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Volume, texture, and molecular mechanism behind the collapse of bread made with different levels of hard waxy wheat flours

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ABSTRACT

Physico-chemical properties of bread baked by partially replacing normal wheat (*Triticum aestivum* L.) flour (15, 30, and 45%) with two hard waxy wheat flours were investigated. Substitution with waxy wheat flour resulted in higher loaf volume and softer loaves. However, substitution at >30% resulted in excessive post-bake shrinkage and a 'key-hole' shape with an open crumb structure. Bread crumb microstructure indicated a loss of starch granule rigidity and fusing of starch granules. The cells in the interior of the bread did not become gas-continuous and as a result, shrunk as the loaf cooled. Soluble starch content was significantly higher in bread crumb containing waxy wheat flour than in control bread. Debranching studies indicated that the soluble starch in bread made with 30–45% hard waxy wheat flour was mostly amylopectin. Incorporation of waxy wheat flour resulted in softer bread immediately after baking but did not retard staling upon storage.

1. Introduction

Based on the level of amylose in its endosperm starch, wheat (*Triticum aestivum* L.) varieties are classified as full waxy, partial waxy, normal (wild-type) and high-amylose wheat (Graybosch, 1998; Nakumura et al., 1993, 1995). Full waxy wheat has little, if any amylose. A change in the ratio of amylose to amylopectin can result in altered textural attributes in food products, primarily because of differences in swelling and gelling properties. Because of its lack of amylose, waxy wheat can potentially reduce the initial phase of retrogradation i.e. rapid association of amylose molecules (Graybosch, 1998). A number of studies have been conducted to understand the potential of waxy wheat as a shelf-life extender of baked goods. Bread containing waxy wheat was reported to be softer than bread made with wild-type wheat immediately after baking (Graybosch, 2001; Morita et al., 2002a,b; Yi et al., 2009). Reduced amylose wheat used in a French bread formulation resulted in a soft crumb structure (Park and Baik, 2007). Incorporation of 10–50% waxy wheat flour into a white-pan bread formulation resulted in a high loaf volume immediately after baking (Bhattacharya et al., 2002; Graybosch, 2001; Morita et al., 2002a); however, the loaves collapsed upon storage and shrunk excessively within 24 h after baking (Lee et al., 2001; Morita et al., 2002a). The crumb structure of bread containing waxy wheat flour displayed a more open and porous structure compared to the control (Graybosch, 2001; Hung et al., 2007a,b; Lee et al., 2001).

Previous reports on the inclusion of waxy wheat flour in bread and its impact on staling have been inconsistent. When flour from near-isogenic waxy wheat lines was substituted (up to 40%) for wild-type flour in a white-pan bread formulation, the bread showed lower firmness for up to 7 days of storage as compared to the control (Morita et al., 2002a). When durum waxy wheat flour was used (up to 30%), the resulting loaves showed lower firmness than the control (Bhattacharya et al., 2002). In contrast to those studies, when flours from waxy wheat lines were substituted for stronger hard red winter wheat flour (up to 50%), the rate of crumb firming was higher than the control (Graybosch, 2005). Compared to the bread made with commercial normal white flour, the firmness of breadcrumbs with 30% and 50% whole waxy wheat flour was lower after one day of storage but increased quickly after 3 days of storage (Hung et al., 2007a). In a separate study, incorporation of waxy wheat flour in bread was reported to increase the moisture retention capacity of crumb during storage (Park and Baik, 2007).

In addition to the inconclusive conclusion on the impact of waxy wheat flour on bread staling, the reasons why waxy wheat flour causes the collapse of bread loaves upon storage are not clearly

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understood. Objectives of this study were to (i) evaluate the impact on white-pan bread of incorporating 15–45% of total flour weight with hard waxy wheat flour from advanced breeding lines; (ii) understand and explain the underlying mechanism of loaf collapse in bread containing high levels of waxy wheat flour; and (iii) clarify the impact of waxy wheat flour on bread staling.

2. Materials and methods

2.1. Materials

Control wild-type wheat (Karl 92) and two waxy wheats, NX03Y2114 (sample 2114) and NX03Y2489 (sample 2489) from advanced breeding lines were procured from USDA-ARS, Lincoln, NE. The pedigree of sample 2114 was Cimarron/Rio Blanco//Baihou4/L910145/3/Colt/Cody//Stozher/NE86582 and that of sample 2489 was BaiHuo/Kanto107//Ike/3/KS91H184/3*RBL//N87V106. Wheat kernels were tempered to 16% moisture for 18 h and roller-milled into straight-grade flour on an MLU 202 Bühler experimental mill (Bühler Co., Uzwill, Switzerland). The protein contents of the flours were 11.44, 13.01, and 13.25 (%db) for Karl 92, sample 2114 and sample 2489, respectively, and the starch contents were 76.7, 75.0, and 80.0 (%db) for Karl 92, sample 2114 and sample 2489, respectively, as previously reported (Guan et al., 2009).

