Original paper

Effects of Xylanase on Quality of Frozen Dough Steamed Bread

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Effects of xylanase on the quality of frozen dough and steamed bread stored at a freezing temperature for 15, 30, and 45 d were investigated. Results showed that prolonging the frozen storage time can decrease the leavening power and yeast survival rate of frozen dough as well as the specific volume and anti-staling activity of frozen dough steamed bread. By contrast, these properties can be improved significantly through the addition of xylanase. The frozen dough supplemented with 80 mg/kg of xylanase had a low freezable water content (28.51 ± 0.37)%, which was 29.11% lower than that of the control (40.22 ± 0.43)%. The addition of xylanase can also significantly reduce the melting point and melting scope of ice crystals (P < 0.05), which can result in a homogeneous, small ice crystal structure in frozen dough and improve the quality of frozen dough steamed bread.

Keywords: xylanase, frozen dough, steamed bread quality, fermentation ability, freezable water content

Introduction

Developed in the 1950s, frozen dough has become a new trend in processing bread and other baked goods. Freezing dough can reduce costs, standardize product quality, and guarantee the freshness of bread; thus, this practice has been widely accepted by consumers. Other than its use in bread production, frozen dough can also be adopted to manufacture food that fits the Chinese diet, including steamed breads, stuffed buns, dumplings, and spring rolls. Steamed bread is a staple food in China, and their production using frozen dough has become a new trend widely accepted in the country.

However, frozen dough demonstrates water migration, structure relaxation, and recrystallization during quick freezing, frozen storage, and thawing (Xu et al. 2009; Gabric et al. 2011). These phenomena damage the gluten network structure of dough, thereby reducing its fermentability. The arabinoxylan (AX) content of wheat flour is extremely low (approximately 1.5% to 3%, dry weight basis), but it significantly affects the quality of wheat flour, the rheological characteristics of dough, and the quality of baking products. High-molecular weight water-extracted AX (WEAX) is beneficial to the characteristics of bread, whereas the unextracted AX and low-molecular weight WEAX are disadvantageous (Wang et al. 2004; Wang et al. 2002). Numerous studies have been conducted in China and other countries to analyze the effects of xylanase on wet gluten, frozen dough, and quality improvement of bread. The action mechanism of xylanase has also been investigated (Krishnarau and Hoseney 1994; Rouau and Moreau 1993; María Eugenia Steffolani et al. 2012; Cristina et al. 2005). However, the available information about the function of xylanase in steamed bread created using frozen dough is insufficient. In this study, we looked into the protective effects of xylanase on yeast cells and its beneficial effects on frozen dough steamed bread. A theoretical basis for the application of xylanase in freezing fermented flour products in Asia was presented.

Materials and Methods

**Materials** Fancy patent flour (important ingredients: 12.76% of water, 11.85% of protein, 29.59% of wet gluten, and 0.31% of ash) was purchased from Zhengzhou Jin Yuan Surface Industry Co., Ltd. (Zhengzhou, China). Fungal xylanase (VERON191, enzyme activity 1750XylH·g⁻¹, which was verified to ensure the absence of noticeable side activities) was obtained from AB
ENZYMES (Darmstadt, Germany). Highly active dry yeast was purchased from Angel Yeast Co., Ltd. (Yichang, China).

**Preparation of frozen dough and steamed bread** The frozen dough was prepared using a fast fermentation method with fermentation time ranging from 0 min to 45 min.

Flour (100 g), yeast (1 g), water (48 g), and xylanase (0, 2, 4, 6, 8, and 10 mg) were blended using a food mixer (BSA, Guangzhou Wellborn Hotel Equipment Co., Ltd., Guangzhou, China) at a low speed (85 rpm) for 5 min and, subsequently, at a high speed (200 rpm) for 3 min. After the blend was kneaded well and molded into round shapes, the dough was fermented at room temperature for 15 min, was enclosed in Saran Wrap after molding, and was placed inside a freezer (MDF-U460BR, SANYO Electric Co., Ltd., Osaka, Japan) at −35°C for 2 h until the center temperature decreased to −18°C. The dough was stored at −18°C for 15, 30, and 45 d. The dough was thawed and fermented in a fermenting room (SM-32S, Xinmai Machinery Co., Ltd., Wuxi, China) at 37 ± 1°C and under a relative humidity of 75% to 80% for 90 min. Using a steamer (CM1022, Changmei Stainless Steel Products Co., Ltd., Chaoanxian, China), the dough was steamed over boiling water for 20 min.

