Protein and Quality Characterization of Complete and Partial Near-Isogenic Lines of Waxy Wheat

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ABSTRACT

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The objective of this study was to evaluate protein composition and its effects on flour quality and physical dough test parameters using waxy wheat near-isogenic lines. Partial waxy (single and double nulls) and waxy (null at all three waxy loci, *Wx-A1, Wx-B1,* and *Wx-D1*) lines of N11 set (bread wheat) and Svevo (durum) were investigated. For protein composition, waxy wheats in this study had relatively lower albumins-globulins than the hard winter wheat control. In the bread wheats (N11), dough strength as measured by mixograph peak dough development time (MDDT) (r = 0.75) and maximum resistance (R_{max}) (r = 0.70) was significantly correlated with unextractable polymeric protein (UPP), whereas in durum wheats, moderate correlation was observed (r = 0.73 and 0.59, respectively). This may be due to the presence of high molecular weight glutenin subunits (HMW-GS) Dx2+Dy12 at the Glu-D1 locus instead of Dx5+Dy10, which are associated with dough strength. Significant correlation of initial loaf volume (ILV) to flour polymeric protein (FPP) (r = 0.75) and flour protein (FP) (r = 0.63) was found in bread wheats, whereas in durum wheats, a weak correlation of ILV was observed with FP (r = 0.09) and FPP (r =0.51). Significant correlation of ILV with FPP in bread wheats and with % polymeric protein (PPP) (r = 0.75) in durum lines indicates that this aspect of end-use functionality is influenced by FPP and PPP, respectively, in these waxy wheat lines. High ILV was observed with 100% waxy wheat flour alone and was not affected by 50% blending with bread wheat flour. However, dark color and poor crumb structure was observed with 100% waxy flour, which was unacceptable to consumers. As the amylopectin content of the starch increases, loaf expansion increases but the crumb structure becomes increasingly unstable and collapses.

Granule-bound starch synthase (GBSS), well known as waxy protein, is responsible for amylose synthesis in the wheat endosperm (Sivak and Preiss 1995). Hexaploid (bread) wheat (*Triticum aestivum* L.) has a base chromosome number of 2n=6x=42, and contains three nearly identical sets (A, B, and D genomes) of chromosomes. On the other hand, tetraploid durum wheat (*T. durum*) has only A and B genomes. The genes coding for waxy protein (*Wx*) in hexaploid wheat are located on chromosomes 7AS (*Wx-A1*), 4AL (*Wx-B1*), and 7DS (*Wx-D1*). Durum only contains the waxy alleles *Wx-A1* and *Wx-B1*. The molecular weights of *Wx-A1, Wx-B1*, and *Wx-D1* are 60.1, 59.2, and 59.0 kDa, respectively (Fujita et al 1996). Depending on the number of waxy loci carried, waxy lines with one (single null) or two null alleles (double null) are called partial waxy lines (Nakamura et al 1993), whereas complete waxy lines carry *null* alleles at all three waxy loci (null at *Wx-A1/B1/D1*). The lines that carry all these protein loci are called wild types.

Waxy wheats were first reported by Nakamura et al (1995). *Null* or nonfunctional alleles at loci *Wx-A1* and *Wx-B1* are common in wheat lines; however, no *null* alleles at the *Wx-D1* locus have been found in U.S. wheats (Graybosch 1998), though analyses of germplasm collections have permitted identification of *null* lines at this locus (Yamamori et al 1994; Murai et al 1999; Urbano et al 2002).

Different *null* alleles affect the amylose content and other functional properties differently. For instance, the reduction of amylose content due to these *null* alleles were *Wx-B1*>*Wx-D1*>*Wx-A1* (Miura et al 1994, 1999). Wild types (presence of all three *Wx* proteins) contain 25–28% amylose (Graybosch 1998; Yamamori et al 2000), whereas lack of all three *Wx* loci (waxy) contains 0–3% amylose (Nakamura et al 1995; Yasui et al 1997). Variation in amylose-to-amylopectin ratios contribute to differences in physicochemical properties, ultimately affecting quality of end-use products (Hung et al 2006).

Waxy wheats are used in the production of modified starches, as blends in flour for superior noodle quality, and for enhanced breadmaking performance (Graybosch 1998). The effect of waxy wheat starch on retarding bread staling has been studied widely (Lee et al 2001; Morita et al 2002; Hayakawa et al 2004). These studies reported that incorporation of waxy starch resulted in retention of more moisture in bread crumb and extended shelf-life of baked products. Nevertheless, optimum amylose-to-amylopectin ratio for production of good quality end-use products is still being debated (Hung et al 2006). Substitution of 50% waxy wheat flour resulted in lower loaf volumes and high levels of starch retrogradation (Lee et al 2001; Hayakawa et al 2004), whereas 40% substitution resulted in larger loaves with improved dough structure (Morita et al 2002).

