International Journal of Natural and Engineering Sciences 3 (3): 89-96, 2009
ISSN: 1307-1149, www.nobel.gen.tr

Dough-Mixing Characteristics of Wa xy Wheat Flours and Breadmaking Qualities of Waxy Wheat Glutens

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Received: July 04, 2009
Accepted: August 10,2009

ABSTRACT

Potential uses of recently developed waxy wheats have been under heavy investigation. The objectives of this study were to investigate the dough-mixing characteristics and breadmaking qualities of vital glutens isolated from waxy wheat flours. Kernel properties of the waxy wheats were comparable with the partial waxy and nonwaxy control wheats. However, the waxy wheats yielded about 5% less flour. Amylose contents of the waxy, partial waxy and nonwaxy flours were, respectively, <1.5, 21.3, and 27.6%. All of the waxy flours contained reduced levels of total starch, whereas several waxy flours possessed elevated levels of pentosans. Dough-mixing characteristics of the waxy flours were inferior to the controls. The waxy flours had shorter mixing times with lower mixing tolerances. In a starch-stress baking test, the vital glutens isolated from the waxy and control flours yielded breads with comparable loaf volumes and crumb grain scores. This indicates that vital glutens isolated from waxy wheats can perform similarly to the vital glutens isolated from partial waxy or nonwaxy wheat flours when used in bread formulations for the purpose of dough-strengthening.

Keywords: Waxy wheat, vital gluten, breadmaking quality

INTRODUCTION

Waxy or amylose-free wheats (*Triticum aestivum*) have been developed in recent years by several groups of researchers [1-8]. It was discovered in 1994 that some hexaploid wheats were deficient in one or two of the three possible waxy proteins in their starches. Waxy protein is another name given for granule-bound starch synthase (EC 2.4.1.21), the enzyme associated with amylose synthesis [9]. The waxy proteins, namely *Wx-A1*, *Wx-B1*, and *Wx-D1*, are respectively coded by the homologous genes located on the chromosome arms 7A, 4A, and 7D [1, 9]. Wild-type (nonwaxy) wheats contain all three waxy proteins and produce ~25% amylose in their starches, whereas waxy wheats lack all three waxy proteins with essentially no amylose or <1% amylose in their starches. On the other hand, reduced-amylose (partial waxy) wheats are deficient in one or two of the three possible waxy proteins and thus contain amylose ranging from 15 to 25% in their starches [5, 10].

Hexaploid waxy wheats were first produced in Japan by genetically eliminating the three waxy proteins through the cross-breeding technique, where Bai Huo, a Chinese wheat cultivar that lacks the *Wx-D1* protein, was crossed with Kanto 107, a Japanese cultivar that lacks the *Wx-A1* and *Wx-B1* proteins [1]. Later, hexaploid waxy wheats were also developed by cross-breeding in Canada [2] and in the USA [5, 8]. In addition, mutagens have been used to produce waxy wheats, where partial waxy wheats Kanto 107 and Ike, which are null in *Wx-A1 and Wx-B1* proteins, were separately treated with the mutagen ethyl methanesulphonate to remove their *Wx-D1* proteins [3-4]. Besides the hexaploid waxy wheats, developments of tetraploid (durum) waxy wheats by cross-breeding [7] and diploid waxy wheats by mutagenesis [6] were reported.

Potential uses of waxy wheats have been the focus of interest for many wheat breeders and cereal scientists [5, 10-13]. Waxy wheat flours are reportedly not suitable for the production of breads, noodles, and tortillas as they often cause a gummy internal structure in those products [13-19]. However, blends of waxy wheat flours with nonwaxy or partial waxy wheat flours at various levels may find uses in bakery products for shelf-life extension, extruded products for volume expansion, frozen doughs and bakery foods for cold-temperature stability, and in the production of maltodextrins with reduced rancid off-flavor [5, 10-13, 19]. Also, waxy wheat flours are very likely to be used by the wet-milling industry for the production of waxy wheat starch. For the time being, wet-milling to co-produce waxy starch and vital gluten appears to be the most potential area of utilization for waxy wheats [5, 20-21].

