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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 inconsistent 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 (%).