2.2. Dough mixing characteristics

Dough characteristics were measured using a 10 g mixograph according to AACC 54–40 A (AACC International, 2000). Water absorption was initially calculated based on protein content by using AACC 54–40A, but was finally optimized for each sample based on series of mixograms (Guan et al., 2009).

2.3. Gas generation from flours using Risograph

Gas generated from liquid ferment of flours was measured by using a modified AACC 89-01 method (AACC International, 2000). Instant yeast (0.4 g) (Lesaffre Yeast Corp., Milwaukee, WI) and distilled water (15 mL) were added to each flour (10 g) and mixed for 1 min in Risograph (RDesign, Pullman, WA) containers by using a glass rod, which was left in the container. The containers were connected to the Risograph and the rate and the total amount of carbon dioxide released from liquid ferment was measured at 30 °C over a 90-min period.

2.4. Enzyme digestion of flours and release of D-glucose

Enzyme digestion of flours was done using a modified Englyst method (Englyst et al., 1992). The enzyme mixture was prepared by adding 3.0 g of pancreatin (P-7545, Sigma Aldirch, St. Louis, MO) to 20 mL of distilled water, mixing for 10 min and centrifuging at 4000× *g* for 10 min. An aliquot (15 mL) of supernatant was transferred into a solution of 60 mg of amyloglucosidase (A-7255, Sigma–Aldrich, St. Louis, MO) in 1.7 mL distilled water. Flour samples (0.60 g) were suspended in 10 mL of distilled water and incubated for 30 min at 37 °C. Subsequently, 10 mL of 0.25 N sodium acetate and 5 mL of the enzyme mixture were added to the suspension which was then incubated up to 180 min at 37 °C with continuous mixing. At time intervals of 20, 40, 60, 90, 120 and 180 min, 0.25 mL of solution was transferred into 25 mL glass tubes containing 10 mL of 66% ethanol. The tubes were centrifuged at 4500× *g* for 10 min. The supernatant (0.1 mL) was transferred into 10 mL glass tubes and 3.0 mL glucose oxidase–peroxidase (GOPOD, Megazyme Kit, Wicklow, Ireland) was added immediately. The tubes were incubated at 40 °C for 20 min, and the absorbance was measured against a reagent blank at 510 nm.

2.5. Bread baking

Pup-loaf bread was baked using the AACC 10–10B (AACC International, 2000) straight dough method with 90-min fermentation time. The baking formula (flour basis) was 100.0 g flour (14% mb), 6.0 g sucrose, 3.0 g shortening (Crisco®, Orville, OH), 2.0 g yeast, 1.5 g salt, 50 mg L-ascorbic acid (Merck, Darmstadt, Denmark) and 0.5 g diastatic malt (King Arthur Flour, Norwich, VA). For breads made with 15–45% levels of waxy wheat flour, Karl 92 flour was partially replaced on a dry weight basis with one of the two hard waxy wheat flours (2114 or 2489). Additionally, pup-loaf breads were baked using 100% waxy wheat flour. Four loaves of bread were baked for each formulation.

Loaf weight and loaf volume (rapeseed displacement AACC 10-05, AACC International, 2000) were measured immediately, 1 h and 24 h after removal from the oven, and specific volume data were reported. The loaves were double bagged in polypropylene bags and stored at room temperature. On day 1 and day 7 after baking, two loaves of each formulation were sliced into 1″ thick slices. The two slices from the middle were analyzed. Characteristics of bread crumb were determined using C-Cell (Calibre Control Intl., Warrington, UK), an image analysis instrument, to obtain an image of the slice and data on number of gas cells, gas cell volume, cell wall thickness and slice brightness. Moisture content of the slices was determined by AACC 44-15A (AACC International, 2000).

2.6. Texture analysis

Firmness was measured by a modified AACC 74-09 method (AACC International, 2000). Bread slices were tested using a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, N.Y.) with a 36 mm cylindrical probe. Each slice was compressed to a 7 mm distance. Firmness was calculated as the peak force at 7 mm. Firmness values reported were the average of three measurements.