The wet-milling properties of waxy wheat flours [20-23] and physicochemical properties of their isolated waxy starches have been widely investigated [6, 11, 24-29]. However, no published data are available on the breadmaking characteristics of vital glutens isolated from waxy wheat flours. Being a valuable co-product of the commercial wheat starch production, vital wheat gluten is commonly used in the bakery products to improve product quality by increasing flour protein content and/or quality [20, 30-35]. Vital wheat gluten is also used in the production of breakfast cereals and snacks; pizza, meat and cheese analogs; breading and batter mixes; and in specific meat, fish and poultry products [32, 36]. The objectives of this study were therefore to investigate the dough-mixing characteristics of waxy wheat flours and breadmaking qualities of vital glutens isolated from several hexaploid waxy wheat flours.

MATERIALS AND METHODS

Materials

Experimental waxy wheat lines used in this study were obtained from Dr. C.F. Konzak (Northwest Plant Breeding Co., Pullman, WA) and Dr. R.A. Graybosch (USDA/ARS, University of Nebraska, Lincoln, NE). The waxy wheat samples were randomly coded as follows: Wx-1, Wx-2, Wx-3, Wx-4, Wx-5, Wx-6, and Wx-7. Karl 92 (nonwaxy) and Ike (partial waxy) wheats, included as controls, were provided by Dr. J. Martin (Hays Branch of State Agricultural Experiment Station, Kansas State University, Manhattan, KS).

The waxy wheat lines were produced by either mutagenesis of Ike partial waxy wheat, or by crossing Ike or Kanto 107 partial waxy wheat with Bai Huo partial waxy Chinese wheat. Some of the waxy wheats were further backcrossed with Klasic wheat. Commercial wheat starch and vital wheat gluten samples were obtained from Midwest Grain Products, Inc., Atchison, KS. Standard bread flour (Regional Baking Standard, RBS-98) with 12.0% protein (14% mb) was provided by Dr. O.K. Chung, USDA/ARS Grain Marketing and Production Research Center (GMPRC), Manhattan, KS. All chemicals used were reagent-grade and other materials were of the highest purity available.

Physicochemical characterization of samples

Physicochemical characterization of the wheat samples and their flours were performed as described in detail by Sayaslan and co-workers [21]. Briefly, wheat samples were first cleaned (Carter-Day Dockage Tester, Minneapolis, MN) and then their test weights (Burrows Equipment Co., Evanston, IL) and single kernel characteristics (Single Kernel Characterization System - SKCS 4100, Perten Instruments North America, Inc., Reno, NV) were determined. All wheat samples were tempered at 15.5% moisture for 20 h and roller-milled to straight-grade white flours on a Buhler experimental mill (Buhler Co., Uzwil, Switzerland). Moisture and ash contents of the wheats, flours and wet-milling fractions were determined by the American Association of Cereal Chemists (AACC) methods 44-15A and 08-01, respectively [37]. Protein contents (Nx5.7) of the samples were measured by combustion method on an FP Protein/Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Total starch and damaged starch contents of the flours were determined by the AACC methods 76-13 and 76-31, respectively [37], using assay kits from Megazyme International Ireland Ltd, Wicklow, Ireland. Amylose contents of flours and α-amylase activities of wheat kernels were measured using, respectively, amylose/amylopectin and Ceralpha α-amylase assay kits from Megazyme International Ireland Ltd, Wicklow, Ireland. Mixograms of the flours and flour-starch-gluten blends were obtained by the AACC method 54-40A [37] using a 10-g mixograph (National Manufacturing Co., Lincoln, NE). Gluten index (GI) and falling number of the flours were determined, respectively, on Glutomatic and Falling Number instruments (Perten Instruments North America, Inc., Reno, NV) by the AACC methods 38-12 and 56-81B, respectively [37].

Insoluble polymeric protein (IPP) contents, sodium dodecyl sulfate (SDS) sedimentation volumes, and total pentosan contents of the flours were determined as described elsewhere [21].

