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Characteristics of remixed fermentation dough and its influence on the quality of steamed bread

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ABSTRACT

In this study, the effects of the amount of remixed flour on the properties of remixed fermentation dough and the quality of Chinese steamed breads were investigated. The hardness, chewiness and whiteness of steamed bread increased when the amount of remixed flour was higher than 10 g/100 g, whereas the specific volume of steamed bread significantly decreased. SEM analysis demonstrated that the gas cells of the steamed bread remained as a discrete spherical or oval-like entity only at 10 g/100 g level of remixed flour. Time-domain NMR showed that water migrated from T_{22} population to T_{21} population with increasing the amount of remixed flour. The XRD results indicated that starch in the steamed bread with remixed flour was gelatinized. A significant decrease of both the rate and extent of starch hydrolysis of the steamed bread was observed when flour was remixed.

1. Introduction

Chinese steamed bread is the most popular traditional fermented wheat food in China, representing approximately 40% of wheat consumption in China (Kim, Huang, Zhu, & Rayas-Duarte, 2009). Two types of steamed bread, northern and southern styles, are generally recognized based on the production process, the composition of ingredients and consumption regions (Zhu, 2014). Most research focuses on northern style steamed bread, since it is an important staple food in the wheat-growing area of northern China. The northern style steamed bread has a higher arch domed shape, dense structure and a very chewy eating quality (Zhu, 2014). The traditional procedure for northern style steamed bread making includes mixing of the dough, full fermentation, neutralization, remixing and molding, proofing, and steaming. Remixing is the critical and unique stage for making northern style steamed bread (socalled Qiang Mian mantou). After the dough is fully fermented, more flour is mixed in at a ratio of additional flour up to 40% by weight, which is called remixed fermentation dough. Much of the steamed bread in rural China is still produced this way. Sometimes the remixing step is also used in industry in recent years.

The quality of steamed bread can be affected by many factors. Since the 1990s, considerable studies have explored the influence of various wheat varieties and ingredients on steamed bread quality (Huang, Yun, Quail, & Moss, 1996; Kim et al., 2009; Lin, Liu, Bi, & Li, 2012; Sun, Zhou, Zhi, & Li, 2007). In recent years, the development of traditional biotechnology for improving steamed bread quality has become an attractive subject. However, most of these studies focus on the technological role of traditional and novel starters in dough fermentation (Li, Li, Deng, & Liu, 2014; Wu et al., 2012; Yeh, Wu, Charles, & Huang, 2009). The effects of the traditional processing procedure on the properties of wheat dough and Chinese steamed bread are little known. The remixed fermentation dough gives the unique quality of the northern style steamed bread, and its technological role need to be elucidated. It has been reported that there is a significant influence of the dough hydration level on the bread quality (de la Hera, Rosell, & Gomez, 2014). When remixed fermentation dough is used, the remixed flour can significantly affect the water distribution and the micro-structure of dough. Therefore, understanding the effects of remixed flour on the properties of dough system and the end-product quality is essential for guiding the production of the wheat products.

In light of the unique properties of the remixed fermentation dough in making the traditional northern style steamed bread, the effects of amount of remixed flour on the dough characteristics and quality of Chinese steamed breads were investigated.

2. Materials and methods

2.1. Materials

Wheat flour with 10.1 g/100 g protein, 0.35 g/100 g ash, 0.75 g/100 g fat and 14.2 g/100 g moisture was used for this

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study, which was supplied by Jinyuan Flour Co., Ltd. (Zhengzhou, China).

2.2. Steamed bread making process

The steamed bread was made by three steps. Firstly, full fermentation dough is prepared with 500 g wheat flour, 225 g water and 4 g yeast. The ingredients were mixed in a mixing machine (SZM5, Xuzhong Co. Ltd., Guangzhou, China) for 15 min. Then, the dough was fermented at 30 °C and 85% relative humidity. Secondly, after the fermentation, 0 g, 50 g, 100 g and 150 g (0 g/100 g, 10 g/100 g, 20 g/100 g and 30 g/100 g wheat flour on the basis of the wheat flour used in the first step) wheat flour were remixed with the full fermentation dough for 15 min, then the dough was sheeted 20 times on the surface pressure machine (JCXZ, Dongfu Jiuheng Instrument Technology Co. Ltd., Beijing, China) and split into 100 g portions. The chunks were formed into round shape by hand and fermented at 30 °C and 85% relative humidity for 35 min in a controlled fermentation cabinet (JXFD 7, Dongfu Jiuheng Instrument Technology Co. Ltd., Beijing, China). Thirdly, the proofed doughs were steamed for 25 min in a pot using a steam tray and boiling water (JYC-21HEC0, Joyong, Jinan, China). After cooling at room temperature for 1 h, the quality of steamed bread was evaluated.