**Determination of yeast fermentation capacity** According to GB/T 20886-2007, 75 g of frozen dough was first thawed and was then immediately placed in a column-shaped glass container. This container was sealed and connected to a narrow-mouthed bottle filled with liquid through an exhaust pipe. The amount of drainage (V, mL) within 2 h was recorded using a self-made fermentation device (Fig.1).

\[ \text{Fermenting force (mL)} = V \] \hspace{1cm} \text{Eq. 1}

**Determination of yeast survival rate** According to GB/T 20886-2007, 20 g of thawed dough was scattered in 180 mL of sterile water, was magnetically stirred for 30 min, and was then left to stand for 15 min. The clear supernatant (0.5 mL) was stained with methylene blue for 10 min. The stained supernatant (0.1 mL) was then placed on a blood count sheet (XB-K-25, Kangjian Medical Plastic Factor, Jiangyan, China) and left to stand for 5 min, after which yeast cells were counted immediately under a microscope (XSP-C204, Chongqing Photoelectric Instrument Co., Ltd., Chongqin, China). The live yeast cells were colorless and transparent, whereas the dead yeast cells were stained blue. The calculation principles for yeast based on the grid line included the counting of yeast cells above and to the right of the gridline. The yeast cells below and to the right of the gridline were not counted.

\[ \text{Yeast survival rate} = \frac{\text{number of active cells}}{\text{total cell count}} \] \hspace{1cm} \text{Eq. 2}

**Determination of specific volume of steamed bread** According to GB/T 21118-2007 (China), after steaming, the circular steamed bread was left to stand at room temperature for 1 h. Millet displacement method was used to test volume using a bread volume measuring instrument (JMTY, Hangzhou Daji Plastic Factor, Jiangyan, China). Specific volume (mL/g) = displaced millet volume (mL)/steamed bread mass (g) \hspace{1cm} \text{Eq. 3}

**Determination of steamed bread aging index** After steaming, the steamed bread (150 g) wrapped with plastic to prevent moisture evaporation was left to stand at room temperature for 1 h. The bread was then cut into 15-mm thick slices with a knife. Each steamed bread was cut into four pieces, and the two middle pieces were used for texture profile analysis (TPA). The steamed bread that was cooled at room temperature for 1 h was subsequently wrapped with a preservative film to prevent moisture evaporation and was left to stand at room temperature for 24 h. TPA was performed according to the abovementioned standard. The test parameters are as follows: detector, P/32; pre-test speed, 2.00 mm/s; test speed, 1.00 mm/s; post-test speed, 1.00 mm/s; strain, 60.00%; and time, 10.00 s.

\[ \text{Hardening rate of steamed bread (g/h)} = \frac{\text{Hardness of 24 h} - \text{Hardness of 0 h}}{24} \] \hspace{1cm} \text{Eq. 4}

A low hardening rate of steamed bread leads to a high anti-staling effect (Zhao and Wang 2002).

**Determination of moisture content** Moisture content was determined according to GB 5009.3-2010 (China).

**Determination of freezable water** Freezable water content was estimated according to the methods proposed by Laaksonen and Roost (2000) and Lodi and Vodovotz (2008). The samples (15 mg) were obtained from a 45-d frozen dough centered, tabletted, sealed, and placed inside a differential scanning calorimetry (DSC) sample room. An empty crucible was used for comparison. The samples were equilibrated at −15°C for 5 min and were then transported from the freezer to the DSC instrument (DSC204F1, NETZSCH -
Effects of Xylanase on Quality of Frozen Dough Steamed Bread

Gerätebau GmbH, Selb, Germany). The scanning range was from −15°C to 5°C, and the heating rate was 1°C/min. The DSC curve was analyzed to obtain the ice crystal melting starting point ($T_s$) and peak point ($T_m$), the ice crystal melting temperature range ($T_f$−$T_i$), and the ice crystal melting enthalpy ($\Delta H_m$).