Isolation of vital wheat glutens

Vital wheat glutens were isolated from the control and waxy wheat flours by the dough-washing (Martin) procedure described by Sayaslan and co-workers [21]. The isolated wet glutens were partly frozen in the freezer, cut into ~1 cm^3 pieces with a piece of scissors, placed in jars, and freeze-dried (Flexi-Dry/MP, TMS Systems, Inc., Stone Ridge, NY). The freeze-dried glutens, which typically contained <5% moisture, were ground in a Thomas-Wiley intermediate mill (Thomas Scientific Co., Philadelphia, PA) to pass through a 420-µm opening sieve and stored at -20°C in tightly closed glass containers.

Breadmaking qualities of vital wheat glutens

Breadmaking qualities of the freeze-dried and ground vital gluten fractions from the control and waxy wheat flours, called test glutens throughout this work, were evaluated by the so-called starch-stress bake test procedure [38]. For this baking test, the flour portion of the formula was a blend of a standard bread flour (RBS-98), commercial wheat starch, and test gluten. To prepare the blends, the standard RBS-98 bread flour with 12.0% protein (14% mb) was first blended with the commercial wheat starch at a ratio of 1:1 to reduce the protein level of the flour from 12.0% to ~6% (14% mb). The test gluten was then added to the flour-starch mixture to raise its protein level back to ~12% (14% mb). Pup-loaf breads were baked by the AACC Method 10-10B, a straight-dough procedure with 90 min fermentation [37]. Loaf volumes were measured using a rapeseed displacement apparatus and crumb grain scores were assessed by a trained panel.

Statistical analysis

The means of the data were compared by the Fisher's least significant difference (LSD) test in one-way analysis of variance (ANOVA), using the Statistical Analysis System software (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Wheat kernel and flour characteristics

Physicochemical properties of the wheats and their flours used in the study are listed in Table 1. Hard red winter wheat cultivars Karl 92 and Ike were included in the study as the controls, the former being a nonwaxy and the latter a partial waxy wheat cultivar. A total of seven waxy wheat lines were studied, four of which were classified as hard and three of which classified as soft by the SKCS. As given in Table 1, all waxy wheats contained quite high levels of protein, ranging from 13.2 to 16.1%. The control samples of Karl 92 and Ike contained 12.9 and 14.2% protein, respectively. The test weights, single kernel weights, and kernel diameters of the wheats were all within the acceptable limits [21].

Table 1. Physicochemical characteristic of wheats and their flours

Sample	Kernel		Single kernel characteristics					Flour									
	Protein (%)[a] (Nx5.7)	Test weight (kg/hL)	Moisture (%)	Weight (mg)	Diameter (mm)	Hardness Index	Class	Yield (%)	Protein (%)[a] (Nx5.7)	Ash (%)[a]	Starch (%)[b]	Damaged Starch (%)[a]	Amylose (%)	IPP Content (%)[c]	SDS sed.vol. (mL)[d]	Total pentosans (%)[a]	Color (L^*)
Karl 92	12.9	77.5	12.7	34.4	2.5	71.3	Hard	71.9	12.1	0.49	79.1	6.2	27.6	42.4	3.7	1.60	91.8
Ike	14.2	76.1	11.4	27.7	2.1	74.8	Hard	70.8	12.9	0.44	79.7	4.8	21.3	37.7	3.5	1.78	90.9
Wx-1	14.0	79.2	13.2	33.8	2.5	80.7	Hard	65.8	13.3	0.59	75.3	10.9	1.5	35.1	3.1	2.81	91.2
Wx-2	14.8	-	10.9	28.9	2.2	74.1	Hard	69.7	14.0	0.53	74.3	8.0	1.3	37.9	3.1	2.21	91.1
Wx-3	16.1	79.2	11.9	38.0	2.5	39.7	Soft	64.8	15.1	0.44	75.7	3.1	1.1	37.1	2.2	2.22	93.1
Wx-4	13.8	79.2	12.0	39.4	2.5	34.4	Soft	65.6	13.1	0.45	74.7	3.0	0.7	40.5	2.7	1.49	93.4
Wx-5	13.9	79.2	12.2	42.0	2.5	30.8	Soft	66.8	13.1	0.41	76.6	3.0	0.9	39.4	2.7	1.60	93.6
Wx-6	13.3	77.3	12.9	42.1	2.8	76.7	Hard	66.1	12.0	0.46	73.6	5.8	1.2	38.3	2.8	1.92	91.3
Wx-7	13.2	76.7	12.7	40.0	2.8	68.2	Hard	67.1	12.0	0.48	74.6	5.1	1.4	29.7	1.8	1.99	91.4
$LSD_{0.05}$	-	-	-	-	-	-	-	-	-	-	*2.4*	*0.7*	*0.5*	*2.3*	*0.3*	*0.26*	*0.2*

[a]14% moisture basis; [b]Dry basis; [c]Insoluble polymeric protein as percentage of total protein in flour (Nx5.7); [d]Sodium dodecyl sulfate sedimentation volume after correction for flour protein content (SDS sedimentation volume in mL / flour protein content in %).