Starch-based properties such as pasting and gelatinization differ between waxy, partial waxy, and nonwaxy wheats (Yasui et al 1996; Kiribuchi-Otobe et al 1997; Baik et al 2003). Effects of seven *Wx* protein null types on starch properties were studied using near-isogenic lines (Miura et al 2002; Wickramasinghe et al 2003). However, except for starch properties, there is limited information about the quality characteristics of partial waxy and complete waxy wheats (Takata et al 2007). There is a need to study waxy wheat protein composition effects on dough and end-use product quality (Hung et al 2006) using near-isogenic lines (Graybosch et al 2003).

The goal of this study was to evaluate the effect of waxy and partial (single and double null) waxy proteins on flour quality and physical dough testing parameters using two sets of near-isogenic lines developed in a durum wheat cultivar (Svevo) and a bread wheat line (N11).

MATERIALS AND METHODS

Development of Waxy Wheat Lines

The bread wheat cultivar Kanto 107 (Wx-A1/-B1 null) and the bread wheat accession MG 689 (Wx-D1 null) were used to obtain the partial and complete set of durum and bread wheat, and were

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used in crossing with the durum wheat cultivar Svevo and the bread wheat line N11 (Urbano et al 2002). Partial waxy lines of the Svevo set contain two single nulls at the *WX-A1* and *WX-B1* loci along with one complete waxy and the parent line. The N11 set has a complete waxy, a normal parent line, and a partial waxy (three lines as single nulls at *Wx-A1, B1,* and *D1* and three double null combinations at *Wx-A1B1/A1D1/B1D1*). Complete description of the production of the lines was reported elsewhere (Urbano et al 2002). Briefly, all of these lines were developed for at least six generations, backcrossed to the same parent and thus are near-isogenic lines. These waxy wheats were developed at the Institute of Plant Genetics, CNR, Bari, Italy, and the Department of Agrobiology and Agrochemistry, University of Tuscia, Viterbo, Italy. They were grown in Viterbo, Italy, during 2005-2006. A brief description of the lines used in this study is provided in Table I.

Initial Physical Tests and Milling Yield

Approximately 200–300 g of grain samples were subjected to the same handling, storage, and milling procedures. The goal was to avoid any postharvest external effects on the samples that would adversely influence sample performance during analysis. All grain samples were aspirated to remove foreign material. The cleaned grain was then weighed and stored at –20°C in clearly labeled polyethylene bags until milled.

Single Kernel Characterization System (SKCS) and Milling

Samples were thawed to room temperature and ≈300 kernels of each wheat sample was passed through the 4100 Single Kernel Characterization System (SKCS) (Perten Instruments, Huddinge, Sweden) to obtain moisture content, hardness index, and individual kernel size and weight values before milling (Martin et al 1993). SKCS data was reported as the average of 300 kernels of wheat. All SKCS measurements were made in duplicate and final data was reported as the average of two 300-kernel samples each. Observed grain moisture content was used to calculate the amount of moisture needed for tempering before milling. Grain samples were tempered to 15.5% moisture and allowed to rest for ≈16 hr before milling. A Brabender Quadrumat Jr. mill (C.W. Brabender, Duisburg, Germany) was used to mill the samples according to Approved Method 26-50.01 (AACC International 2010). Break flour, reduction flour (including red dog fraction), and bran fractions (including shorts) were collected separately and labeled. Bran (including shorts) and break flour fractions were stored at –20°C until used. Reduction flour (including red dog fraction) was discarded after milling yield was determined.

Flour Protein and Moisture Content

Flour protein content (FP) was determined by nitrogen combustion method (Approved Method 46-30.01) (AACC International 2010) using a FP-2000 nitrogen/protein analyzer (Leco Corporation, St. Joseph, MI). A factor of 5.7 was used to convert the total nitrogen into protein content. Final protein content was reported on a 14% moisture basis. Flour moisture content was determined using the air-oven method (AACC Approved Method 44-15.02). Final moisture content was calculated as described in AACC Approved Method 44-01.01.