Content of freezable water ($F_w$) = \frac{\Delta H_m}{\Delta H_f} × 100 \quad \ldots \text{Eq. 6}

where $F_w$ is the content of freezable water (%), $\Delta H_m$ is the ice crystal melting enthalpy (J/g), $\Delta H_f$ is the water melting enthalpy (334 J/g), and $W$ is the water content of frozen dough (%).

**Determination of Xylan Content in Dough**

**Drawing of the xylene standard curve:** The standard solution of 100 μg·mL$^-1$ was prepared with distilled water and D-xylene. Consequently, 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the solution were separately placed into test tubes with a stopper; distilled water was added to a volume of 3 mL. Subsequently, 3 mL of 0.1% FeCl$_3$ acid solution (soluble in concentrated hydrochloric acid) and 0.3 mL of 1% orcinol–alcohol solution (soluble in anhydrous ethanol) were added to all tubes sequentially. The solutions were mixed using a vortex oscillator. The test tubes were placed in a boiling water bath for 40 min for color development. Afterwards, the tubes were taken out and subsequently cooled with flowing water rapidly. Distilled water was again added to a volume of 10 mL. The absorbance value of the water-soluble xylene at 670 nm was determined, and the absorbance value of the water-soluble xylan at 670 nm was calculated.

Formula: Content of total xylan or water-soluble xylan (%) = \left(\frac{c × 0.88 × 10}{(m(1 - w)) × n} × 100\right) \quad \ldots \text{Eq. 7}

where $c$ is the concentration of xylose obtained by the standard curve (μg·mL$^-1$), 0.88 is the ratio of xylan to xylose, $m$ is the weight of the sample (mg), $w$ is the moisture content in the sample (%), and $n$ is the dilution ratio ($n = 20$ in this experiment).

**Data processing**

Three independent trials ($n = 3$) with triplicate sample analyses were performed. The data are presented as mean±standard deviations. The statistical analysis was performed using SPSS 17.0 and Excel 2007, and ANOVA was conducted according to the standard procedures. $P < 0.05$ indicates a significant difference.

**Results and Discussion**

**Effect of xylanase on the fermentation capacity of frozen dough**

The maximum fermenting power reached 289 mL for the frozen dough supplemented with 60 mg/kg of xylanase and was stored in freezing temperature for 15 d. Such value was 27.31% higher than that of the blank, and the difference was significant ($P < 0.05$). The fermenting power failed to increase; instead, it decreased with increasing xylanase levels. The maximum fermenting power for the frozen dough supplemented with xylanase at 60 mg/kg and stored in freezing temperature for 30 d attained 253 mL. Such value was 19.34% higher than that of the blank, and the difference was significant ($P < 0.05$). For the frozen dough supplemented with xylanase at 60 mg/kg and stored in freezing temperature for 45 d, the maximum fermenting power reached 220 mL. This value was 17.65% higher than that of the blank, with a significant difference ($P < 0.05$) (Fig. 2). The effect of xylanase on the fermentation ability of frozen dough was significant. The fresh dough was flowing water rapidly. Distilled water was added to 10 mL. The absorbance value of the water-soluble xylose at 670 nm was measured, and its content was calculated.

Determination of total xylan content: 50 mg of the samples were weighed accurately and placed in a 50-mL centrifugal pipette; 10 mL of 1 mol·L$^-1$ hydrochloric acid solution was then added. The pipette was sealed and placed in a boiling water bath for 150 min. After cooling, 20 mL of distilled water was added into the pipette, and the mixture was shaken well. The mixture was subsequently centrifuged under 4000 r·min$^{-1}$ for 15 min. Afterwards, 1.0 mL of the mixture was transferred into another pipette. Sequentially, 2 mL of distilled water, 3 mL of 0.1% FeCl$_3$ acid solution, and 0.3 mL of 1% orcinol–alcohol solution were added. The solution was mixed using a vortex oscillator. The pipette was then placed in boiling water bath for color development. After 40 min, the pipette was taken out and cooled with flowing water rapidly. Distilled water up to 10 mL was added to the solution. The absorbance values at 670 and 580 nm (eliminating the disturbance of a small amount of hexose through the absorbance difference under double wavelengths) were determined, and the content of the water-soluble xylan was calculated.
determined influenced by xylanase as well. In particular, the effect of the added quantity of xylanase on the fermentation ability of fresh dough showed a similar trend to that of frozen dough ($P < 0.05$) (Fig. 2).