2.7. Soluble carbohydrate in bread crumbs

Bread samples were analyzed for soluble carbohydrate (starch) content and molecular weight distribution. Soluble carbohydrate content was determined by a modified AACC 76-13 method (AACC International, 2000) (Megazyme Kit, Wicklow, Ireland). Soluble starch was extracted by mixing 100 mg of freeze-dried bread with 1.5 mL of water in a 2.0 mL microcentrifuge tube. The sample was vortexed for 45 s and centrifuged at 12,000× *g*. The supernatant (1.0 mL) was immediately transferred to a test tube containing 3.0 mL of thermostable α-amylase (300 U) in MOPS buffer (50 mM, pH 7.0). The contents of the test tube were vigorously mixed and incubated in a boiling water bath for 6 min with intermediate stirring at 2 and 4 min intervals. The test tube was placed in a 50 °C water bath and sodium acetate buffer (4.0 mL, 200 mM, pH 4.5), followed by amyloglucosidase (0.1 mL, 20 U) were added. The contents were thoroughly mixed and the test tube was incubated in a 50 °C water bath for 30 min. The volume of the test tube contents was adjusted to 10.0 mL with distilled water and centrifuged at 3000× *g* for 10 min. An aliquot (0.1 mL) of the supernatant was transferred to a test tube to which 3.0 mL of glucose oxidase peroxidase (GOPOD) reagent was added. The tubes were incubated in a 50 °C water bath for 30 min. Absorbance of the samples was taken at 510 nm against the reagent blank and D-glucose was used as the reference standard. Percent soluble starch was calculated based on the starch content of the flour. An average of three replicates was reported as total soluble carbohydrate (%).

Molecular weight distribution of soluble carbohydrate was determined by gel permeation chromatography (GPC). Freeze-dried soluble starch was dissolved in 1.0 mL of dimethyl sulphoxide (DMSO) in 2.0 mL microcentrifuge tubes to obtain a final concentration of 0.1% starch. The GPC analysis was performed with a Polymer Laboratory (Amherst, MA) PL-GPC 220 Integrated GPC/SEC fully automated system as previously described (Cai et al., 2010).

2.8. *Thermal properties of bread*

Thermal properties of bread crumb were determined by using differential scanning calorimetry (DSC) (Q100 DSC, TA Instruments, New Castle, DE). Freeze-dried bread samples (10 mg) from day 1 and day 7 and distilled water were added to the DSC pan in a 1:2 ratio (w/w). The pan was hermitically sealed and allowed to equilibrate at 25 °C for 1 h. The samples were then heated from 10 °C to 140 °C at 10 °C/min. An empty DSC pan was used as a reference. Onset, peak and completion temperatures along with enthalpy were determined. Each sample was analyzed in duplicate and average values were reported.

2.9. *Scanning electron microscopy (SEM)*

A small piece (<1 mm^3) of freeze-dried bread crumb was fixed on specimen stubs using carbon paste. The samples were coated with gold–palladium by a sputter coater (Denton Vaccuum, LLC., Moorestown, NJ). The samples were viewed at 300× and 1000× resolution with a scanning electron microscope (S-3500N, Hitachi Science Systems, Ltd., Japan) operating at an accelerating voltage of 20 kV. Each sample was analyzed two times.

2.10. *Confocal laser scanning microscopy (CLSM)*

Slides of freeze-dried bread samples were prepared based on the methods of Lee et al. (2001) and Schober et al. (2004). A small piece (<1 mm^3) of freeze-dried bread crumb was placed on a microscopic slide and a weakly alkaline solution of flourescein 5(6)-isothiocyanate (FITC) (Sigma–Aldrich, St. Louis, MO) was added to the sample. The slide was air-dried at room temperature in a dark environment for 1 h. Prior to analysis, immersion oil was dropped on the sample and the sample was covered with a cover slip. A Zeiss LSM 5 Pa CLSM (Ziess, Gottingen,Germany) was used to view the microstructure of the bread crumb. Fluorescence emission imaging of FITC was done using the 488 nm line of a 458/488/514 argon gas ion laser to excite FITC. Overlaid images of birefringent starch granules and florescent protein matrix were used to compare the internal structures of different bread samples. Each sample was analyzed two times.

2.11. *Statistical analysis*

Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis, MN) was used to perform ANOVA and honest significance difference (HSD) analysis. The level of significance was $p < 0.05$ for all data analyses.

3. Results and discussion

3.1. *Flour and dough properties*

Protein content of Karl 92, waxy wheat samples 2114, and 2489 were 15.47, 13.88 and 12.82%, respectively (previously reported by Guan et al., 2009). As previously reported (Guan, 2008), the optimized mixograph data indicated that the wild-type wheat flour (Karl 92) had a longer peak time (4.82 min) than the two waxy wheat samples (4.22 and 3.41 min for samples 2114 and 2489, respectively). Additionally, sample 2114 had higher peak height (59.5%) and sample 2489 had lower peak height (47.4%) when compared to wild-type Karl 92 wheat flour (55%). Water absorption capacity for Karl 92, sample 2114 and 2489 were 60.8, 66.4, and 57.7%, respectively. The high water absorption capacity of sample 2114 is probably due to its high arabinoxylan content and small flour particle size (Garimella Purna, 2010). In this study, the blends of Karl 92 wheat flour and each waxy wheat flour were further examined by mixograph and the optimum water absorption and mixing time were determined from a series of mixographs (Table 1). When the normal wheat flour was replaced by 45% waxy wheat flour, the water absorption increased when sample 2114 was added to the blend, but slightly decreased when sample 2489 was added. The optimum mixing time decreased with increasing incorporation of waxy wheat flour.

