1 Quality characteristics of northern-style Chinese steamed bread prepared
2 from soft red winter wheat flours with waxy wheat flour substitution

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13 **Keywords**

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Abstract

Quality characteristics of northern-style Chinese steamed bread (CSB) prepared from two soft red winter (SRW) wheat flours blended with 0-30% waxy wheat flour (WWF) were analyzed to estimate the influence of starch amylose content. The increased proportion of WWF in blends raised mixograph absorption with insignificant changes in protein content and dough strength-related parameters. WWF incorporation generally increased specific volume and crumb softness of CSB. The analysis of covariance revealed that CSB quality attributes were little affected by protein content and dough strength-related parameters, indicating that starch amylose content was largely responsible for the changes in CSB quality. Flour blends with 5-10% WWF, of which starch amylose content was 22.4-24.7%, produced CSB with superior crumb structure compared to other blends, but insignificant changes in surface smoothness, stress relaxation and total score compared to the respective control wheat flours. Flour blends with 15% WWF to produce a starch amylose content of 21.4-22.7% exhibited reduced staling of CSB with total scores comparable to the respective control wheat flours. CSB prepared from blends with more than 10% WWF exhibited a higher soluble starch content, indicative of reduced starch retrogradation, than that prepared from wheat flours without WWF during storage for 3 days.

1. Introduction

Chinese steamed bread (CSB), a staple of wheat-based traditional fermented Chinese food, has been consumed for almost two millennia in China. The basic ingredients for making CSB are wheat flour, water and yeast or sourdough. The shelf-life of CSB is only 1-3 days when stored at room temperature, and becomes shorter at a higher storage temperature or a reduced storage relative humidity (Qin et al., 2007). CSB shelf-life extension faces a great challenge as it is quick staling. CSB staling, also referred as hardening, is commonly defined as the loss of freshness (mouth-feel, flavor and moisture loss) during storage, lowering eating quality and marketability. Increased crumb firmness over time is a common indicator of CSB staling and a most important characteristic noted by consumers.

Wheat varieties are classified as waxy, partial waxy, normal or high-amylose wheat based on starch amylose content (Graybosch, 1998; Nakumura et al., 1995). As it is lacking in amylose molecules, waxy wheat starch has a lower pasting temperature and a lower setback value, absorbs more water, and exhibits less syneresis as compared to normal wheat starches (Baik and Lee, 2003; Sasaki et al., 2000). Waxy wheat is known to be more resistant to retrogradation during storage than wheat of normal starch endosperm, and could be used to retard the staling of wheat products (Sasaki et al., 2000).

A number of studies have been conducted to understand the potential of waxy wheat flour (WWF), including as a source for blending flour to improve shelf-life stability and the processing quality of wheat products (Baik and Lee, 2003;

Bhattacharya et al., 2002; Guo et al., 2003; Lee et al., 2001; Morita et al., 2002; Yi et al., 2009). The uses of WWF have been tested in several products including bread (Bhattacharya et al., 2002; Morita et al., 2002) and noodles (Baik and Lee, 2003; Guo et al., 2003). WWFs produced bread with softer crumb texture, even after storage, than wheat flours of normal type starch (Morita et al., 2002). However, bread baked from WWFs exhibited a highly porous crumb structure and a gummy texture, and tended to collapse during storage (Garimella Purna et al., 2011; Morita et al., 2002; Jonnala et al., 2010). Noodles made from WWFs were sticky and soft, and failed to maintain the integral structure of the noodle strands (Baik and Lee, 2003). Since 100% WWF is not suitable for making bread or noodles, attempts have been made to use blends of waxy wheat and normal wheat flours for the production of bread and noodles with improved quality. Previous studies on the effects of WWF addition on bread staling have been in disagreement. Bhattacharya et al. (2002), Hayakawa et al. (2004) and Morita et al. (2002) observed a reduction in the crumb firmness of bread with the addition of WWF even after storage. In contrast, Graybosch (2001) found an increase in crumb firmness of bread made from flour blends containing WWF during storage. In a separate study, the incorporation of WWF resulted in softer bread immediately after baking, but didn't retard staling during storage (Garimella Purna et al., 2011). The inconsistent effects of WWF substitution on crumb firmness during storage may be due to the differences in baking formulations and processes, storage conditions and characteristics of bread baked from waxy wheat and normal wheat flour blends.

Up until now, information about the shelf-life extension of CSB with incorporated WWF is still very limited. Qin et al. (2007) reported that the quality of northern-style CSB cannot be improved by the addition of WWF, but the firmness of fresh and re-steamed CSB decreased with an increasing content of WWF at proportions up to 25% when compared to the control wheat flour, without elaborating much on the staling of CSB during storage and the associated changes in crumb characteristics.

The objectives of this study were to identify the effects of starch amylose content on the quality and staling of northern-style CSB using flour blends of waxy wheat and normal wheat flours and to determine the optimal proportion of WWF for the production of CSB with improved quality.

2. Materials and methods

2.1. Materials

A SRW waxy wheat breeding line, NX10MD2268 Waxy 1711 (Waxy 1711) procured from the USDA-ARS, Lincoln, NE, and two SRW wheat varieties, Kristy and OH04-264-58, with normal starch endosperm, were selected to produce flour blends of varying starch amylose content with 0, 5, 10, 15, 25 and 30% WWF. Kristy and OH04-264-58 were similar in protein content and dough strength.