**Effect of xylanase on the survival rate of yeast**  
Adding xylanase to frozen dough can improve the yeast survival rate (Fig. 3). The yeast survival rate of dough frozen for 15 d ($P < 0.05$) was increased when it was added with xylanase ranging from 40 mg/kg to 100 mg/kg. The survival rate of yeast in the blank group was 97.01%, whereas the maximum survival rate reached 99.24% when xylanase at 60 mg/kg was added. This finding indicates that xylanase caused a 2.08% higher survival rate of frozen dough than that of the blank. With increasing xylanase levels, the survival rate of yeast did not increase but decreased instead. The yeast survival rate of dough frozen for 30 d ($P < 0.05$) was significantly affected by the added xylanase ranging from 40 mg/kg to 100 mg/kg. The survival rate of yeast in the blank group was 96.54%, whereas the maximum survival rate of the frozen dough reached 98.45% when xylanase was added at 60 mg/kg. This observation entails that the survival rate of the frozen dough was 1.13% higher than that of the blank. The survival rate of yeast did not increase with increasing xylanase levels; it decreased instead. Similarly, the yeast survival rate of dough frozen for 45 d ($P < 0.05$) was significantly affected by the added xylanase ranging from 40 mg/kg to 100 mg/kg. The survival rate of yeast in the blank group was 95.85%, whereas the maximum survival rate of the frozen dough reached 98.06% when xylanase was added at 80 mg/kg. This finding indicates that the survival rate of the frozen dough was 2.31% higher than that of the blank. The survival rate of yeast did not increase but gradually decreased with increasing xylanase levels.
The effects of xylanase on the fermentation capacity and survival rate of yeast were compared. We determined that the specific trends were not completely consistent although the general ones were consistent. This inconsistency was caused by the dependence of yeast fermenting power not only on the survival rate, but also on yeast fermentation activity. Weak fermentation activity results in low fermenting power even with high survival rate (Laaksonen and Roost2000). This condition indicates that xylanase affects the survival rate and fermentation activity of yeast. In the experiment, xylanase significantly affected the survival rate of yeast of frozen dough, whereas it insignificantly influenced that of the fresh dough.

Effect of xylanase on specific volume

Specific volume is an important index that reflects the quality of steamed bread, which is satisfactory at a volume ranging from 2.3 mL/g to 2.6 mL/g (GB/T21118-2007, China). In this study, the specific volume of the steamed bread made using frozen dough was lower than the satisfactory value (Fig.4).

Adding xylanase can improve the specific volume of steamed bread. When the steamed bread was made using a frozen dough stored at −18°C for 15 d, the specific volume of the blank group was 2.11 mL/g. The maximum specific volume of the steamed bread was 2.56 mL/g when 60 mg/kg of xylanase was added, and this value was 21.33% higher than that of the blank. When the steamed bread was made with frozen dough stored at −18°C for 30 d, the specific volume of the blank was 1.91 mL/g, and the maximum specific volume of the bread was 2.36 mL/g when 60 mg/kg of xylanase was added. This maximum value was 23.56% higher than that of the blank. When the steamed bread was made with frozen dough stored at −18°C for 45 d, its maximum specific volume was 2.21 mL/g with 80 mg/kg xylanase, and the specific volume of the blank group was 1.76 mL/g. The maximum value was 25.57% higher than that of the blank.

Xylanase significantly affected the specific volume of the steamed bread produced using a frozen dough. Similarly, the fresh dough was significantly affected by xylanase, whose effect on the fermentation ability of the dough showed a trend similar to that of frozen dough (P < 0.05) (Fig. 4). Adding the correct amount of xylanase to the dough can improve the product specific volume probably because the appropriate hydrolysis of xylan by xylanase can cause the moisture to move from xylan to gluten and can increase the moisture liquidity, making it a free water with weak binding force. This circumstance improves the fermentation ability of the dough. Selinheimo et al. (2006) also reported that xylanase can promote the transfer of moisture from xylan to gluten and that the change in the moisture distribution in dough can significantly affect its machining properties. Long-term freezing may destroy the gluten network, killing the yeast, or may cause the water in the dough to be uneven, thereby decreasing the specific volume of the steamed bread. This possibility needs further investigation.