Table 1
Mixing time and level of water absorption used for dough making (based on series of four mixographs).

Replacement	Waxy flour 2114		Waxy flour 2489	
	Absorption (%)	Time (min)	Absorption (%)	Time (min)
0% (Control[a])	62.0	5.5	62.0	5.5
15%	62.0	5.0	62.0	5.0
30%	63.0	5.0	61.0	4.75
45%	63.5	4.75	61.0	4.5

[a] Karl 92 was base flour.

3.2. *Bread volume and microstructure of crumb*

3.2.1. *Volume*

Changes in specific loaf volume (cc/gm) are given in Fig. 1. Immediately after baking, the specific volume of bread loaves containing waxy wheat flour was significantly higher ($p < 0.05$) than the Karl 92 control, and was highest for bread loaves containing 100% waxy wheat flour. These results are consistent with findings by previous researchers (Morita et al., 2002a,b; Yi et al., 2009) who reported an increase in volume of breads baked with waxy wheat. The higher loaf volume in waxy wheat breads could be due to higher gas (carbon dioxide) production during fermentation of waxy wheat flours (Fig. S1A). In our study, the starch in waxy wheat flour was more readily digestible than starch in wild-type flour (Fig. S1B). Liquid ferment from waxy wheat flour 2489 released approximately 100% more carbon dioxide than wild-type flour during the 120-min fermentation time (Fig. S1A). Higher amounts of damaged starch in waxy wheat (Bettge et al., 2000 and Garimella Purna, 2010) provided readily fermentable sugars during yeast fermentation (Lee et al., 2001). However, it should be noted

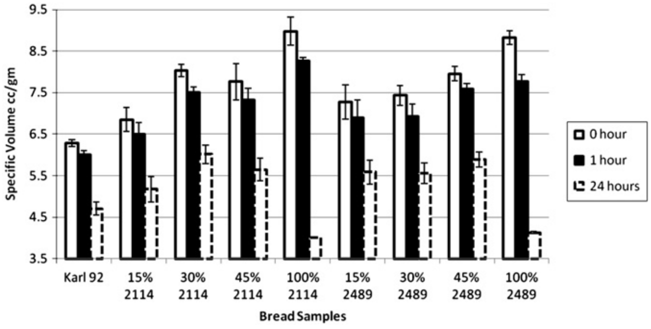


Fig. 1. Changes in bread specific volume after baking ($N = 4$).

that sucrose provided readily fermentable sugar in the bread formula whereas no sucrose was added in the Risograph experiment. The differences in specific loaf volumes were not significantly different ($p > 0.05$) between the two waxy wheat samples at all substitution levels, although there were differences between the two waxy wheat samples with respect to gas production in a liquid ferment (Fig. S1A) and their dough mixing properties (Table 1).