Wheat grain was tempered to 15% moisture prior to milling for 12 h, and was milled to obtain flour of about 70% extraction using a Miag Multomat Mill (Buhler, Inc., Braunschweig, Germany).

2.2. Proximate analysis and rheological properties of flours

Flour moisture, ash content and protein content were determined according to AACC Approved Methods 44-16.01, 08-01.01 and 46-30.01, respectively (AACC International, 2010). The determination of total starch content was performed according to AACC Approved Method 76-13.01 (AACC International, 2010) using a Megazyme Total Starch Assay Kit (Megazyme International Ltd., Wicklow, Ireland). The amylose content of flour was measured according to the procedure described by the manufacturer for the Megazyme Amylose/Amylopectin Assay Kit (Megazyme International Ltd., Wicklow, Ireland). Flour color was determined using a Hunter Lab Mini Scan XE plus colorimeter, Model 4500L (Hunter Associates Laboratory, Inc., Reston, VA, USA), and expressed with L* (100 for perfect whiteness/zero for blackness), a* (+redness/-greenness), and b* (+yellowness/-blueness).

Dough mixing properties were measured using a 10 g Mixograph (National Manufacturing Co., Lincoln NE, USA) following AACC Approved Method 54-40.02 (AACC International, 2010). Dough mixograph absorption was initially calculated based on flour protein content using AACC Approved Method 54-40.02, but was finally optimized for each sample based on a series of mixograms. The parameters recorded were midline peak time (MPT, min) and midline peak value (MPV, %Torque), and were obtained from the mixogram and collected using MixSmart Software Version 3.8 (National Manufacturing Co., Lincoln, NE, USA). Wet and dry gluten contents of flour were determined using a Perten 2200 Glutomatic System

(Perten Instruments AB, Huddinge, Sweden) according to AACC Approved Method 38-12.02 (AACC International, 2010). The sodium dodecyl sulfate sedimentation (SDSS) test was performed according to the method described by Axford et al. (1979) with minor modifications in flour weight to 3 g (14% moisture basis) and a sedimentation time of 20 min.

2.3. Pasting profile determination

Flour pasting properties were measured using a Rapid Viscosity Analyzer (RVA) following AACC Approved Method 76-21.01 (AACC International, 2010) with minor modifications. Flour (3.5 g, 14% moisture basis) was weighted in a canister, to which 25 mL water containing 1 mM AgNO₃ was added in order to eliminate α -amylase activity that could mask the actual pasting behavior of the flour samples (Crosbie et al., 1999). Parameters including peak viscosity (Rapid Viscosity Analyzer Unit, RVU), breakdown value (RVU), final viscosity (RVU), setback value (RVU) and pasting temperature (°C) were recorded.

2.4. Preparation and quality evaluation of Chinese steamed bread

Northern-style CSB was prepared from flour blends according to the method described by Ma and Baik (2016). The CSB formulation included flour (100 g, 14% moisture basis), instant yeast (1.5 g), and water (78% of mixograph absorption). Mixing times were calculated based on MPT according to the method described by Ma and Baik (2016). Flour, water and yeast were mixed to achieve optimal dough and

gluten development using a 100 g mixer (National Manufacturing Co., Lincoln, NE, USA). After fermentation for 1 h at 32°C and 85% relative humidity, dough was mixed again using a 100 g mixer, sheeted 20 times through a pair of rolls with a 7 mm gap, and then rounded to a dome shape 5.5 cm in height. Dough was proofed for 15 min at 32°C and 85% relative humidity, and steamed for 20 min in a commercial steamer (Southbend, NC, USA). After cooling for 15 min at 24°C, the quality attributes of CSB were evaluated following the method described by Ma and Baik (2016). CSB total score (100) includes 20 for specific volume (volume/weight), 5 for spread ratio (width/height), 10 for surface smoothness, 15 for crumb structure, 5 for external color, 5 for crumb color, 5 for brightness and 35 for stress relaxation. Stress relaxation (SR) was calculated from the two compression forces (P1 and P2) by a texture analyzer (TA.XT2i, Stable Micro Systems, Haslemere, Surrey, UK) as described by Huang et al. (1995) using the equation $SR = (P1 - P2) / P1 \times 100$.

2.5. Moisture content, firmness, soluble starch content and thermal properties of Chinese steamed bread

After cooling at 24°C for 1 h, CSB was kept in an airtight plastic Ziploc bag until analysis at 24°C. The moisture content, firmness, soluble starch content and thermal properties of CSB crumb at 0 day, 1 day and 3 days after preparation were determined.

The moisture content of CSB crumb was determined by oven drying at 105°C for 24 h. The firmness of CSB crumb was determined by a compression test using a

texture analyzer (TA.XT2i, Stable Micro Systems, Haslemere, Surrey, UK) equipped with a 50 kg load cell and a 3.6 cm diameter cylindrical acrylic probe. A 28 mm-thick, horizontal-cut slice of CSB was obtained from the center part of CSB using an electric knife. The slice was placed on a flat metal plate and compressed to 50% of its original thickness at a speed of 1.0 mm/sec. The maximum force of the sample was recorded as the firmness of CSB. The firmness value reported was the average of three measurements.