2.3. Steamed bread evaluation

Steamed bread was sliced transversely to obtain uniform slices of 15 mm thickness. Two slices taken from the center were evaluated. Hardness and chewiness of the crumb was performed using a Texture Analyzer (TA.XT2i, Stable Micro Systems, Ltd., Godalming, UK) equipped with a 35 mm diameter aluminum cylindrical probe with pre-test speed 1 mm/s, test speed 5 mm/s, post-test speed 5 mm/s and trigger force 5 g. The deformation level was 75% of the sample height. Steamed bread specific volume was measured using the rape seed displacement method and the whiteness was determined by whiteness meter (WGB-IV, TASAN Co. Ltd., Hangzhou, China) (Li, Li, Deng, Bian, & Liu, 2014; Sim, Noor Aziah, & Cheng, 2011).

2.4. Scanning electron microscopy (SEM)

The remixed dough and steamed bread samples were prepared for SEM examination by the methods reported previously (Kim, Morita, Lee, & Moon, 2003). In brief, the samples were freeze dried, and then fractured into sizes of about $1 \times 1 \times 0.5$ cm using a knife. The morphology of the samples was evaluated by the SEM (Quanta 200, FEI, Hillsboro, USA) operating at an accelerating voltage of 15 kV.

2.5. Nuclear magnetic resonance (NMR) analysis

Spin–spin relaxation time (T_2) was determined using the NMR system to observe the water migration in dough system. The relaxation time measurements were performed on a Niumag Desktop Pulsed NMR Analyzer (MicroMR-CL-I, Shanghai Niumag Electronics Technology Co. Ltd., Shanghai, China). Transverse relaxation (T_2) was measured using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. The parameters of the NMR test are as follows: the number of points selected to measure on every sample was 163,238, the number of echos was 2000, the number of scans was 4, and the relaxation time decayed was 1 s. The CPMG data were fitted using T_2-fit program (Ningbo Jianxin Machinery Co., Ltd., Ningbo, Zhejiang, China).

2.6. In vitro digestion of starch in steamed bread

To evaluate the digestibility of steamed bread, an *in vitro* starch digestion model simulating gastric and small intestinal conditions was used and modified (Bordoloi, Singh, & Kaur, 2012). In brief, 10 g of steamed bread were added to a jacketed glass reactor and digested in 100 ml simulated gastric fluid (SGF, containing 0.4 g pepsin, pH 1.2). The reactor jacket was maintained at 37 ± 1 °C in water bath. After 30 min, pepsin was inactivated by changing the pH to 6.8 using 1 M NaOH. Then 14 ml simulated intestinal fluid (SIF) (containing 1 g pancreatin, amyloglucosidase and invertase, respectively) was added to the reaction mixture to simulate digestion in the small intestine for 120 min. Aliquots were withdrawn at 1, 5, 10, 20, 30, 45, 60, 90, 120 min of digestion during the small intestinal phase and then immediately analyzed for reducing sugars. The reducing sugar was analyzed by the 3,5-dinitrosalicylic acid method using a maltose standard curve (Miller, 1959).

2.7. X-ray diffraction (XRD) analysis

Wide angle X-ray scattering patterns of remixed dough and steamed bread samples (vacuum freeze drying and milled to 100 mesh powder) was performed on Bruker D8-Advance XRD instrument (D8 Advance, Bruker AXS Inc., Germany). The diffractograms were collected under the conditions of 40 kV, 35 mA, with the scanning angle (2θ) from 4° to 40° at a scanning rate of 4°/min.

2.8. Statistical analysis

The results reported in this article are the average values ± S.D. and the significant differences between two samples were analyzed by the Duncan's multiple-range test ($P < 0.05$) using SPSS software (SPSS 19.0, SPSS Inc., Chicago, U.S.A.).