Effect of xylanase on the staling characteristics of steamed bread

Steamed bread staling mainly refers to the loss of freshness during storage. During this process, the hardness of the steamed bread increases, its elasticity and strength decrease, whereas its nutrients are lost (Zhao and Wang 2002). Hardening rate and resilience change are common indicators of the properties of steamed bread staling. The addition of xylanase can strengthen the anti-staling effect of steamed bread. In this study, the anti-staling effect of xylanase on the steamed bread was at the maximum when 60 ~ 80 mg/kg of xylanase was added to the dough frozen for 15, 30, and 45 d (Fig.5). Steamed bread aging can be attributed to numerous reasons, such as water loss, starch recrystallization, glass transition, as well as gluten and starch interaction. Xylooligosaccharides, which are produced by xylanase, may be involved in the formation of gluten protein and may influence the interaction of gluten protein. This interaction influences water loss,
starch crystallization, glass transition, and other processes.

*Effect of xylanase on ice crystal melting characteristic and freezable water content of frozen dough* Freezable water comprises free water and a part of bound water. Water at different states presents varied crystallization features, and the volume of water gain is 9% during freezing. Abnormal expansion damages the 3D network structure of the proteins in yeast cells. Freezable water content can be observed in the area under the peak of the DSC curve (Bhattacharya *et al.* 2003; Lu and Grant 1999; Inoue and Bushuk 1991).

Table 1 shows that in the experiment, the melting temperature of ice varied between −3.5°C and −2°C. Compared with the blank
Effects of Xylanase on Quality of Frozen Dough Steamed Bread

Effects of Xylanase on Quality of Frozen Dough Steamed Bread

415

group, xylanase significantly affected the melting starting point and ice crystal melting range ($P < 0.05$). The low melting starting point resulted in small ice particles. The narrow ice crystal melting scope resulted in uniform ice particles and low amount of damage on gluten structure and yeast cells. The melting peak value of the test group ranged from 0°C to 1.5°C, which was significantly lower than that of the control group.

The freezable water content of the frozen dough decreased with increasing xylanase content (Fig. 6). The freezable water content of the test group was significantly lower than that of the control group. The lowest freezable water content was (28.51 ± 0.37)% when xylanase was added at 80 mg/kg, which was 29.11% lower than that of the controlled (40.22 ± 0.43)% with significant difference ($P < 0.05$).

The reconfiguration of macromolecules (e.g., gluten transformation and starch recrystallization) and moisture redistribution may change the degree of “binding” of the water molecules to these macromolecules. Xylanase can add water to the highly molecular material and may help identify the mechanisms causing freeze damage to the dough during frozen storage.

Effect of Xylanase on the Xylan Content in Dough

In the experiment, xylan reduced the dough elasticity through the cross-linking effect between ferulic acid and protein and changed the formation of gluten through competitive water uptake. As a result, the content of wet gluten was reduced, and ductility became weak. Water-soluble xylan performed a positive function in the bread production, but total xylan was detrimental to the bread quality, which was mainly determined by its content, solubility, and structure difference in flour. The excessive hydrolysis of xylan by xylanase may cause the dough to be flabby, sticky, and wet. The addition of an appropriate amount of xylanase can improve bread quality (Pan et al. 2008).

According to the standard curve equation, $y = 0.0233x – 0.0045$, where $y$ is the absorbance difference at 670 and 580 nm, the xylan contents in the dough were determined by $x$ or the xylose concentration ($\mu$g·mL$^{-1}$) as shown in Table 2. The table indicates that the content of total xylan was 1.97% and that of the water-soluble xylan was 0.63% in raw flour. The content of water-soluble xylan in blank dough sample slightly increased because of a small amount of xylanase in the dough. Xylanase had a good contact with the substrate in water during flour mixing, partially degrading the xylan.