The soluble starch content of CSB crumb was determined following AACC Approved Method 76-13.01 (AACC International, 2010) with minor modifications. A piece of CSB crumb was lyophilized and ground to powder using a Udy cyclone Mill (Udy Co., Fort Collins, CO, USA) fitted with a 0.5-mm perforated screen for the determination of soluble starch content as an indicator of starch retrogradation. The soluble starch of CSB crumb was extracted by mixing 100 mg of lyophilized CSB with 1.5 mL of water in a 2.0 mL centrifugal tube according to the procedure described by Garimella Purna et al. (2011). The sample was mixed for 45 sec using a vortex and centrifuged at 12,000 x g for 10 min. The supernatant (1 mL) was immediately transferred to a test tube and the soluble starch content of CSB crumb was determined according to the procedure described by the manufacturer for the Megazyme Total Starch Assay Kit (Megazyme International Ltd., Wicklow, Ireland). Percent soluble starch was calculated based on the starch content of the flour (on a dry basis). An average of three replicates was reported as the total soluble starch (%).

Thermal properties of CSB crumb were determined by using differential

scanning calorimetry (DSC) (Pyris DSC7, Perkin Elmer Co., Norwalk, CT, USA). Lyophilized CSB crumb (10 mg) from day 0, day 1 and day 3 and distilled water (20 μ L) were placed in a stainless steel DSC pan. The pan was sealed and allowed to equilibrate at 24°C for 24 h. An indium standard was used for temperature and enthalpy calibration. An empty DSC pan was used as a reference. The samples were heated from 20°C to 140°C at 10°C/min. The transition enthalpy of gelatinization was calculated from the peak area and expressed as J/g on a dry basis. Each sample was analyzed in duplicate and average values were reported.

2.6. Quality of re-steamed Chinese steamed bread

After cooling at 24°C for 1 h, CSB was kept in an airtight plastic Ziploc bag and stored at refrigerator temperature (4°C) for 3 days. Then, the CSB was re-steamed for 10 min in a commercial steamer. Quality attributes including specific volume, surface smoothness, crumb structure, stress relaxation, total score and crumb firmness of CSB were determined as described above.

2.7. Statistical analysis

All determinations were performed at least in duplicate. Analysis of variance (ANOVA) and least significant difference (LSD) tests were performed using the Statistical Analysis System 9.3 (SAS Institute, Cary, NC, USA). In order to estimate the effect of starch amylose content independently of protein characteristics, flour protein content, SDSS volume, MPT and wet/dry gluten contents were used as

covariates in the analysis of covariance (ANCOVA) following the method described by Guo et al. (2003).

3. Results and discussion

3.1. Flour characteristics

The flour composition, protein characteristics and dough mixing properties of SRW wheat flours and their WWF blends with WWF are summarized in Table 1. The starch amylose contents were 25.7, 24.4 and 5.1% for OH04-264-58, Kristy and Waxy 1711, respectively. Starch amylose contents of the flour blends decreased with increasing proportions of WWF. SRW variety OH04-264-58 had a higher total starch content (74.3% on a 14% moisture basis), while Kristy had a lower total starch content (70.9%) when compared to Waxy 1711 (73.2%). Therefore, the changes in total starch content of the two control wheat flours blended with WWF were inconsistent with increasing proportions of WWF (Table 1).

The protein contents of the flours were 9.8, 9.8 and 11.0 % (14% moisture basis) for OH04-264-58, Kristy and Waxy 1711, respectively. There were slight increases in both protein content and wet/dry gluten content of the flour blends with increasing proportions of WWF (Table 1). SDSS volumes of OH04-264-58, Kristy and Waxy 1711 were 42.0, 43.5 and 45.5 mL, respectively. SDSS volumes of the flour blends were slightly changed by WWF substitution without being significantly different among flour blends.

The L*, a* and b* values of WWF and two control wheat flours were 92.1-92.5,

0.1-0.2 and 8.9-9.5, respectively. Accordingly, the L*, a* and b* values of flour blends were not significantly affected by WWF substitution. The flour ash content was 0.35, 0.36 and 0.45 % (14% moisture basis) for OH04-264-58, Kristy and Waxy 1711, respectively. The ash content of flour blends ranged from 0.35% to 0.39%, showing 0.03-0.04% increases with WWF substitution.

The optimized mixograph absorptions were 58.0, 58.6 and 62.0% for OH04-264-58, Kristy and Waxy 1711, respectively. The higher absorption of WWF as compared to the normal flours was probably due to its higher protein content and the greater water absorption capacity of waxy starch than normal starch. The mixograph absorptions of the flour blends increased with the increased incorporation of WWF, probably due to the higher water affinity of amylopectin than amylose. As the MPTs of the two control wheat flours (4.5 min) were the same as that of waxy 1711 (4.5 min), the MPTs of the flour blends were no different compared to those of the two control wheat flours. Both Kristy and OH04-264-58 had lower MPVs (46.4 and 41.7, respectively) than Waxy 1711 wheat flour (50.4). The flour blends exhibited slight increases in MPVs with the incorporation of WWF.

3.2. Pasting properties

Pasting properties of the flour blends are listed in Table 2. The pasting temperatures of the flour blends decreased with the increased incorporation of WWF, which was probably due to the lower pasting temperature of waxy starch than that of normal wheat starch (Baik and Lee, 2003; Sasaki et al., 2000). The peak viscosity and

final viscosity of the flour blends exhibited decreasing trends with increasing proportions of WWF. The flour blends exhibited RVA pasting profiles with two peaks. These observations are consistent with previous studies (Guo et al., 2003; Sasaki et al., 2000). The two peaks indicate that the starch granules of WWF and normal wheat flour in the flour blends swelled and developed viscosity at different temperatures. The increase in viscosity during cooling was attributed to leached amylose which could rearrange and form a thin amylose gel layer (Flipse et al., 1996). The flour blends with lower amylose contents were also lower in the amount of leached amylose, resulting in a decreased degree of gelation and resultant viscosity during cooling.