Subunit Identification Using Lab-on-a-Chip Method

Identification of high molecular weight glutenin subunit (HMW-GS) composition of all flour samples was performed using the Lab-on-a-Chip method as described by Uthayakumaran et al (2006). Approximately 150 mg of flour was used for sequential extraction of albumins and globulins (two 5-min extractions using Tris-HCl buffer containing KCl + EDTA, pH 7.8), gliadins (two 5-min extractions using 50% isopropanol), and glutenins (single extraction for 30 min using 50% isopropanol + 2% DTT + 2% SDS, pH 7.0). Each extraction was performed with vortexing followed by centrifugation at 13,400 × *g* for 2 min except for glutenin, which was centrifuged for 10 min. After extraction of albumins and globulins and gliadins, the remaining pellet was used as initial material for glutenin extraction.

Each clarified glutenin extract (4 μL) was mixed with 2 μL of sample buffer (Agilent Technologies, Palo Alto, CA) heated to 100°C for 5 min and 84 μL of deionized water was added. This mixture (6 μL) was applied to one of 10 sample wells on the Agilent protein labchip. Proteins thus extracted were analyzed in the Agilent 2100 Bioanalyzer with a Protein 230 chip. Each sample contained an internal standard comprising an upper marker of 240 kDa and a lower marker of 4.5 kDa. Each chip included a reference ladder of proteins consisting of 7, 15, 28, 46, 63, 95, and 150 kDa markers, plus the lower and upper markers (4.5 and 240 kDa), against which protein mobilities were compared for each analysis. Samples of well-known subunit composition with closely related subunits were selected as controls to compare the apparent molecular sizes and run on 2 of the 10 sample wells on each chip to facilitate the identification and comparison of HMW-GS. The standards used were Chinese Spring (null, 7+8, 2+12), Karl-92 (1, 7+8, 5+10), and Jagger (1, 17+18, 5+10).

Size-Exclusion High-Performance Liquid Chromatography

Flour protein compositions were determined quantitatively using the SE-HPLC extraction procedure described by Gupta et al (1993). Briefly, a 10-mg sample was used to extract total (TPP), extractable (EPP), and unextractable polymeric protein (UPP) fractions. TPP was extracted with 2% SDS buffer for 5 min by vortexing, sonicated for 15 sec (6W output) and centrifuged (Eppendorf 1514, Westbury, NY) for 20 min at 13,400 × *g*. A similar procedure was followed for EPP (with the exception of sonication) and the residue was used for the unextractable fraction. UPP was extracted for 10 min and centrifuged for 20 min (13,400 × *g*) after sonication for 25 sec. Protein samples were stabilized before analysis by heating the samples in a water bath at 85°C for 5 min and cooled by storage in ice.

Stabilized protein extracts were injected (20 μL) onto a Phenomenex Biosep-SEC-S4000 (300 × 7.8 mm) size-exclusion column (Phenomenex, Torrance, CA) connected to the Agilent 1100 HPLC system. Data analysis was performed using the Agilent ChemStation software program. A variable wavelength detector set to 214 nm was used. The column temperature was 40°C. A 1:1 solvent mixture of A (0.05% trifluoroacetic acid [TFA] in acetonitrile, v/v) and B (0.05% TFA in deionized water, v/v) was used. All the solvents used were HPLC grade (Fisher Scientific, Pittsburgh, PA). A flow rate of 1.0 mL/min was employed with a total runtime of 28 min.

The percentage of UPP in the total polymeric protein (TPP) was calculated as (peak 1 [unextractable] area/peak 1 [total] area) ×100, where peak 1 (total) refers to the sum of peak 1 (extract-

TABLE I
Description of Svevo Durum Waxy Wheat and N11 Bread Waxy Wheat Lines

Sample Name	Type of Wheat	Variation
Svevo set (null, 7+8)		
Wheat	Durum wheat	Parent line
Waxy	Complete waxy	Null at A1/B1
Waxy A1	Partial waxy	Null at A1
Waxy B1	Partial waxy	Null at B1
N11 set (1, 7+8, 2+12)		
Wheat	Bread wheat	Parent line
Waxy	Complete waxy	Null at A1/B1/D1
Waxy A1	Partial waxy	Null at A1
Waxy B1	Partial waxy	Null at B1
Waxy D1	Partial waxy	Null at D1
Waxy A1/B1	Partial waxy	Null at A1/B1
Waxy A1/D1	Partial waxy	Null at A1/D1
Waxy B1/D1	Partial waxy	Null at B1/D1

able) and peak 1 (unextractable). The percentage of total extractable protein present in polymeric form (PPP) was calculated as (peak 1 area/total area) × 100. Percentage of same in the flour was calculated as (% of peak 1 × % of flour protein)/100.