All wheat samples were milled to straight-grade white flours and their proximate analyses are given in Table 1. Flour yields of the wheats ranged from 64.8 to 71.9%. The waxy wheats gave ~5% less flour than did the control wheats. Also, total starch contents of the waxy wheat flours were lower (~6%) than those of the controls. The amylose levels of the waxy wheat flours were determined to vary from 0.7 to 1.5%, as opposed to 21.3 and 27.6% amylose in partial waxy Ike and nonwaxy Karl 92 wheat flours, respectively. Total pentosan contents of several waxy wheat flours were found to be elevated. Similar results for waxy wheats and flours also were reported previously [20-23, 39-41].

Dough-mixing characteristics and bread making qualities of vital glutens

Protein quantity and quality are among the primary factors responsible for the variation in the baking qualities of wheat flours. A large portion of the commercially produced vital gluten (~70%) is used as a dough-strengthener in bread flour formulations [34], although wheat gluten has gained increasing importance as an industrial protein in recent years. Numerous analytical methods and baking-test procedures have been developed for the evaluation of viscoelastic and cohesive properties of gluten proteins as they relate to their breadmaking potentials [42]. Among the analytical approaches, various modifications of the SDS sedimentation volume, GI, and mixograph measurements in flours have been widely investigated and mostly correlated with flour or gluten breadmaking quality [38, 42-46].

Table 1 contains the IPP contents, SDS sedimentation volumes, and total pentosan levels of the flours. The results of those tests implied that the gluten proteins in the waxy flours were of less elastic nature than those of the gluten proteins in the partial waxy and nonwaxy control flours. The SDS sedimentation volumes of the flours from the waxy wheats were significantly lower than those of the controls. The IPP contents of the several waxy wheat flours were lower as well. The IPP contents of flours were reported to strongly correlate with dough-mixing time and tolerance and bread loaf volume [47]. Furthermore, the mixograms of the flours at their optimum water absorption levels (Figure 1) confirmed that the gluten proteins in the waxy flours were weaker as the waxy wheat flours displayed shorter mixing times and lower mixing tolerances compared to the control flours. It was reported that mixograms of flours could be considered a short-time evaluation tool capable of distinguishing between glutens of acceptable and unacceptable baking quality [46].

The baking data and mixograms of the blends prepared for the starch-stress bake test are given in Table 2 and Figure 2, respectively. In contrast to the moderate mixing time for the standard flour (RBS-98), longer mixing times were indicated by the mixograms of all the blends containing the test glutens. This is partly because of the contribution of the standard flour and due somewhat to the larger particle sizes of the test glutens that required more time to hydrate and develop into a gluten network.

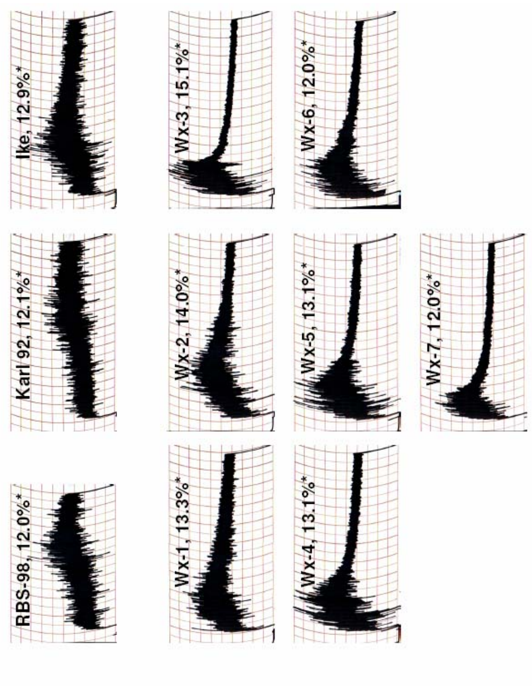

Figure 1. Mixograms of flours at optimum water absorption levels (*Protein contents of flours on 14% moisture basis)