Breakdown and setback values of the flour blends also exhibited decreasing trends with increasing proportions of WWF in both control wheat flours. These results are consistent with Guo et al. (2003) who reported that breakdown and setback values of the flour blends decreased along with an increased amount of WWF.

For OH04-264-58 and its blends, setback values of the flour blends were significantly lower than that of control wheat flour; however, no significant differences were observed in setback values between Kristy and its blends. Gluten and water-soluble components had significant influences on the pasting properties of the flour blends. In our previous study, setback value was significantly correlated with surface smoothness, crumb structure, stress relaxation and total score of CSB made from SRW wheat flours, whereas all other RVA parameters failed to show any relationships with CSB quality attributes (Ma and Baik, 2016). The differences in

setback values between the two control flours when blended with WWF could explain their differences in quality attributes of CSB.

3.3. Covariate effects of protein characteristics on quality of Chinese steamed bread

Flour blends of normal and waxy starch endosperm were used to determine the influence of starch amylose content on CSB quality, since this approach is more practical for food processing and flour milling industries than fractionation and reconstitution methods. When normal wheat flour and WWF were blended in different proportions, both starch amylose content and other flour characteristics were affected, making it difficult to identify the unique effects of amylose content on CSB quality. In this study, flour characteristics including protein content, SDSS volume, MPT and wet/dry gluten content were treated as covariates of starch amylose content and their effects on CSB quality attributes were determined.

Covariate effects were tested for the specific volume, surface smoothness, crumb structure, stress relaxation, total score and firmness of CSB. The analysis of covariance indicated that none of the covariate effects were significant at the 5% level. Accordingly, it is believed that the changes in CSB quality with WWF substitution can be largely attributed to the variation in starch amylose content.

3.4. Influence of waxy wheat flour substitution on Chinese steamed bread quality

No significant differences were observed in brightness, external color or crumb color of CSB between the flour blends with varying proportions of WWF, as expected from the similar L*, a* and b* values between the two base flours and WWF.

3.4.1 Appearance

CSB made from blends of OH04-264-58 with 0 to 30% WWF showed no significant differences in spread ratio. CSB made from Kristy with 10 to 30% WWF appeared to be flatter than CSB made from Kristy without WWF substitution, but CSB made from Kristy with 5% WWF was similar to that prepared from the control wheat flour. Flour blends with 10 to 30% WWF have reduced amylose contents and consequently exhibit reduced leaching of amylose molecules from starch granules during steaming, which results in the weakened cross-linking of amylose molecules and starch in the starch-gluten network (Guo et al., 2014; Martin and Hoskeney, 1991). An overly loose starch-gluten network would have an adverse effect on the stability of gas cells (Park et al., 2005), and the subsequent coalition of some gas cells would lead to flatter CSB. In addition, the flattened shape of CSB made from flour blends may be due to softer dough, owing to the greater amount of water (0.3-1.2%) used in the CSB formulation of flour blends than that of control flour.

The smoothness score of CSB decreased with the inclusion of 20% or more WWF in blends due to the appearance of wrinkles and dimples on the surface, but no apparent changes in smoothness were observed with up to 15% WWF substitution (Fig. 1). Garimella Purna et al. (2011) reported that waxy starch granules swell during baking, making the gas cell walls impermeable and resulting in high post-bake

shrinkage due to the negative internal pressure. The cell walls of CSB expand during steaming, but fail to rupture. During cooling, the cell walls shrink due to negative internal pressure, causing CSB to have a wrinkled or dimpled appearance. In addition, CSB made from flour blends of Kristy and WWF exhibited better surface smoothness than that made from flour blends of OH04-264-58 and WWF. This difference may be due to the different pasting properties of the two control flours blended with WWF (Table 2).

3.4.2 Specific volume and crumb structure

The specific volume of CSB containing WWF was significantly greater than that of CSB prepared from the control wheat flours, but no significant differences were observed among flour blends containing 15 to 30% WWF in both varieties (Table 3). Qin et al. (2007), however, reported that the specific volume of northern-style CSB was not affected by the proportion of WWF in the experiment employing flour blends with 0-40% WWF for making CSB. This discrepancy might be due to the differences in CSB formulation and the proportion of WWF used.

The specific volume of CSB is an important quality parameter, as it is related to gas retentive ability and elasticity of dough. Specific volume should not be too large or too small as it affects crumb structure. In general, an excessively small specific volume relates to a very compact and dense grain structure, while an excessively large specific volume gives a very open and airy grain structure. The crumb structures of CSB made from control wheat flours blended with varying proportions of WWF are shown in Fig. 1. CSB with 5-15% WWF exhibited comparable crumb structure to that

prepared from control flours, but more open crumb structure was observed in CSB containing more than 20% WWF. These results agree with Bhattacharya et al. (2002) and Garimella Purna et al. (2011) who reported that pub-loaf bread containing high levels of WWF exhibited an open crumb grain. On the one hand, Garimella Purna et al. (2011) observed that the greater gas production in dough systems with WWF could result in large gas cells which might expand during bread baking, creating an open crumb structure in the resultant bread. On the other hand, Guan (2008) noted that gas cells coalesce during bread baking with WWF incorporation as the excessive swelling of waxy wheat starch granules occurs, resulting in an open crumb structure. Therefore, amylose molecules could play an important role in the formation of CSB crumb structure and prevent the collapse of CSB during cooling. Decreased proportions of amylose molecules with WWF incorporation produced CSB with a more open-structured crumb.