After the addition of xylanase, the content of water-soluble xylan increased significantly. When 100 mg·kg$^{-1}$ of xylanase was added, the content of water-soluble xylan in the dough sample increased from 0.67% without adding it to 1.17%. However, an increase in the content of water-soluble xylan in the dough is not necessarily good. An excessive content of water-soluble xylan may

<table>
<thead>
<tr>
<th>Xylanase level (mg/kg)</th>
<th>$T_0$ (°C)</th>
<th>$T_e - T_0$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$\Delta H_m$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-2.50 ± 0.14$^a$</td>
<td>3.55 ± 0.21$^a$</td>
<td>-0.05 ± 0.07$^a$</td>
<td>53.125 ± 0.27$^a$</td>
</tr>
<tr>
<td>20</td>
<td>-2.90 ± 0.14$^b$</td>
<td>3.45 ± 0.21$^b$</td>
<td>-0.25 ± 0.07$^b$</td>
<td>45.96 ± 0.31$^b$</td>
</tr>
<tr>
<td>40</td>
<td>-2.95 ± 0.21$^b$</td>
<td>2.30 ± 0.14$^b$</td>
<td>-1.1 ± 0.00$^b$</td>
<td>39.78 ± 0.54$^b$</td>
</tr>
<tr>
<td>60</td>
<td>-3.25 ± 0.07$^b$</td>
<td>2.50 ± 0.00$^b$</td>
<td>-1.15 ± 0.07$^b$</td>
<td>38.30 ± 0.31$^b$</td>
</tr>
<tr>
<td>80</td>
<td>-3.00 ± 0.28$^b$</td>
<td>2.25 ± 0.21$^b$</td>
<td>-1.15 ± 0.07$^b$</td>
<td>38.01 ± 0.49$^b$</td>
</tr>
<tr>
<td>100</td>
<td>-3.00 ± 0.00$^b$</td>
<td>2.30 ± 0.00$^b$</td>
<td>-1.12 ± 0.00$^b$</td>
<td>42.17 ± 0.20$^b$</td>
</tr>
</tbody>
</table>

Each value is expressed as a mean±standard deviation ($n = 3$). The values within a column superscripted with different letters significantly vary ($P < 0.05$).

Fig. 6. Effect of xylanase on freezable water content of frozen dough (45 d)

Table 1. Effect of xylanase on ice-melting properties and water content of frozen dough

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Table 2. Content of water-extractable xylan of dough with different levels of xylanase

<table>
<thead>
<tr>
<th>Xylanase level (mg·kg⁻¹)</th>
<th>0 d</th>
<th>15 d</th>
<th>30 d</th>
<th>45 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.67 ± 0.02</td>
<td>0.66 ± 0.03</td>
<td>0.65 ± 0.06</td>
<td>0.65 ± 0.17</td>
</tr>
<tr>
<td>20</td>
<td>0.75 ± 0.00</td>
<td>0.74 ± 0.00</td>
<td>0.71 ± 0.14</td>
<td>0.69 ± 0.24</td>
</tr>
<tr>
<td>40</td>
<td>0.86 ± 0.02</td>
<td>0.83 ± 0.02</td>
<td>0.80 ± 0.21</td>
<td>0.78 ± 0.11</td>
</tr>
<tr>
<td>60</td>
<td>0.95 ± 0.04</td>
<td>0.91 ± 0.17</td>
<td>0.89 ± 0.10</td>
<td>0.85 ± 0.17</td>
</tr>
<tr>
<td>80</td>
<td>1.04 ± 0.07</td>
<td>1.00 ± 0.07</td>
<td>0.92 ± 0.14</td>
<td>0.90 ± 0.13</td>
</tr>
<tr>
<td>100</td>
<td>1.17 ± 0.03</td>
<td>1.12 ± 0.03</td>
<td>1.10 ± 0.24</td>
<td>1.07 ± 0.26</td>
</tr>
<tr>
<td>Flour</td>
<td>0.65 ± 0.06</td>
<td>1.97 ± 0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Xylan content in xylose (dry basis). Each value is expressed as a mean±standard deviation (n = 3). The values within a column superscripted with different letters significantly vary (P < 0.05).*

References