3. Results and discussion

3.1. Effects of the amount of remixed flour on the steamed bread quality

The effects of the amount of remixed flour on the quality of steamed bread are shown in Table 1. It was demonstrated that the higher amount of remixed flour (>10 g/100 g) could significantly increase the hardness and chewiness of steamed bread. This proved the chewy eating quality of the northern style steamed bread. It should be noted that as no additional water was added in the remixed flour procedure, the relative water content decreased when the full fermented dough remixed with higher amount of flour, which could be the reason for the unique quality of northern style steamed bread. It was reported that an increase of the hardness was observed when decreasing the water content in the recipe of making bread (de la Hera et al., 2014).

The specific volume of steamed bread significantly decreased as the amount of remixed flour higher than 10 g/100 g. The lower specific volume resulting in denser crumb and more compact gas cells (de la Hera et al., 2014; Li, Li, Deng, Liu, 2014). Thus, the decreased specific volume of steamed bread maybe related to the poor leavening and disrupted gluten network structure due to the unavailability of enough water in the dough. The steamed bread prepared by the remixed fermentation dough showed higher whiteness. The improved whiteness of the steamed bread may be due to the changed interior microstructure and composition (Li, Li, Deng, Bian, et al., 2014). Based on the data shown in Table 1, it seems that the remixed fermentation dough would be appropriate for the improved chewiness and whiteness. Consumers in northern of

Table 1
Quality of steamed bread prepared by remixed fermentation dough.

Amount of remixed flour (g/100 g)	Hardness (g)	Chewiness (g)	Specific volume (mL/g)	Whiteness
0	1836 ± 104[a]	1462 ± 104[a]	2.42 ± 0.18[a]	49.4 ± 0.1[a]
10	2074 ± 131[a]	1587 ± 91[a]	2.35 ± 0.15[a]	52.2 ± 0.2[b]
20	3271 ± 250[b]	2999 ± 233[b]	2.01 ± 0.05[b]	54.5 ± 1.5[c]
30	7382 ± 301[c]	3763 ± 262[c]	1.74 ± 0.14[c]	54.3 ± 0.3[c]

Means with different superscript letters within the same column are significantly different ($P < 0.05$).

China prefer steamed bread with a white crumb and surface, very chewiness eating quality and higher specific volume.

3.2. SEM analysis

Microstructural observations help explain the observed quality properties of steamed bread. The microstructures of full fermentation dough with different amount of remixed flour were observed by SEM (Fig. 1). The proteins constituted the amorphous matrix with embedded starch granules and voids existed among the gluten network and starch granules in all dough samples. As shown in Fig. 1(A), the control dough without remixed flour showed the typical structure of some starch granules embedded in a gluten network. In the remixed fermentation dough, the micrograph of the dough with 10 g/100 g and 20 g/100 g remixed flour addition (Fig. 1B and C) was similar to the control. In the dough with 30 g/100 g remixed flour (Fig. 1D), the microstructure presented a more dense structure as compared to the control samples. In addition, too much remixed flour increased the surface connectivity between starch granules and gluten, and the gluten strands in the dough cannot be clearly observed, indicating that too much remixed flour was against in developing gluten network. The discontinuous gluten matrix implied that the resistance and extensibility of dough were disturbed (Keeratipibul et al., 2013; Sim et al., 2011), which could further influence the textural properties of steamed bread.

The microstructures of steamed bread samples with and without remixed flour are compared and analyzed by SEM (Fig. 2). The gelatinized starch granules were immersed in a continuous matrix formed by the heat-induced denatured protein during steaming. Apparent distinctions were observed on the morphology of gas cell between control and samples treated with remixed flour. The damaged gas cell wall and the resulted coalescence of individual larger gas cells could be found in the control steamed bread without remixed flour (Fig. 2A). Perhaps this outcome is related to the inferior gluten network disrupted during the full fermentation process (Keeratipibul et al., 2013).