Quality of Dough Mixing Properties

Dough mixing characteristics were determined with a computerized 10-g mixograph (National Manufacturing, TMCO division, Lincoln, NE). Data analysis was performed using the MixSmart software program (v.3.40, National). Flour samples were mixed using Approved Method 54-40.02 (AACC International 2010) with slight modifications according to Gupta et al (1993). A 2% NaCl solution by flour weight on a 14% moisture basis was used at constant water addition (65%). Mixing properties such as mixograph dough development time (MDDT), peak height, peak width, width at 8 min, and peak slope were measured. Mixing was performed in duplicate for all samples with a runtime of 10 min.

Dough Extensibility Tests

Extensibility characteristics of dough were evaluated using a micro-extensibility test described by Suchy et al (2000), modified for use with the 10-g mixograph. Tests were conducted twice with each replicate. Final data was reported as an average of three replicates for each sample. A texture analyzer (TA-XT2, Texture Technologies, Scarsdale, NY) was equipped with a Kieffer dough and gluten extensibility rig as a probe. Water absorption results along with MDDT analyzed using the mixograph were used to develop dough suitable for the extensibility test. A 2% NaCl solution (flour weight basis) was also used as in the mixograph. Dough collected from the mixograph was rolled gently into a ball and placed in a plastic container, covered with a Ziploc plastic bag and kept in the proofing chamber (at 30 ± 2°C temperature and 95 ± 1% rh) for 20 min. After the resting period, dough was hand-rolled into elongated cylinder-shaped rods of ≈3.4 in. × 0.8 in. × 0.5 in. size with as little manipulation of the dough as possible, and placed over 8–10 channels of a Teflon-coated block that was prepared by placing thin Teflon strips coated in mineral oil in the channels. Mineral oil was used to prevent the dough from sticking to the Teflon strips and the Teflon block during the experiment. The upper half of the block was placed and clamped tightly by removing the excess dough on both sides of the block. The dough thus clamped was rested at 30 ± 2°C and 95 ± 1% rh for 40 min. The developed dough was used immediately for the extensibility test without any equilibration time. Testing was done as rapidly as possible to avoid temperature variation. Test conditions employed were pretest speed of 2.0 mm/sec, test speed of 3.3 mm/sec, post-test speed of 10.0 mm/sec, and trigger force of 5 g. Distance was adjusted according to sample and varied at 75–120 mm. R_{max} (mN), extensibility (mm), and area under the curve (g-mm) were recorded for different dough samples.

Test Baking

Bread loaves were baked according to the breadmaking test for 10 g of flour as in Shogren and Finney (1984). Bread loaves were made in duplicate for each wheat sample and the average data was reported. Bread formulation included flour (100%), sugar (6%), shortening (3%), yeast (2%), salt (1.5%), and water as required. All ingredients were mixed together to optimum gluten development and the mixing times were according to the previous mixograph analysis. Dough temperature was 27 ± 1°C. Developed doughs were sheeted, folded twice, and placed in an open container and fermented for 120 min in a proofing chamber maintained at 30 ± 1°C and 95 ± 1% rh. Punch times were 69, 103, and 120 min during the fermentation. The doughs were then resheeted and molded using a 10-g molder. Doughs were proofed for 40 min at 30°C. The baking was performed at 232°C for 13 min. Initial loaf volumes were assessed using rapeseed displacement (Approved Method 10-05.01) (AACC International 2010) after cooling the baked bread for 2 hr. All bread loaves of developed lines were compared against the bread made from parent lines.

Internal control flour was used in the blended samples to replace 50% waxy wheat flour in breads according to treatments. The blended flour had a flour protein content of 11.9% (on 14% moisture basis) and 12.4% flour moisture content. The blended flour had a flour absorption of 61.7%, a bake mix time of 6 min, an average loaf volume of 940 mm, and an average crumb grain of 3.8 (0–6 scale) from pup-loaf test baking. Baked data for internal control flour was reported from the average of three replicates.

The term initial loaf volume (ILV) was used in this study because the loaf volumes measured after 2 hr of baking would be expected to collapse the structure, losing as much as 25% of the volume within the first 24 hr, as previously reported by Barsby et al (2001).

