket. Test conditions: experimental temperature 38 °C, dough weight 150 g, weight 2 kg, test time 3 h. Each sample was repeated at least twice, and the average was taken.

3. Results and discussion

3.1 Basic ingredients of high-gluten flour, barley bran and wheat bran

Table 1: Compositions of the wheat flour, barley bran and wheat bran

Sample	Moisture (%)	Protein (%)	Ash content (%)	Total dietary fiber (%)
High-gluten flour	14.28	13.14	0.43	2.36
barley bran	9.89	12.62	4.4	31.3
wheat bran	14.43	11.61	4.1	30.4

As shown in Table 1, the basic components of barley bran and wheat bran are measured after passing through a 40-mesh sieve. The ash content and total dietary fiber content of barley bran and wheat bran are much greater than those of high-gluten flour, and the protein content Slightly lower than high-gluten flour, the moisture content of wheat bran is similar to that of high-gluten flour, and the water content of barley bran is lower.

3.2 Growth and acid production of Lactobacillus plantarum in bran sourdough

3.2.1 Changes in the number of viable bacteria during sourdough fermentation

The number of viable bacteria during the fermentation process of *Lactobacillus plantarum* in high-gluten flour, barley bran and wheat bran matrices is shown in Figure 1. During the fermentation time of 0 to 24 h, no matter in the high-gluten flour matrix or in the gluten In the skin matrix, the number of viable lactic acid bacteria increased with the increase in fermentation time. This shows that Lactobacillus plantarum can grow well in high-gluten flour and bran matrix.

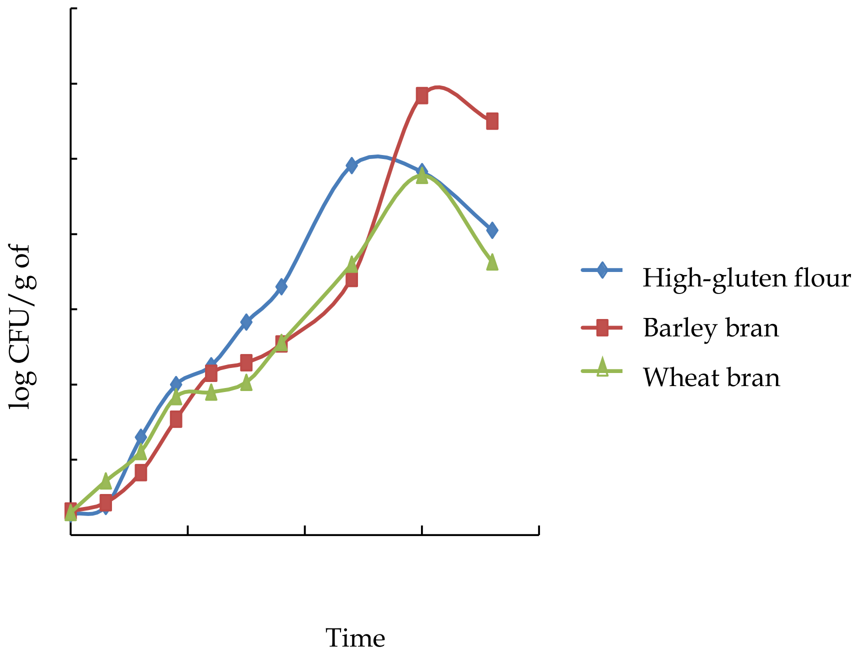

Figure 1: Viable count of Lactobacillus plantarum in wheat flour and bran substrates