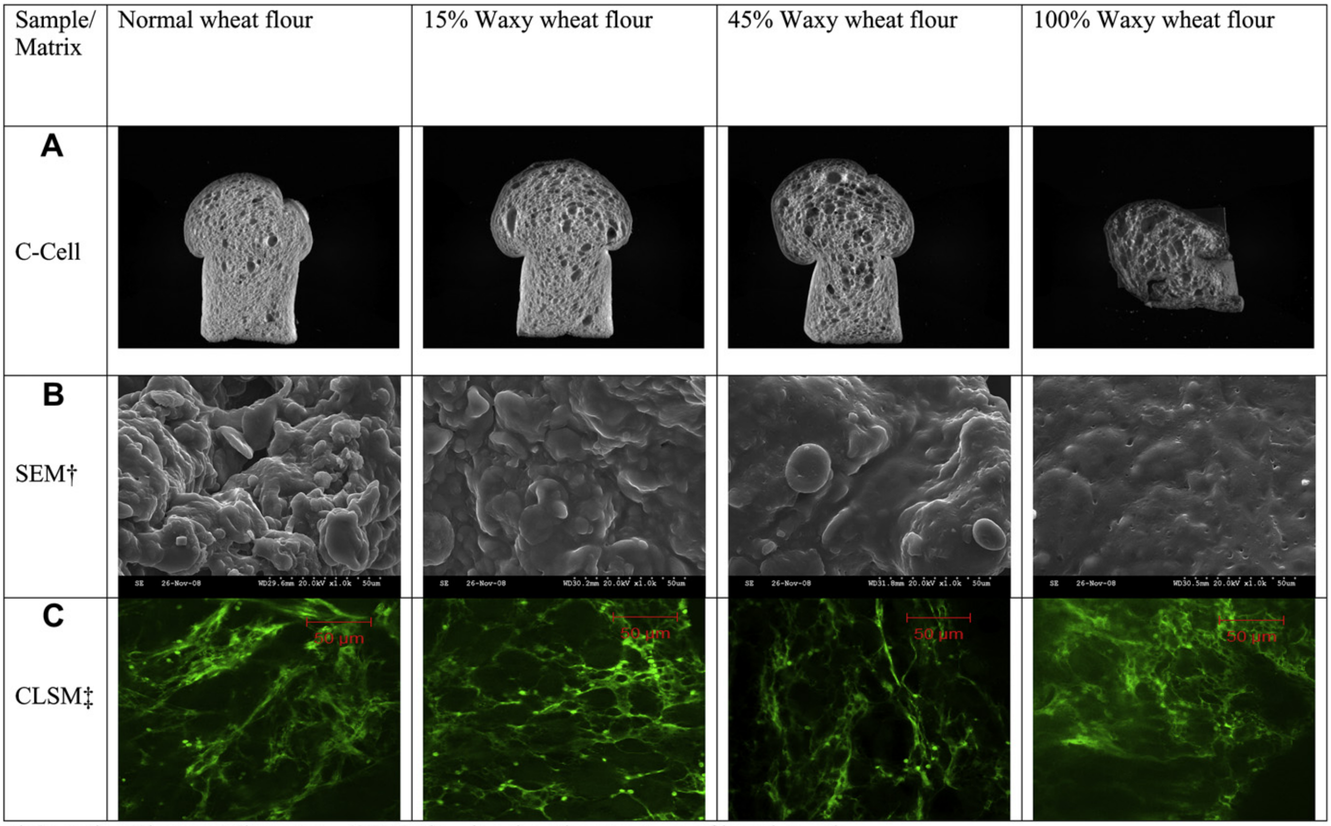
3.2.2. *"Keyhole" effect*

There was a considerable decrease in specific volume from 0 min (immediately after baking) to 24 h after baking for all formulations. The decrease in specific volume with time was higher in formulations containing higher levels of waxy wheat flour. Excessive shrinkage of loaves containing 45 and 100% waxy wheat flour resulted in a "keyhole" effect (Fig. 2A). Bread made with 100% waxy barley starch has been reported to collapse (Hoseney et al., 1978) or shrink excessively (keyhole) (Ghiasi et al., 1984) after baking. Kusunose et al. (1999) studied the impact of wheat, potato and tapioca starches in bread and attributed excessive post-baking shrinkage in bread containing tapioca starch to its lower pasting temperature and fusing of starch granules into a continuous network. Waxy wheat starch granules have a lower pasting temperature than wild-type starch granules (Guan, 2008). Moreover, waxy wheat starch swells rapidly and the granules lose structural integrity and disintegrate at temperatures around 70 °C (Guan, 2008). SEM showed that starch granules maintained integrity in Karl 92 control bread crumb (Fig. 2B) but waxy wheat bread crumb, especially those containing 100% waxy wheat, had a fused starch granule network (Fig. 2), similar to the microstructure of bread made from tapioca and waxy barley (Ghiasi et al., 1984; Kusunose et al., 1999). The fusing of starch granules became more evident as the level of waxy wheat in bread crumb increased. Additionally, CLSM showed the protein network in bread crumb made with high levels of waxy wheat appears to be elongated between the starch granules (Fig. 2C).

From dough to bread, there is a phase inversion during which foam structure of dough is converted into sponge i.e. bread (Bloksma, 1981). During mixing, air is incorporated in the form of small nuclei/cells into the dough (Baker and Mize, 1946; MacRitchie, 1976). The gas cells are surrounded by a starch gluten matrix, and this matrix acts as a cell wall. These gas cells expand during proofing as they are filled with fermentation gases, and during baking, the gas cells expand with increasing temperature (He and Hoseney, 1991). Up to this point, dough is considered to be a closed cell foam that retains CO_2 (Hoseney, 1986). During the later stages of baking, cross linking of proteins along with gelatinization of starch leads to a rupture in the cell wall, allowing the gas to escape from crumb to crust (Bloksma, 1981). After baking, the leached amylose forms a gel between the swollen starch granules during cooling and could be responsible for the setting or rigidity of the loaf. Baked bread is considered to be an open celled sponge that is permeable to air (Baker and Mize, 1946). We postulate that when dough containing waxy wheat flour is baked, fusing of waxy starch granules prevents the cell walls from becoming gas-continuous. The cell walls are impermeable. When high amounts of carbon dioxide are produced during baking, the cell walls expand to their maximum but fail to rupture thereby continuing to maintain their foam structure. During cooling, the cell walls shrink due to negative internal pressure and result in a keyhole effect.