CSB made from OH04-264-58 containing 5 to 10% WWF exhibited the best crumb structure, while the best crumb structures of CSB made from Kristy was observed with flour blends containing 0 to 10% WWF. This observation indicates that an optimal range of starch amylose content for producing CSB with good crumb structure exists. Flour blends with 5 to 10% WWF, of which the starch amylose content ranged from 22.4 to 24.7%, produced CSB with superior crumb structure compared to other flour blends, without causing significant changes in surface smoothness, stress relaxation and total score of CSB compared to the respective control wheat flours.

3.4.3 Stress relaxation

The stress relaxation score of CSB exhibited a significant decrease with incorporation of WWF, but no significant differences were observed among flour blends containing 0-15% WWF in both varieties. Our previous study showed that MPT was the most influential determinant of stress relaxation score, followed by protein content and setback value (Ma and Baik, 2016). In this study, however, CSB quality attributes were slightly affected by protein content and dough strength-related parameters including MPT, SDSS volume and wet/dry gluten content. This observation suggests that the stress relaxation of CSB was mainly affected by dough gluten strength when the amylose content ranged from 21.4% to 25.7%, whereas it was largely determined by starch properties when the amylose content was less than 21.4%. Guo et al. (2014) reported that no significant trend was observed in the stress relaxation of CSB made from reconstituted flours with constant gluten and water soluble, but different starch properties. This discrepancy may be due to the different ranges of starch amylose content in the flours used in the respective studies.

3.4.4 Total score

The total score of CSB containing 20-30% WWF significantly decreased, but CSB containing 0-15% WWF was comparable to that prepared from control flours in total score. This result agrees with Qin et al. (2007) who reported that the total score of CSB with WWF incorporation was not significantly different compared to the respective control wheat flours when the proportions of WWF were less than 15%.

385 Additionally, the variations in surface smoothness, stress relaxation score and
386 total score among OH04-264-58 and its blends were larger than those among Kristy
387 and its blends. In our previous study, setback value was significantly correlated with
388 surface smoothness, stress relaxation and total score of CSB (Ma and Baik, 2016). In
389 this study, the variation in setback values among OH04-264-58 and its blends was
390 larger than in those among Kristy and its blends (Table 2). Therefore, the large
391 differences in CSB quality attributes between the two control wheat flours blended
392 with WWF may be due to the differences in their pasting properties.

394 *3.5. Influence of waxy wheat flour substitution on staling of Chinese steamed bread*

395 CSB was tested for firmness, moisture content, thermal properties and soluble
396 starch content of crumb on days 0, 1 and 3 after preparation to evaluate the effects of
397 WWF substitution on the staling of CSB.

398 *3.5.1 Firmness*

399 On day 0, CSB made from OH04-264-58 containing 15-30% WWF was
400 significantly softer than that made from control flour; CSB made from Kristy
401 containing 5-30% WWF was also significantly softer than that made from control
402 flour (Table 4). The reduction in firmness of CSB at day 0 with increasing proportions
403 of WWF was likely due to the decrease in amylose content and the consequent
404 formation of a weak gel and crumb structure. These results disagree with Qin et al.
405 (2007) who reported that the firmness of CSB increased when the proportions of
406 WWF were more than 25%. This inconsistency might result from differences in CSB

formulation and changed trends in the volume of CSB made from flour blends containing WWF. The amount of water used for the preparation of CSB by Qin et al. (2007) was 55% of the flour weight regardless of the added proportion of WWF. In this study, the amount of water used for making CSB was optimized based on the mixograph absorption. Mixograph absorptions of the flour blends increased with increasing proportions of WWF, and consequently the amount of water used for making CSB was increased. An amount of water less than that required for making CSB probably resulted in the production of small-sized CSB with a compact volume. Qin et al. (2007) observed an increase in volume for CSB containing low levels of WWF, but a decrease for CSB containing high levels of WWF. The decreased volume of CSB due to WWF substitution was likely responsible for the increased firmness.

With increased storage time as observed on day 1, there was a sharp increase in the firmness of all CSB. CSB prepared from control wheat flours still exhibited the highest firmness values, whereas CSB containing 15-30% WWF remained significantly softer than the respective control wheat flours. A further increase in the firmness of CSB was observed at day 3, with still comparatively lower firmness values for CSB containing 15-30% WWF. While the increase in firmness of CSB during storage was inevitable, the incorporation of 15-30% WWF slowed the increase in firmness considerably. These results indicate that less than 10% WWF substitution was too low to influence CSB firmness, but 15-30% WWF substitution retarded the staling of CSB. Our results are consistent with two previous studies. Bhattacharya et al. (2002) reported that up to 30% substitution with waxy durum wheat flour resulted

in a decrease in the firmness of bread stored for 5 days, and Morita et al. (2002) showed that up to 40% substitution with WWF resulted in a decrease in the firmness of bread stored for 7 days. On the contrary, an increase in the crumb firmness of bread prepared with up to 50% WWF substitution was observed when stored for 4 days even though the volume of bread containing WWF was greater than that containing control wheat flour (Graybosch, 2001). These inconsistent observations could be due to the differences in bread formulation and production, storage conditions and the characteristics of bread baked from the flour blends in the respective studies.