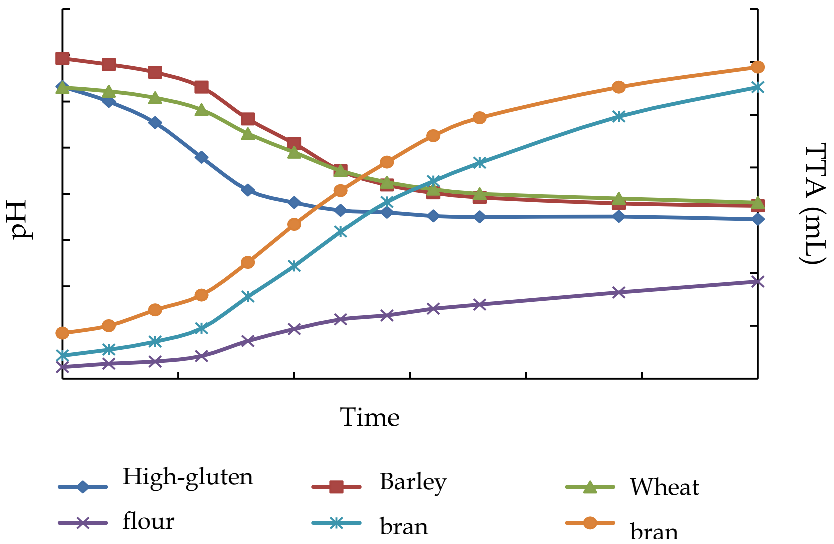

Figure 2: pH and TTA values of fermented wheat flour and bran substrates

pH reflects the concentration of H+, that is, the concentration of dissociated acid, in the sample. Its size is related to the nature and amount of acid in the sample as well as the amount and buffering capacity of the buffer in the sample. Total acidity refers to the total amount of all acidic components in food, including free acids that dissociate into H+, as well as undissociated bound states and acid salts. The total acidity in a sample obtained through standard alkali titration is also called titratable Acidity (TTA).

Figure 2 shows the changes in pH and TTA of high-gluten flour, wheat bran and barley bran during lactic acid bacteria fermentation. The pH value is closely related to the growth of lactic acid bacteria in the matrix, reflecting the different growth stages of lactic acid bacteria in the matrix. It can be seen from the figure that the initial pH of high-gluten flour and barley bran matrix is lower than that of smaller wheat bran. This is related to the different chemical compositions in different matrices. The pH of high-gluten flour drops the fastest during the fermentation process, and the lag period is extremely short. It immediately enters the logarithmic phase (2~10 h). After 10 h, the pH value is basically stable; while the lag period for the growth of lactic acid bacteria in the bran matrix is relatively It is longer and begins to enter the logarithmic phase (4~16 h) around 4 h of fermentation, and the pH tends to be stable after 16 h. Whether high-gluten flour or bran is used as the matrix, lactic acid bacteria can grow well, and the growth rate in high-gluten flour is greater than that in bran.
Throughout the fermentation process, the TTA value of the bran group was always greater than that of high-gluten flour, especially the barley bran group, which is related to the ash content in high-gluten flour, barley bran and wheat bran. Ash not only affects the acid production of lactic acid bacteria, but also has a certain buffering capacity for acid (Kim et al. 2009). The three ash contents from low to high are: high-gluten flour, wheat bran, and barley bran (Table 1).

3.3 Effect of bran sourdough on pH and TTA of bread dough

The pH and TTA values of bread dough added with unfermented (fermentation time 0 h) barley or wheat bran sourdough increased with the amount added and were both higher than the blank group (pH: 5.51; TTA: 5.25). The increase in pH may be due to the increase in bran content in the dough, which reduces free hydrogen ions; Kim and colleagues (2009) found that the TTA value of whole wheat flour is much larger than that of ordinary wheat flour, and the high ash content of whole wheat flour has good buffering capacity for acids. As shown in Table 3-2 and Table 3-3, as the fermentation time of wheat gluten sourdough increases and the addition amount increases, the pH of the bread dough decreases significantly, and the total acidity TTA value gradually increases. This is closely related to the growth of lactic acid bacteria in the bran matrix. In the early stage of fermentation (0~8 h), the lactic acid bacteria enter the early logarithmic stage after a short stagnation period, the total number of viable bacteria continues to increase (as shown in Figure 3-1), and the amount of acid production also continues to accumulate. When the addition amount is 20% and 30%, The pH of barley or wheat bran sourdough bread dough fermented for 8 h was significantly lower than that of the corresponding unfermented groups (fermentation time was 0 h).

Table 2: Effect of fermentation time and amount of bran sourdough on the pH of bread dough

Fermentation Time	Barley bran sourdough addition amount (%)			Wheat bran sourdough addition amount (%)		
	10	20	30	10	20	30
0	5.57^{a}	5.80^{b}	5.82^{b}	5.73^{a}	5.86^{b}	6.02^{c}
8	5.50^{a}	5.31^{c}	5.05^{d}	5.70^{a}	5.37^{d}	5.28^{e}
16	5.19^{e}	4.86^{f}	4.63^{g}	5.42^{d}	4.87^{f}	4.69^{g}
24	5.14^{e}	4.76^{h}	4.53^{i}	5.10^{h}	4.71^{g}	4.48^{i}

In the middle stage of fermentation (8~16 h), the growth of lactic acid bacteria is in the logarithmic growth phase. At this time, the bacterial activity is high, and the growth rate is fast. The total number of viable bacteria continues to increase and reaches a high level. Metabolic acid production increases and accumulates in large quantities. The pH Significantly reduced, TTA value increased significantly. When the sourdough fermentation time was 16 h, the TTA values of bread dough with 20% and 30% barley bran sourdough increased by 9.7% and 37.5% respectively, while the wheat bran group increased by 42.9% and 40.7% respectively; fermentation at 24 h, the growth of lactic acid bacteria enters a stable phase. At this time, the total amount of cell metabolites was still increasing, and the acid accumulation was the largest. Therefore, the bread dough with 30% bran sourdough had the lowest pH and the highest TTA value.