Also, water-solubles lost during the isolation of starch and gluten through wet-milling might have influenced the mixing characteristics of the blends. As expected, the test gluten isolated from the quite strong Karl 92 flour formed a strong dough when added to the mixture of the RBS-98 flour and commercial starch. The Ike test gluten gave a less strong dough. However, the test glutens isolated from the waxy flours reduced the dough-mixing times and tolerances due probably to their poor viscoelastic properties, implying their inferior gluten quality. However, the starch-stress baking test of the isolated test glutens from the waxy wheat flours, controls, and a commercial gluten sample produced breads with comparable loaf volumes and crumb grain scores (Table 2, Figure 3).

In the starch-stress baking test, ~50% of the proteins in the standard base flour were replaced with the isolated test glutens.

A certain level of discrepancy is noticeable between the instrumentally obtained data, i.e. mixograms, SDS sedimentation volumes, and IPP contents, and the results of the baking test. As discussed in detail elsewhere [20-21], elevated levels of pentosans in the waxy wheat flours are very likely to interfere with gluten agglomeration via different mechanisms [48-54], leading to weaker glutens or less elastic doughs. Since some of the pentosans were removed during wet-milling to isolate the glutens, isolated waxy wheat glutens performed better in the baking test, which was in accordance with the observations during the wet-milling of waxy wheat flours [20-23].

It thus seems that it is not the waxy character per se that renders the waxy wheat gluten proteins weaker when assessed instrumentally, it is more likely the interference of pentosans with gluten agglomeration during dough-mixing. Recent works [55-56] on waxy flour-dough model systems and water absorption experiments showed that waxy starches absorbed more water than did normal starches during dough-mixing, which in turn influenced the water distribution between the starch and gluten leading to altered waxy wheat dough rheology. It was also observed [55] that the stickiness of waxy wheat doughs subsided by mixing the dough with 2% NaCl solution or by adding hemicellulase. These findings support the above hypothesis that it is not the waxy character per se that render the waxy wheat gluten proteins weaker, it is more likely the elevated levels of pentosans and altered water distribution between the gluten and starch in waxy wheat flour doughs, both of which affect the gluten hydration and development and rheology of the doughs. Taking into consideration that actual baking trials are the ultimate tests in the judgment of breadmaking qualities of flours, vital wheat glutens isolated from the waxy wheat flours are of comparable potential with the conventional vital glutens when used in bread formulations as dough-strengtheners.

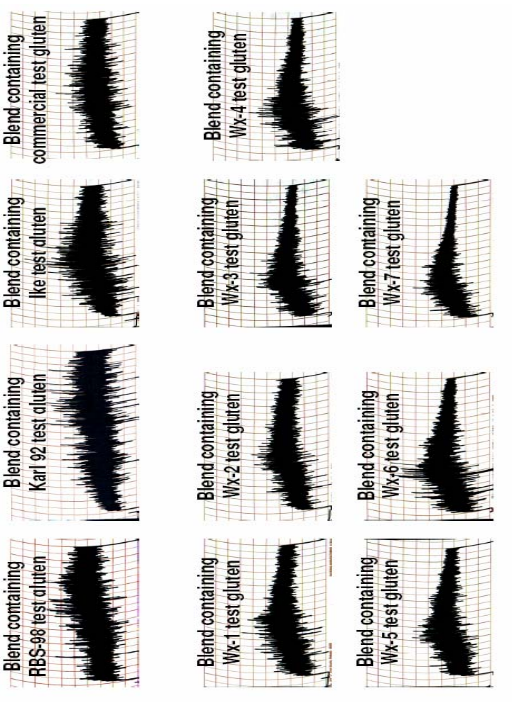

Figure 2. Mixograms of blends consisting of RBS-98 flour, commercial wheat starch, and test gluten (~46/46/8, w/w/w) at optimum water absorption levels (Protein contents of blends averaged 12.2±0.2%, 14% moisture basis)