3.5.2 Moisture content

The crumb moisture contents of CSB made from OH04-264-58 and Kristy were 39.5 and 40.1%, respectively. The moisture contents of CSB made from flour blends were 39.6-40.5%. There was no significant difference in the moisture contents of CSB crumb among control wheat flours and flour blends with WWF. The steaming process must make similar moisture contents of CSB even though the amount of water used in the CSB formulations was different. The moisture content of CSB crumb made from flour blends during storage was not different from that of the control wheat flour, indicating that the moisture loss over time was not fully responsible for the increased crumb firmness of CSB. This observation agrees with the reports by Sha et al. (2007) and Sheng et al. (2015) that moisture loss did not play a key role in crumb firmness of CSB.

3.5.3 Thermal properties

Thermal properties of CSB crumb are shown in Table 4. On day 0, there were

inconsistent differences in the DSC enthalpies of CSB crumb among flour blends and the respective control wheat flours. The starch retrogradation enthalpy of CSB crumb exhibited a substantial increase during the first day in all of the flour blends followed by a gradual but significant increase during the next two days. Sharp increases in the crumb firmness and starch retrogradation enthalpy of CSB on the first day of storage were observed and leveled off with further storage time, indicating that the starch retrogradation of CSB crumb is related to the staling of CSB during storage. This observation is consistent with Sha et al. (2007) and Sheng et al. (2015) who reported that starch amylopectin retrogradation is an important factor in CSB crumb firmness during storage.

As DSC measures the melting enthalpy of retrograded amylopectin molecules, CSB made from flour blends containing WWF during storage is expected to have greater DSC enthalpy than CSB made from control wheat flours; this is due to its much higher proportion of amylopectin molecules than the control wheat flours. However, no evident trend was observed for the enthalpy of CSB made from WWF blends, and CSB containing WWF maintained a softer crumb texture than that made from the respective control wheat flours.

Bhattacharya et al. (2002) showed that the increase in enthalpy over time was much lower in bread containing WWF than in the control counterparts, and they concluded that waxy wheat starch is resistant to retrogradation. On the other hand, Lee et al. (2001) showed that the increase in starch retrogradation enthalpy over time was much higher in bread with added waxy starches than in bread baked from regular

starch and gluten blends. Park and Baik (2007) reported that there were inconsistent differences in starch retrogradation enthalpies among wheat genotypes with different starch amylose contents after 1-day and 2-day storage. Additionally, Garimella Purna et al. (2011) reported that there were no differences in enthalpy values among breads containing WWF and the control wheat flour when stored for 7 days. These discrepancies were probably due to the differences in crumb moisture in the respective studies. Zeleznak and Hoseney (1986) reported that the retrogradation of starch amylopectin is dependent on the amount of water present. In this study, there were no differences in the crumb moisture contents among flour blends and the respective control wheat flours even during storage for 3 days. The rates of starch retrogradation were expected to be variable among CSBs similar in crumb moisture content but different in starch amylopectin content. It appears that the DSC enthalpy of starch retrogradation is not a suitable indicator of the staling of CSB prepared from flour blends containing different proportions of WWF. This result agrees with Park and Baik (2007) who reported that the DSC enthalpy of starch retrogradation failed to distinguish differences in the staling of bread containing starch with variable amylose content.

3.5.4 Soluble starch

Soluble starch content of CSB crumb, an indicator of starch retrogradation, could be used to assess the extent of staling of starch-based foods (Sha et al., 2007). The soluble starch content of CSB crumb increased with increasing proportions of WWF for fresh (0 day) CSB. Significant differences in soluble starch content were observed

between CSB made from flour blends containing 15-30% WWF and that prepared from control wheat flours. Our results agree with Garimella Purna et al. (2011) who showed that soluble starch content was significantly higher in bread crumb containing WWF than in control bread after 1-day storage. Starch amylopectin has a greater solubility than starch amylose, and the soluble starch of bread containing 30-45% hard WWF was mostly amylopectin (Garimella Purna et al., 2011). Sha et al. (2007) observed that amylopectin was the predominant component of soluble starch leached from the fresh CSB. Therefore, the increase in soluble starch content could be due to the increased amount of amylopectin in the flour blends.

From day 0 to day 1, a sharp decrease in the soluble starch content of all CSB was observed (Table 4), which could be due to the retrogradation of amylopectin in CSB. On day 3, the soluble starch content of all CSB showed a further decrease, but CSB containing 10% or more WWF still maintained higher soluble starch content than that prepared from the control wheat flours. With an increase in the proportion of WWF in the flour blends, the soluble starch content of CSB increased and the firmness of CSB crumb decreased. With WWF incorporation, the amount of amylose released from starch granules during steaming was reduced. Released amylose could form a gel between swelled starch granules (He and Hoseney, 1991) and is thought to be responsible for the rigidity of the bread (Hug-Iten et al., 2003). The combination of less amylose and more soluble starch from amylopectin could result in a soft crumb structure for CSB containing WWF on day 0, as well as after storage for 3 days. Based on the above results, it can be concluded that flour blends containing more than

10% WWF exhibit reduced CSB staling as the result of reduced starch amylopectin retrogradation indicated by the increased soluble starch content.