Table 3: Effect of fermentation time and amount of bran sourdough on the TTA of bread dough

Fermentation Time	Barley bran sourdough addition amount (%)			Wheat bran sourdough addition amount (%)		
	10	20	30	10	20	30
0	5.90[a]	6.50[b]	6.80[c]	5.30[a]	5.50[bc]	5.90[d]
8	6.34[d]	6.65[e]	7.65[f]	5.40[ab]	6.74[e]	7.30[f]
16	6.60[e]	7.20[g]	9.35[h]	5.55[c]	7.86[g]	8.30[h]
24	6.76[c]	7.65[f]	9.40[h]	6.00[d]	9.30[i]	9.40[i]

3.4. Effect of bran sourdough on rheological properties of bread dough

3.4.1 Effects of bran sourdough fermentation time and addition amount on the dynamic rheological properties of bread dough

As shown in Figure 3 and Figure 4, at the same fermentation time, as the amount of barley bran sourdough addition increased, both dough G′ and G″ showed a downward trend, in which 10% barley bran was added. Sourdough bread dough has the highest G′ and G″. This may be because the gluten network structure in the dough is destroyed by dietary fiber and other ingredients contained in barley bran, resulting in a reduction in the elastic modulus and viscous modulus of the dough (Ning 2019). In the sourdough group with the addition of 10% barley, after 8 h of fermentation, the elastic modulus of the dough increased and the elasticity was improved; after 16 h of fermentation, both G′ and G″ of the dough significantly decreased, and the viscoelasticity decreased significantly. The addition amount was 20 %, as the fermentation time increases, the bread dough G′ first increases and then decreases, and the barley bran sourdough was fermented for 8 h.

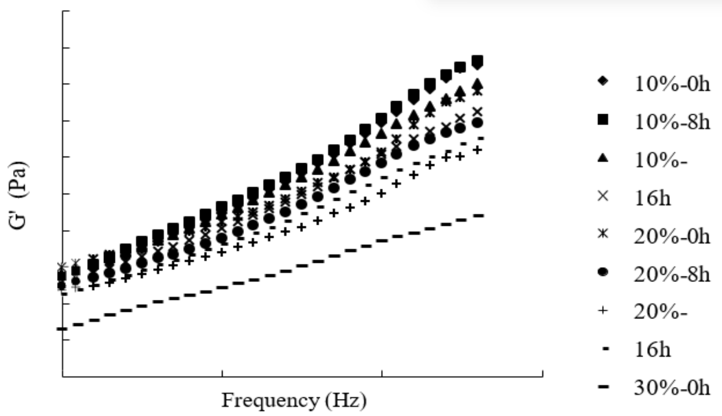

Figure 3. Effects of barley bran sourdough on G′ of bread dough

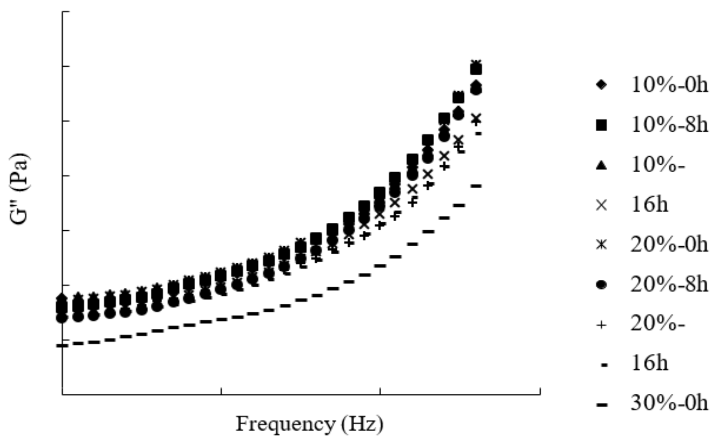

Figure 4. Effects of barley bran sourdough on G″ of bread dough

When the bread dough G′ is larger than the unfermented dough (fermentation time is 0 h); G″ is also larger when fermented for 8 h, even close to the bread dough with 10% bran sourdough added. When the addition amount is 30%, The elastic modulus of dough fermented for 8 h is smaller than that of unfermented dough, while G″ is greater than G′ in dough fermented for 16 h, and the viscous properties exceed the elastic properties. It is known from this that the 20%-8 h barley bran sourdough dough has good viscoelastic properties, indicating that at an appropriate amount of addition, sourdough fermentation can improve the viscoelasticity of the dough and improve the damage of fiber to the viscoelasticity of the dough.