When the steamed bread with 10 g/100 g remixed flour was taken into consideration, although channels formed by coalesce of the gas cells through the small hole on the cell walls, individual gas cells remained as a separate discrete entity, which were mostly in spherical or oval-like shapes. When the flour was remixed, a tighter new network structure was reformed by cross-linking among gluten proteins and it remains each bubble as a separate

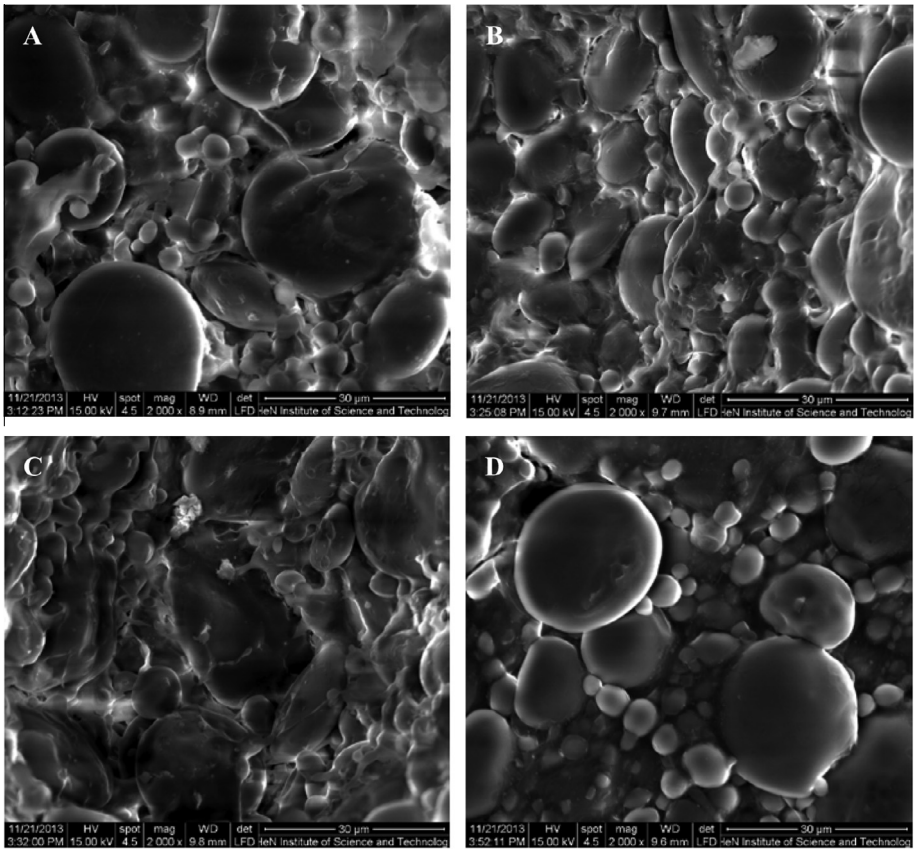

Fig. 1. Microstructure of remixed fermentation dough with 0 g/100 g (A), 10 g/100 g (B), 20 g/100 g (C) and 30 g/100 g (D) remixed flour.