3.6. Influence of waxy wheat flour substitution on re-steamed Chinese steamed bread quality

The total score and firmness of re-steamed CSB were measured after storing at 4°C for 3 days. Quality attributes of re-steamed CSB were not significantly different from fresh CSB (data not shown), but re-steamed CSB exhibited a slightly higher firmness value than that of fresh CSB (Table 4). The re-steamed CSB containing 15-30% WWF still exhibited significantly lower firmness values than that prepared from control wheat flours. Retrograded starch in stored CSB was largely reverted by re-steaming. Higher firmness values of re-steamed CSB than fresh CSB can be explained by the fact that starch retrogradation is not the only factor involved in CSB staling (Sha et al., 2007). These results agree with Sheng et al. (2015) who reported that quality attributes of re-steamed CSB were not significantly different from freshly prepared CSB with only a slight increase in crumb firmness.

3.7. Optimal starch amylose content

Flour blends containing 15-30% WWF produced softer CSB than that made from control wheat flours (Table 4). However, flour blends with 20-30% WWF produced CSB with lower scores of stress relaxation, crumb structure, smoothness and total score than that prepared from control wheat flours (Fig. 1 and Table 3). Flour blends

containing 15% WWF with resulting 21.4-22.7% amylose content exhibited reduced the staling of CSB with total scores comparable to the respective control wheat flours.

4. Conclusions

The incorporation of WWF into the CSB formulation affected quality attributes and staling of CSB. Increasing proportions of WWF in the flour blends raised the mixograph absorption with little to no effect on protein content and dough strength-related parameters including SDSS volume, MPT and wet/dry gluten content. The analysis of covariance indicated that both protein content and dough strength-related parameters had little effect on CSB quality attributes, and that starch amylose content was largely responsible for the changes in CSB quality. Substitution with less than 10% WWF was too low to influence the firmness of CSB, but resulted in a higher specific volume without significantly affecting other CSB quality attributes. The incorporation of more than 10% WWF resulted in a retardation of the staling of CSB resulting from the reduced starch amylopectin retrogradation indicated by the increased soluble starch content. Flour blends containing 15% WWF, of which the amylose content was 21.4-22.7%, produced CSB with a softer crumb texture regardless of storage time, without causing a decrease in the total score of fresh and re-steamed CSB.

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Table 1Characteristics of soft red winter wheat flours blended with varying proportions of waxy wheat flour^a

Proportion (%) ^b	Amylose ^c (%)	Starch ^c (%)	Protein (%)	Wet gluten (%)	Dry gluten (%)	SDSS volume (mL)	Mixograph			658
							Water absorption (%)	Midline peak time (min)	Midline peak value (% Torque)	
OH04-264-58										
0	25.7	74.3	9.8c	23.6b	8.6b	42.0c	58.0	4.5a		41.7a
5	24.7	74.0	9.8c	23.5b	8.6b	43.3b	58.2	4.5a		41.9a
10	23.7	74.0	10.0bc	23.2b	8.5b	43.0b	58.3	4.5a		41.6a
15	22.7	73.9	10.0b	24.0b	8.5b	43.8ab	58.3	4.5a		41.9a
20	21.7	73.8	10.1ab	26.3a	9.7a	44.5a	58.6	4.5a		42.4a
25	20.6	73.8	10.2ab	26.8a	9.4a	43.5ab	58.7	4.5a		42.7a
30	19.6	73.7	10.3a	27.1a	9.4a	44.3a	59.5	4.5a		42.2a
Kristy										
0	24.4	70.9	9.8b	26.7b	9.1c	43.5ab	58.6	4.5a		46.4a
5	23.4	71.0	9.9b	27.1b	9.3bc	43.3b	58.6	4.5a		46.5a
10	22.4	71.1	10.0ab	27.6b	9.3bc	44.5a	58.8	4.5a		46.5a
15	21.4	71.2	10.1ab	27.3b	9.6bc	44.0a	58.8	4.5a		46.2a
20	20.5	71.3	10.1ab	27.8b	9.8ab	44.5a	59.0	4.5a		46.9a
25	19.5	71.5	10.2a	30.1a	10.3a	43.0b	59.1	4.5a		46.1a
30	18.5	71.6	10.2a	28.5ab	10.0ab	43.5ab	59.3	4.5a		46.5a

^a Results are expressed on a 14% moisture basis. ^b Proportion of waxy wheat flour to total wheat flour. ^c Amylose content and starch content of flour blends were calculated from the measured amylose content and starch content of normal and waxy wheat flours.

Values in the same column followed by the same letter are not significantly different at $P<0.05$.

Table 2

Pasting properties of soft red winter wheat flours blended with varying proportions of waxy wheat flour

Proportion (%) ^a	Peak viscosity (RVU)	Breakdown value (RVU)	Final viscosity (RVU)	Setback value (RVU)	Pasting temperature (°C)
OH04-264-58					
0	281a	123a	310a	152a	71.8a
5	272b	123a	291b	142b	71.0a
10	264c	111b	292b	139b	70.2ab
15	261c	110b	290b	139b	69.9bc
20	259c	111b	279c	131c	69.4bcd
25	247d	103bc	272c	128cd	69.3cd
30	234f	95c	262c	123d	69.0d
Kristy					
0	252a	113a	258ab	119a	70.2a
5	245ab	101b	259ab	115ab	69.4ab
10	247ab	99bc	264a	115ab	69.3b
15	241b	99bc	254bc	112ab	69.0bc
20	233c	95bc	249c	110b	68.6c
25	228c	94bc	252bc	117a	68.6c
30	225c	93c	246c	114ab	68.6c

^a Proportion of waxy wheat flour to total wheat flour.