3.2.3. *C-Cell*

Bread containing high levels of waxy wheat flour had an open crumb grain (Fig. 2A). C-Cell results show that as the level of waxy



† Scanning Electron Microscopy (SEM) showing the microstructure of bread crumb
‡ Confocal Laser Scanning Microscopy (CLSM) showing the protein matrix in bread crumb

Fig. 2. Changes in bread structure with inclusion of waxy wheat flour (sample 2114) (24 h after baking).

wheat flour in the bread formulation increased, gas cell volume increased and the number of cells decreased (Table S1). Control bread (Karl 92) had a higher number of cells, but the volume of the cells was lower (Table S1, Fig. 2A). Thus the control bread contained a higher number of smaller size cells than bread containing 45% waxy wheat. Enzyme digestibility data indicated that compared with the control (Karl 92) flour, the starch in waxy wheat flours was more readily digestible by enzymes which could have contributed to the increased gas (carbon dioxide) released by the yeast in the liquid ferment (Fig. S1). Gas produced during fermentation is typically transported to gas nuclei that were formed during dough mixing (Gan et al., 1990), and the greater gas production in dough systems with waxy wheat flour could result in large gas cells. Those large gas cells expand during baking, creating an "open" crumb structure in the resultant bread. Alternatively, gas cells can coalesce during bread making when waxy wheat flour is added because of the excessive swelling of waxy wheat starch granules. Guan (2008) noted that waxy starch granules swell excessively and lose granule integrity upon heating in excess water. Excessive swelling of waxy starch could be the cause of open crumb structure.

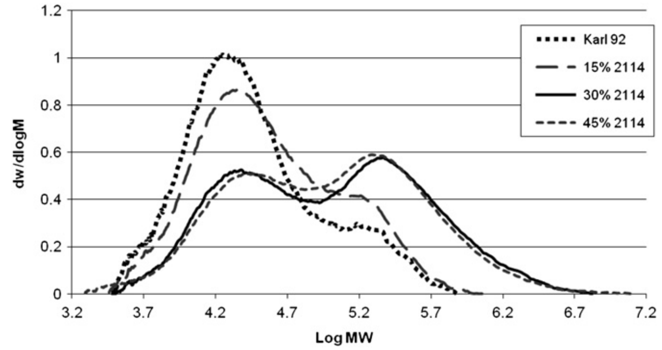


Fig. 4. Molecular weight distribution of soluble starch profile in breads made with normal wheat flour (Karl 92) and with inclusion of waxy wheat flour (sample 2114) after 1 day of storage.

3.3. Soluble starch and structure

In general, the firmness decreased (Fig. 3A) and solubility of starch increased (Fig. 3B) as the percentage of waxy wheat increased (Fig. 3B). This could be because amylopectin has greater solubility than amylose in 1- and 7-day old bread. The control formulation had more amylose than the formulations containing waxy wheat flour. GPC showed differences between the control and the waxy wheat sample 2114 (Fig. 4). Similar results were obtained for sample 2489. Soluble starch from control bread (Karl 92) had a prominent peak in the low molecular weight region plus a shoulder peak in the higher molecular weight region. As the level of waxy wheat flour was increased from 15 to 45%, the distribution became bimodal, with the peaks being almost equally intense in the low and high molecular weight regions. The same phenomenon was observed for both waxy varieties. An increase in the replacement level of waxy wheat flour resulted in an increase in soluble starch in the bread crumb. The increase in soluble starch content could be due to the ease of fragmentation of waxy starch granules (Guan, 2008). Our results indicate that most of the soluble starch observed at high molecular weight was amylopectin (Fig. 4), which agrees with previous studies (Schoch and French, 1947; Ghiasi et al., 1979). Overall, amylose content (the low molecular weight peak) decreased when wild-type wheat flour was partially replaced with