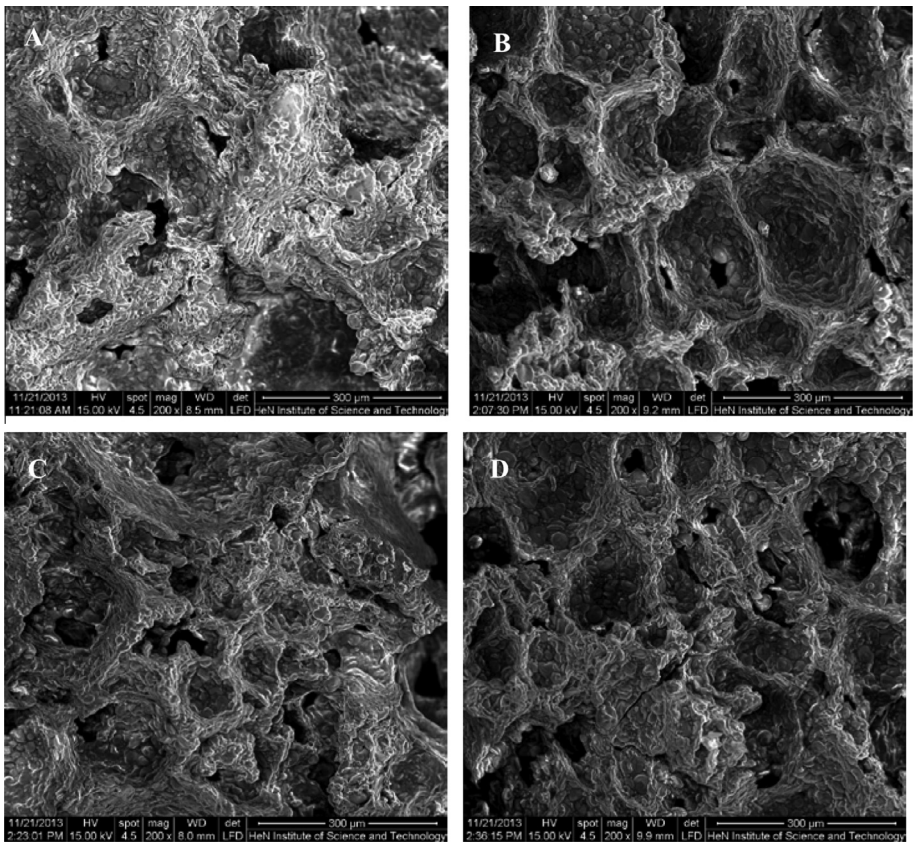

Fig. 2. Microstructure of steamed bread prepared from remixed fermentation dough with 0 g/100 g (A), 10 g/100 g (B), 20 g/100 g (C) and 30 g/100 g (D) remixed flour.

discrete entity, resulting in formation of stable morphology. It was reported that gum adsorbed around the bubbles increased the stability of gas cells by forming thick layer on their surface (Ozge Ozkoc, Sumnu, & Sahin, 2009). It should be noted that the interior structure of steamed bread made with 20 g/100 g and 30 g/100 g remixed flour was a little bumpy. The uneven air cells or pores with thicker wall were smaller and close to each other, resulting in lower specific volume. 20 g/100 g and 30 g/100 g remixed flour resulted in the limited water availability, which hindered the optimal development of gluten network structure and restricted the expansion of gas cells. Then it influenced the textural attributes and microstructure of steamed bread.

3.3. NMR analysis

The amount and state of water play an important role in the properties of dough and their products (de la Hera et al., 2014). Pulsed NMR was used to investigate the water migration of dough systems subjected to different amount of remixed flour (Fig. 3). *X*-axis in T_2 relaxation time curve represents the water activity of food material. A shorter T_2 relaxation time indicates a lower degree of water freedom. In our system, a typical curve of T_2 relaxation time distributions of dough system showed three CMPG proton populations: T_{21} (0.4–3 ms), T_{22} (3–20 ms), and T_{23} (30–100 ms), which represented tightly bound water, less tightly bound (immobilized) water, and weakly bound (free) water of the moisture in the dough, respectively, which were closer to those detected in dough previously (Li, Hou, Chen, Chung, & Gehring, 2014). When the remixed flour was higher than 10 g/100 g, peak time of T_{22} dramatically reduced. Meanwhile, T_{22} merged into T_{21} and formed a broader peak. As the remixed flour reach 30 g/100 g, the T_{21} and

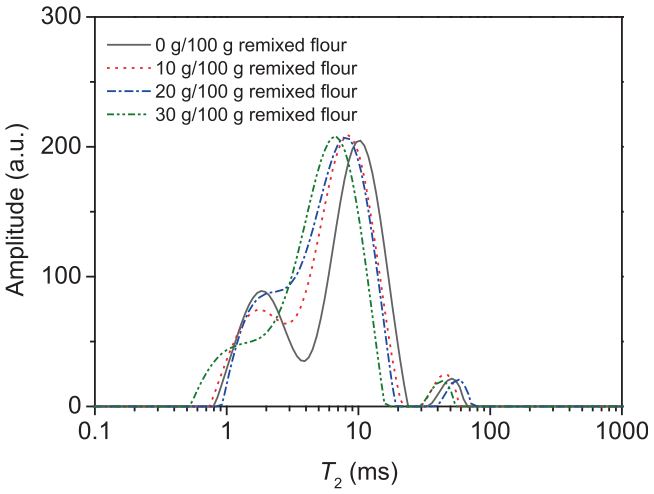

Fig. 3. The relaxation T_2 distribution of the remixed fermentation dough.