RVU, Rapid viscosity analyzer unit.

Values in the same column followed by the same letter are not significantly different at $P<0.05$.

Table 3

Quality attributes of Chinese steamed bread prepared from soft red winter wheat flours blended with varying proportions of waxy wheat flour

Proportion (%) ^a	Specific volume (20)	Spread ratio (5)	Smoothness (10)	Crumb structure (15)	Brightness (5)	Skin color (5)	Crumb color (5)	Stress relaxation (35)	Total score (100)
OH04-264-58									
0	15.7c	3.0b	8.5a	12.0b	4.0a	3.5ab	4.0a	31.4a	82.1ab
5	16.8b	3.0b	8.5a	12.5ab	4.0a	4.0a	3.7a	30.5a	83.0a
10	16.3bc	4.0a	8.0a	13.0a	3.8a	3.2b	3.9a	29.5ab	81.7ab
15	17.9a	3.0b	8.0a	12.0b	3.5a	3.4b	3.7a	29.5ab	81.0ab
20	17.7ab	4.0a	7.0b	11.0c	3.9a	3.3b	4.0a	28.0b	78.9b
25	18.5a	3.0b	6.5c	9.0d	3.5a	3.3b	3.9a	23.0c	70.7c
30	18.4a	3.0b	5.5d	8.0e	3.0b	3.0b	3.7a	24.0c	68.6c
Kristy									
0	16.1c	5.0a	8.9a	13.0a	4.0a	4.0a	4.2a	33.0abc	88.2a
5	17.6b	5.0a	8.7a	12.7a	4.0a	3.8a	4.2a	35.0a	91.0a
10	17.7b	4.0b	8.7a	12.5a	3.6ab	3.5ab	4.0a	34.0ab	88.0a
15	18.1ab	4.0b	8.5ab	11.5b	3.5ab	3.5ab	4.0a	32.2bc	85.3ab
20	18.2ab	4.0b	8.0b	10.0c	3.5ab	3.4ab	4.0a	32.6bc	83.7bc
25	18.1ab	3.0c	8.0b	9.5c	3.0b	3.0b	4.0a	31.8cd	80.4cd
30	18.4a	2.0d	8.0b	8.5d	3.0b	3.0b	4.0a	30.0d	76.9d

^a Proportion of waxy wheat flour to total wheat flour.

Numbers in parentheses in the heading indicate maximum scores.

Values in the same column followed by the same letter are not significantly different at $P < 0.05$.

Table 4

Effects of waxy wheat flour substitution on the firmness, soluble starch and enthalpy of Chinese steamed bread at 0, 1 and 3 days after preparation and on the firmness of re-steamed Chinese steamed bread after 3-day storage

Proportion (%) ^a	Firmness of Chinese steamed bread (N)				Soluble starch (%)			ΔH (J/g)		
	0 day	1 day	3 day	Re-steamed	0 day	1 day	3 day	0 day	1 day	3 day
OH04-264-58										
0	22.0a	78.1a	100.0a	26.8a	2.3d	1.8d	1.6c	0.51b	3.1ab	3.9ab
5	21.9a	80.1a	97.1a	24.8ab	2.5d	1.9d	1.7c	0.42b	2.9b	3.9ab
10	21.0a	70.5b	93.9a	22.8abc	2.9c	2.4ab	2.0b	0.57ab	3.4a	4.4a
15	17.4b	61.2c	84.6b	20.9bc	3.0c	2.4ab	2.0b	0.69a	2.9b	3.5c
20	16.6b	65.6bc	83.3b	19.5c	3.3b	2.2c	2.0b	0.68a	3.0b	4.0ab
25	17.6b	61.6c	85.6b	19.3c	3.5b	2.3bc	2.0b	0.43b	3.1ab	3.8bc
30	13.3c	60.8c	78.0b	16.0d	3.8a	2.5a	2.2a	0.38b	2.8b	3.5c
Kristy										
0	21.7a	75.6a	89.0a	24.9a	1.3d	0.9d	0.8b	0.27b	2.0c	2.8bc
5	18.2b	73.2a	86.7ab	20.3b	1.4d	1.0cd	0.9b	0.25b	1.9bc	2.7bc
10	17.2bc	69.1ab	84.6ab	20.6b	1.5d	1.0cd	0.9b	0.29b	2.1bc	2.6c
15	15.7bc	65.5bc	81.4b	18.2bc	1.8c	1.1bc	0.9b	0.32ab	1.7c	2.6c
20	14.2cd	59.7cd	69.5c	17.0c	1.9bc	1.2ab	1.1a	0.41a	1.9c	2.7c
25	11.4d	56.4d	70.0c	15.1cd	2.1b	1.3a	1.2a	0.32ab	2.6ab	3.4a
30	11.2d	55.9d	68.5c	13.5d	2.5a	1.3a	1.2a	0.24b	2.7a	3.2ab

^a Proportion of waxy wheat flour to total wheat flour.

Values in the same column followed by the same letter are not significantly different at $P < 0.05$.

Figure caption

Fig. 1. Top surface (A and C) and cross sectional (B and D) views of Chinese steamed bread prepared from soft red winter wheat flours blended with varying proportions of waxy wheat flour. A and B were prepared from OH04-264-58, and C and D from Kristy. Values indicate the proportion of waxy wheat flour in flour blends.

Figure