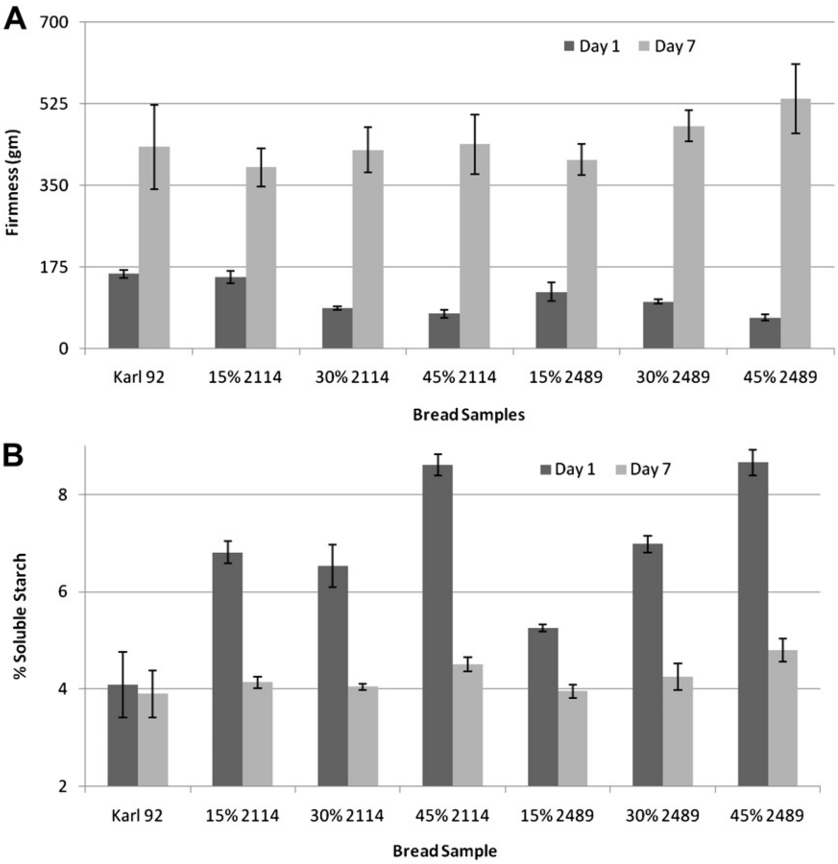


Fig. 3. Changes in (A) firmness and (B) soluble starch of bread samples ($N = 4$) day 1 and day 7 after baking.

waxy wheat flour; therefore, the amount of amylose leached during baking was reduced. Leached amylose forms a gel between swollen starch granules (He and Hoseney, 1991) and is thought to be responsible for the setting or rigidity of the loaf (Hug-Iten et al., 2003; Ghiasi et al., 1979). The combination of less amylose and more soluble starch from amylopectin could result in a soft crumb structure on day 1 and shrinkage after baking for bread that contains a high level of waxy wheat flour.

From day 1 to day 7, the percentage of soluble starch decreased (Fig. 3B), which could be due to retrogradation of amylopectin in bread. On day 7, there was no difference in the percentage of soluble starch between the control, 15% replacement, and 30% replacement, which contained, respectively, ~75, 79 and 83% amylopectin in starch. However, the 45% replacement with ~86% amylopectin in its starch had slightly more soluble starch.

3.4. Texture and effect of waxy wheat on staling

On day 1, bread slices from loaves containing waxy wheat flour were significantly softer than control bread (Fig. 3A). Firmness decreased (Fig. 3A) and bread volume increased (Fig. 1) as the level of waxy wheat flour in the formulation increased. The lower firmness could be due to the lower amylose content in waxy wheat bread formulations. Previous researchers (Biliaderis, 1992; Hug-Iten et al., 2003) have attributed the initial firmness of bread crumb to rapid re-association of the amylose fraction. It was not possible to get consistent firmness values from 100% waxy wheat loaves. The crumb of the 100% waxy wheat loaves was too fragile and the testing surface was too small to measure properly. On day 7, there was not a significant difference in firmness between any of the loaves (Fig. 3A).

Our firmness results from day 1 are consistent with previous studies (Graybosch, 2001; Hung et al., 2007a and b), which reported that loaves made from formulations containing waxy wheat flour were softer than loaves from formulations with wild-type wheat flour. On day 7, there were no significant differences in firmness between bread crumbs containing waxy wheat and control samples. Our results are contrary to two previous studies (Morita et al., 2002a; Bhattacharya et al., 2002). Bhattacharya et al. (2002) reported a decrease in firmness over 5 days when waxy durum wheat flour was substituted at low levels (up to 30%), and Morita et al. (2002a,b) reported a decrease in firmness over 7 days when waxy wheat flour was substituted at low levels (up to 40%). However, some previous studies (Graybosch, 2001, 2005; Hung et al., 2007b) reported an increase in firmness upon storage for bread crumbs made with partial replacement with waxy wheat flour. The differences in staling results could be due to different control and waxy flours used in the baking formulations.

It should be emphasized that, in the present study, bread made with high levels of waxy wheat was softer than the control on day 1 but became as firm as the control upon storage, which correlates with the change in soluble starch (Fig. 3). These results are consistent with previous findings by Ghiasi et al. (1984), who reported that bread loaves containing waxy barley starches were softer than the control on day 1 but had equal firmness after three or five days of storage. Amylose retrogrades rapidly during initial cooling of bread and slow changes in amylopectin are responsible for further firming of bread after day 1 (Kim and D'Appolonia, 1977). Inagaki and Seib (1992) also reported that crumb of bread made with cross-linked waxy barley starch was softer 6 h after baking but firmed much faster during further storage at 25 °C than that of the control bread made with prime wheat starch.