T_{22} peaks seemed to merge into a broad single peak with further decrease peak time. Therefore, both peak times and their distribution patterns shifted with the remixed flour dough systems. The decreased peak position and distribution patterns with increased amount of remixed flour suggested that some water migration occurred from the T_{22} population to the T_{21} population. Relatively less water content occurred when the new flour was remixed and the water migrated between the full fermentation dough and new added flour. It has been reported that as water content decreases, the peak time (T_{22}) decreases from 10 to 3 ms in wheat flour dough (Doona & Baik, 2007).

It is generally believed that the gluten and starch have different water binding capacities. Compared with water populations showed by gluten, the water distributions of the starch showed one merged broad population in the range of 0.4–30 ms (Doona & Baik, 2007), which was similar to the result at higher remixed flour ratio in this study. It indicated that the water migrated between the starch matrix and the gluten network in remixed dough system. Presumably, too much remixed flour decreased the water holding capacity of gluten and redistributed water in the dough system, which was detrimental for gluten to hydrate more and form a continuous network. At a lower remixed ratio, an intact gluten network is formed and dough becomes more extensible and easy to spread during steaming. A higher remixed ratio inhibited the water absorption of gluten to form the network structure, and thus, the internal structures of steamed bread were restrained due to the weak gas-retention capacity of gas cells.

3.4. XRD analysis

The XRD patterns for remixed fermentation dough acquired shortly after remixing are shown in Fig. 4A. All dough samples displayed a prevailing A-type crystalline pattern with the main diffraction doublet at 2θ 17° and 18° and peaks at 15°, 20° and 23° corresponding to wheat starch (Seetapan et al., 2013). After steaming, the original A-type crystalline order reflections were lost and one distinct peak at 19.7° could be observed for all steamed bread samples (Fig. 4B), corresponding to the typical V-type amylase–lipid complexes crystalline pattern (Seetapan et al., 2013). This suggested that the peak did not reveal any significant difference among the four test steamed breads and the native A-type crystallinity was destroyed as a result of the steaming process. The results indicated that wheat starch in the steamed bread was gelatinized and remixing did not inhibit the change of starch crystal type.

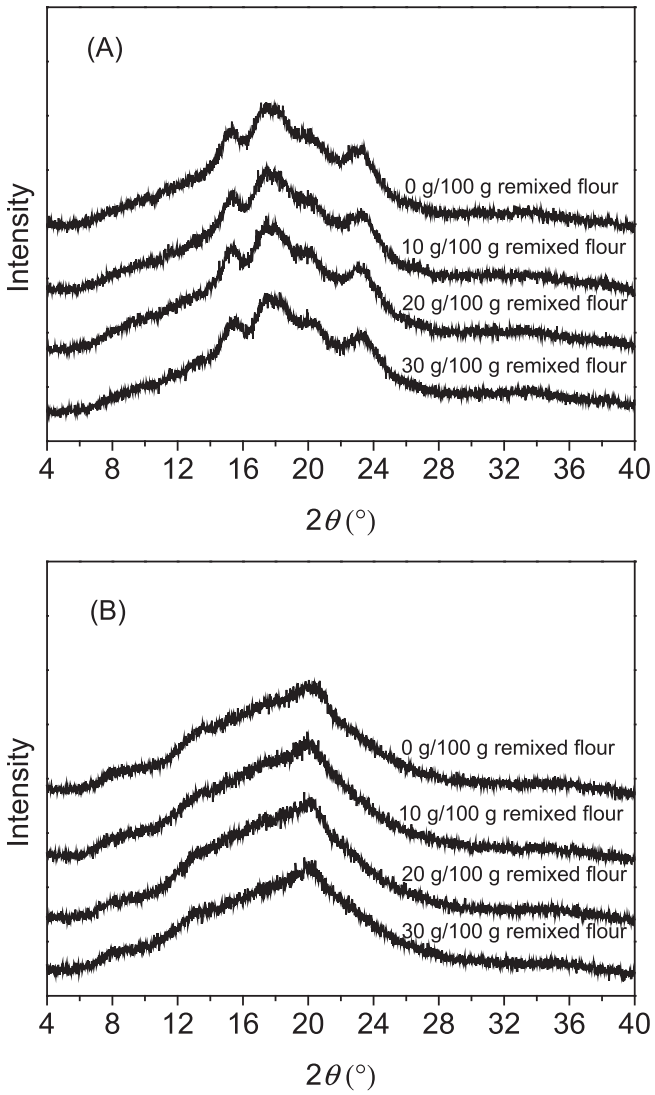

Fig. 4. XRD pattern of (A) remixed fermentation dough and (B) steamed bread.