As shown in Figure 5 and Figure 6, at the same fermentation time, as the addition of wheat bran sourdough increases, both dough G′ and G″ show a downward trend, among which the dough with 10% sourdough added G′ and G″ are the highest. This is also because the dietary fiber and other components in wheat bran destroy the bread dough's gluten network structure and reduce the dough's viscoelastic properties (Li et al. 2023). When the addition amount of wheat bran sourdough is 10%, as the fermentation time increases, the elastic modulus and viscous modulus of the dough show an overall downward trend, and the differences between different fermentation times are obvious; when the addition amount is 20%, the fermentation time The G′ and G″ of the bread doughs fermented for 8 h and 16 h were larger than those of the unfermented group (fermentation time 0 h); when the addition amount was 30%, the viscoelasticity of the dough fermented for 0 h was the best, and the dough fermented for 8 h and 16 h was the best.

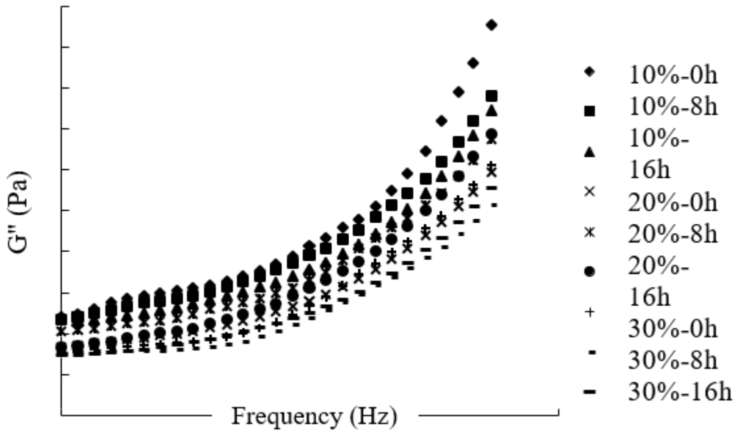

Figure 5. Effects of wheat bran sourdough on G′ of bread dough

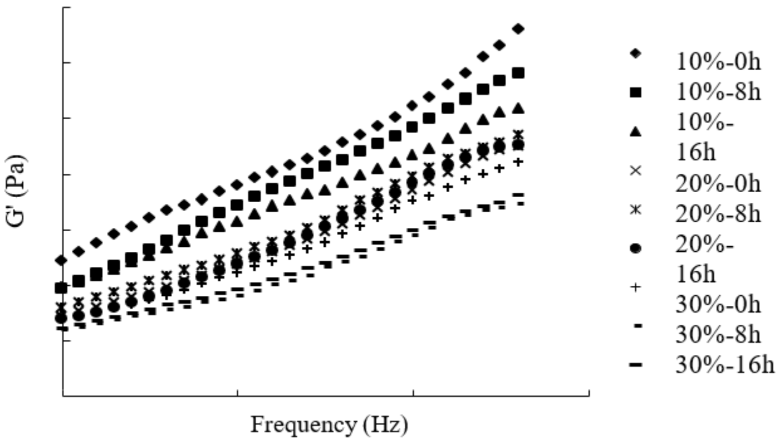

Figure 6. Effects of wheat bran sourdough on G″ of bread dough

The dough G′ and G″ of h were significantly lower than those of the unfermented group. This shows that adding an appropriate amount (20%) of sourdough starter (fermentation for 8 h or 16 h) is beneficial to increasing the viscoelasticity of the dough, while adding too much (30%) or too little (10%) will affect the viscoelasticity of the dough.

4. Conclusion:

The ash content and total dietary fiber content in barley bran and wheat bran are much greater than those in high-gluten flour. The ash content affects the changes in pH and TTA during lactic acid bacteria fermentation. Changes in pH reflect the growth of lactic acid bacteria in the matrix. *Lactobacillus plantarum* can grow well in both high-gluten flour and bran substrates. The pH of the high-gluten flour matrix drops the fastest during the fermentation process. The lactic acid bacteria immediately enter the logarithmic phase (2~10 h) after a very short lag period. After h, the pH value was basically stable; while the lag period for the growth of lactic acid bacteria in the bran matrix is longer, and it begins to enter the logarithmic phase (4~16 h) around 4 h after fermentation, and the pH tends to be stable after 16 h. During the fermentation process, the TTA of the bran matrix was always greater than that of high-gluten flour, especially barley bran. Adding bran sourdough to bread dough also significantly impacted the bread dough's pH and TTA. It could be inferred that the combination of lactic acid bacteria fermentation and amylase or xylanase has a more significant effect, which can effectively improve the baking quality of bread and delay the aging of bread.

Author's contribution

Xiaorong Wei conceptualized and wrote the manuscript and other authors drafted and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Competing Interest

The authors declared no conflict of interest.

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