Table 2
Thermal properties of bread samples measured by differential scanning calorimetry (DSC) ($N = 2$).

Sample	T_{onset} (°C)	T_{peak} (°C)	T_{end} (°C)	ΔH (J/g)
Day 1				
Karl 92	46.1 ± 0.4^{a}	58.2 ± 2.0^{a}	69.4 ± 0.5^{bc}	1.9 ± 0.1^{c}
15% 2114	45.8 ± 1.1^{a}	57.0 ± 0.1^{ab}	71.4 ± 1.1^{a}	2.2 ± 0.3^{abc}
30% 2114	45.6 ± 0.8^{ab}	56.7 ± 0.2^{ab}	69.5 ± 0.7^{bc}	1.9 ± 0.1^{c}
45% 2114	43.9 ± 1.6^{ab}	57.3 ± 0.4^{ab}	72.0 ± 0.5^{a}	2.4 ± 0.1^{a}
15% 2489	44.2 ± 0.3^{ab}	56.2 ± 0.2^{b}	71.3 ± 1.0^{ab}	2.5 ± 0.1^{ab}
30% 2489	45.6 ± 0.9^{ab}	56.5 ± 0.3^{ab}	69.2 ± 1.0^{c}	2.0 ± 0.1^{bc}
45% 2489	43.0 ± 1.8^{b}	55.9 ± 0.9^{b}	72.0 ± 0.2^{a}	2.5 ± 0.3^{a}
Day 7				
Karl 92	44.4 ± 1.7^{a}	55.4 ± 0.7^{b}	70.5 ± 2.1^{ab}	4.0 ± 0.8^{ab}
15% 2114	44.7 ± 1.1^{a}	58.0 ± 0.5^{a}	72.4 ± 0.4^{a}	3.8 ± 0.5^{ab}
30% 2114	43.1 ± 1.5^{a}	54.9 ± 0.6^{b}	71.1 ± 0.7^{ab}	4.4 ± 0.6^{a}
45% 2114	45.2 ± 1.1^{a}	55.8 ± 1.2^{b}	70.6 ± 0.3^{ab}	3.6 ± 0.3^{ab}
15% 2489	44.9 ± 0.5^{a}	55.5 ± 1.0^{b}	70.0 ± 0.2^{b}	3.1 ± 0.1^{b}
30% 2489	44.7 ± 0.2^{a}	55.4 ± 0.7^{b}	70.8 ± 1.1^{ab}	4.2 ± 0.4^{a}
45% 2489	45.4 ± 0.5^{a}	55.4 ± 0.1^{b}	69.9 ± 0.8^{b}	3.4 ± 0.1^{ab}

Mean ± standard deviation values are reported.
Different letters within each day and each column denote significant differences among the samples ($p < 0.05$).

Thermal properties of bread crumb were shown in Table 2. After baking, starch retrogradation is a biphasic phenomenon of starch with rapid association of amylose followed by less rapid recrystallization of amylopectin (Biliaderis, 1992; Hug-Iten et al., 2003). The endothermic peak observed in DSC at onset temperature (T_o) 43.0–46.1 °C was due to the melting of retrograded amylopectin. On day 1, bread crumb containing 45% waxy wheat had higher enthalpy, presumably due to the increased level of amylopectin. However, crumb firmness decreased on day 1 as the level of waxy wheat flour increased, because of the reduced contribution of amylose retrogradation. From day 1 to day 7, there was a smaller increase in enthalpy in bread crumbs containing 45% waxy wheat flour compared with bread crumbs made with wild-type wheat flour, despite the fact that bread crumb containing 45% waxy wheat flour had a higher level of amylopectin. The low retrogradation from waxy wheat flour is consistent with previous researchers (Hayakawa et al., 1997) and with our earlier experimental evidence from DSC analysis of starch-based gels, which indicated a marked resistance of waxy wheat starch to retrogradation (Guan, 2008). Overall, there was no difference in enthalpy values between bread containing waxy wheat flours and the control wheat (Karl 92) on day 7. Additionally, all bread had similar firmness and starch solubilities.

4. Conclusions

Substituting waxy wheat flour in a white-pan bread formulation resulted in increased loaf volume, but significant post-bake shrinkage occurred in formulations with higher levels (>30%) of waxy wheat flour. Disintegration and fusing of starch granules was observed in bread containing high levels of waxy wheat flour. The cells in the interior of the bread did not become gas-continuous, which explains the excessive loaf volume and high post-bake shrinkage. Partial replacement of waxy wheat flour resulted in softer fresh bread immediately after baking but did not retard staling during storage (7 days).

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.jcs.2011.02.008.

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