3.5. In vitro digestion

The hydrolysis of starch of steamed bread *in vitro* digestion is presented in Fig. 5. No hydrolysis of starch occurred under the simulated gastric conditions because of the absence of starch-hydrolyzing enzymes (data not shown). When the SIF was added to the reaction mixture, the starch was rapidly digested within the first 15 min for all samples. However, remixing flour in the starch matrix of full fermented dough led to a significant decrease in both the rate and the extent of final starch hydrolysis (Fig. 5). This affected the final hydrolysis of the starch significantly ($P < 0.05$), with an approx. 35% drop in the hydrolysis at the end of the digestion period when 30 g/100 g flour was remixed.

The rate and extent of starch hydrolysis is dependent upon several intrinsic and extrinsic factors. The food components like proteins or fatty acids have inhibitory effect on the starch hydrolysis (Bordoloi et al., 2012). In particular, the starch granules suspending in the continuous gluten networks and the gluten could act as a barrier when enzymes try to access the starch (Bordoloi et al., 2012; de la Hera et al., 2014). This barrier function became more evident when the surfaces between starch granule and gluten bind closer at higher remixed flour ratio. It should be noted that the starch digestion is not only delayed but also reduced in the presence of remixed flour. Therefore, the inhibition of the enzyme action permanently to prevent further hydrolysis of starch may not be ruled out.

In addition, although the crystalline pattern of the steamed bread samples showed no significant difference, decreasing water availability may influence the degree of gelatinization. It has been reported that limited water availability has an effect on the gelatinization and cooking of the starch (de la Hera et al., 2014). The way of preparing the remixed flour steamed bread could have an

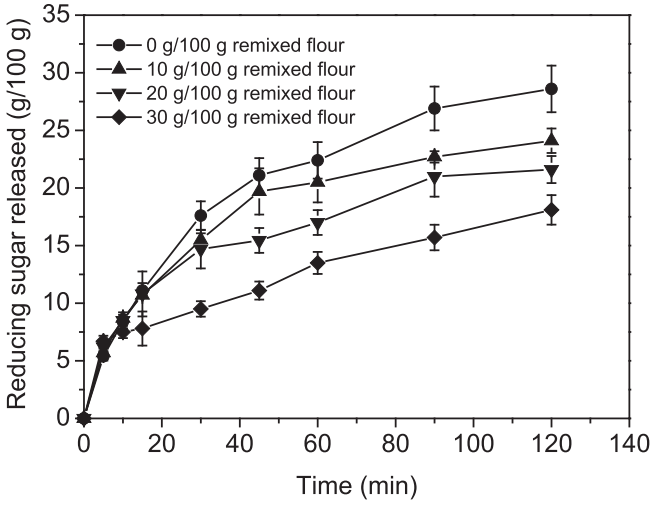

Fig. 5. Reducing sugar released during simulated intestinal digestion of steamed bread.

influence on the water availability for starch gelatinization. The effect became more pronounced at higher amounts of remixed flour due to the increased competition for water molecules between the original and new added starch granules. The addition of remixed flour reduced the swelling of starch granules which result in an alteration in the functional properties of starch, such as the starch digestibility. This observation leads to the conclusion that the remixed fermentation technique influenced starch availability to hydrolytic enzymes.

4. Conclusion

The remixed fermentation dough had a significant influence on the quality of steamed bread. Too much remixed flour decreased the water holding capacity of gluten and redistributed water in the dough system, which was detrimental for gluten to form a continuous network and resulted uneven air cell or pores with thicker wall. The increased amount of remixed flour did not result in any change in the crystalline pattern of the steamed bread due to steaming, whereas it resulted in lower starch hydrolysis.

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