Table 2 Mixograph and baking data for flours and blends

Sample	Flour				Blend (standard flour + starch + test gluten)[a]						
	Protein (%)[b] (Nx5.7)	Mixograph		Mixing tolerance (0→6)	Absorption		Mixing Time		Mixing tolerance (0→6)	Bread	
		Absorption (%)[b]	Mixing time (min)		Mixograph (%)[b]	Baking (%)[b]	Mixograph (min)	Baking (min)		Loaf volume (cc)	Crumb grain Score (0→5)
RBS-98	12.0	63.2	5.0	4	62.8	63.4	6.0	6.7	4	805	4.2
Karl 92	12.1	63.1	6.0	5	64.0	64.2	8.8	10.7	6	708	3.4
Ike	12.9	64.4	3.8	4	62.7	62.4	4.8	6.1	4	785	3.8
Commercial[c]	-	-	-	-	63.4	63.6	5.9	5.7	4	773	3.4
Wx-1	13.3	65.1	1.9	2	63.7	61.4	4.5	4.3	2	785	4.2
Wx-2	14.0	66.4	2.6	1	63.8	61.5	4.5	4.7	3	793	4.2
Wx-3	15.1	68.5	1.6	0	62.0	61.1	3.0	3.4	1	798	3.3
Wx-4	13.1	64.4	2.0	1	63.9	60.6	3.5	3.4	1	880	4.4
Wx-5	13.1	65.3	1.9	1	63.7	60.4	3.8	3.2	1	863	3.8
Wx-6	12.0	62.8	1.8	1	63.8	60.5	3.6	3.3	1	818	4.0
Wx-7	12.0	63.4	1.4	0	63.8	58.5	2.9	2.7	0	803	2.8
$LSD_{0.05}$	-	-	-	-	-	-	-	-	-	41	0.8

[a]Blends consist of RBS-98 flour, commercial wheat starch, and test gluten at ~46/46/8 weight ratio with mean protein content of 12.2±0.2% on 14% moisture basis; [b]14% flour moisture basis; [c]Commercial vital wheat gluten.

CONCLUSIONS

Although kernel properties of the waxy wheats were comparable with the partial waxy and nonwaxy control wheats, their flour yields and total starch contents were somewhat lower. Dough-mixing characteristics of the waxy flours were inferior to the controls as judged by their mixograms. When judged by breadmaking trials, the vital glutens isolated from the waxy flours performed similarly to the controls, indicating their potential as typical dough-strengtheners in bakery formulations.

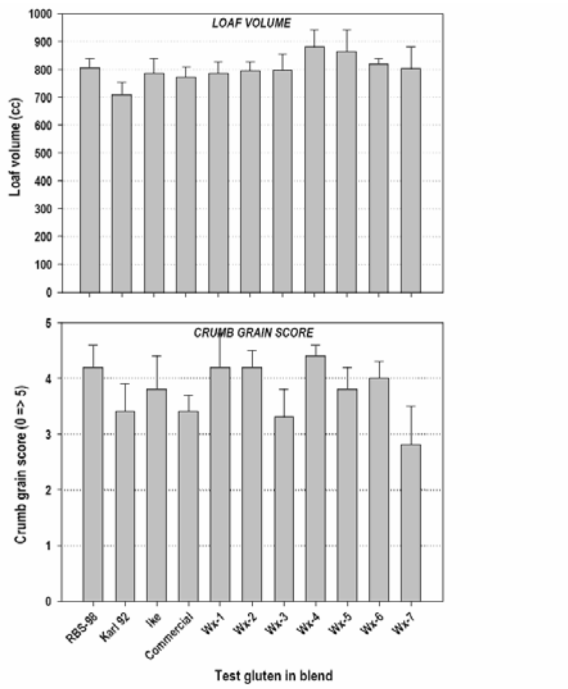

Figure 3. Loaf volumes and crumb grain scores of breads baked with blends containing test glutens (Blends composed of ~46/46/8 weight ratio of RBS-98 flour, commercial wheat starch, and test gluten with average protein content of 12.2±0.2%, 14% moisture basis)

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