C-Cell Image Data Analysis

Bread quality factors such as initial loaf volume, crumb grain, crumb texture, and other cell characteristics were assessed using the C-Cell image analysis technique and the data was analyzed using the C-cell software (Calibre Control International, Warrington, UK). Image analysis of crumb grain was performed on 10-g bread loaves ≈12 hr after baking). Loaves were sliced using a rotary disk blade cutter (unserrated Graef blade), and measurements were conducted on central slices 15-mm thick from each loaf. Two slices from each bread loaf was used for analysis. Thus,

TABLE II
Results from Initial Physical Tests for Hardness Index (HI) from SKCS, Milling Yield from Quadrumat Jr. Mill, and Flour Protein Content Analyzed by Leco Protein Analyzer

		Milling Yield (%)				
	HI	Break Flour	Total Flour	Bran	Protein Content (% flour)	% Flour Moisture
Svevo set						
Wheat	89	43.5	49.3	50.6	14.0c	15.32a
Waxy	81	41.4	47.0	53.0	15.1a	15.25a
Waxy A1$^-$	85	42.1	48.0	52.0	14.4b	14.60c
Waxy B1$^-$	96	33.8	38.7	61.3	12.9d	14.88b
N11 set						
Wheat	98	46.8	53.1	46.9	11.4d	13.6e
Waxy	87	41.3	47.0	53.0	12.3a	14.8ab
Waxy A1	97	43.9	50.0	50.0	12.3a	14.7abc
Waxy B1	91	43.7	50.0	49.9	12.3a	14.3bcd
Waxy D1	95	41.0	46.9	53.1	11.4d	15.1a
Waxy A1/B1	95	41.0	46.6	53.4	11.5b	15.1a
Waxy A1/D1	94	37.4	43.3	56.7	11.4cd	14.0de
Waxy B1/D1	94	39.2	44.8	55.2	11.5bc	14.1cde

[a] Values in each set followed by the same letters in the same column are not significantly different at $P < 0.05$.

the C-Cell data reported was an average of four slices from each wheat sample. Loaf and cell characteristics such as loaf weight, slice area, loaf height, number of cells, wall thickness, and cell diameter measured by image analysis were correlated with quality and protein characteristics.

Statistical Analyses

All extraction runs and analyses were conducted at least in duplicate and in randomized order with mean values being reported. All developed lines in each set were compared statistically with the parent line considered as the control. Analysis of variance (ANOVA) of the results was performed using the General Linear Model procedure of SAS (v.9.1. SAS Institute, Cary, NC). Statistical significance was declared at $P < 0.05$. Linear regression and correlation were also performed using SAS procedures.

RESULTS AND DISCUSSION

Initial Physical Tests

Results from initial physical tests are presented in Table II. Mean hardness index (HI) values were ≈90, which indicates that these samples were very hard. The majority of samples had significantly higher bran % than flour yield. Average flour protein contents of the Svevo set (14.1) were significantly higher than the N11 set (11.8). However, in both sets, complete waxy had higher protein content than other lines. Flour moisture contents of Svevo were also higher than N11.

Complete waxy lines had the lowest HI compared to partial waxy and parent lines in both the waxy sets studied. No variation for HI was observed among partial waxy lines. Mean hardness index values >90 indicate that kernel hardness is independent of starch amylose concentrations (Morris and Konzak 2001).

In both the waxy wheat sets studied, parent lines had significantly higher flour yield compared to the waxy lines, which indicated that flour extraction might be related to the waxy nature of the samples. Waxy wheats tend to produce higher starch damage during milling (Bettge et al 2000) and significantly lower flour yields (Graybosch et al 2003). This can be attributed to the nature of the endosperm in which the higher crystallinity of starch granules that lack amylose influence the milling properties of waxy samples (Graybosch et al 2003). However, this may not completely hold for the present samples, as the lowest flour yield was not found for the lowest amylose (complete waxy) samples in either of the sets studied.

Takata et al (2005, 2007) reported lower flour yields with near-isogenic waxy wheat lines over parent line, but the double null *Wx*-protein phenotypes (AB and BD null) did not affect the flour yield contents. On the contrary, in this study, double waxy nulls (AB/AD/BD nulls) had the lowest flour yields followed by waxy and single nulls. An increase in nonstarch polysaccharide content, such as arabinoxylans (Kato et al 1997) and β-glucan (Yasui et al 1999) resulted in a corresponding decrease in flour yield levels in waxy wheat (Takata et al 2007).

Electrophoretic Characterization of HMW-GS Using Lab-on-a-Chip Method

HMW-GS composition of waxy wheat samples was analyzed using the Lab-on-a-Chip method and results are shown in Fig. 1. Svevo is a durum wheat and contains only A and B genomes. All the Svevo series contained Null and 7+8 HMW-GS, respectively, at A and B genomes. N11, which is a bread wheat cultivar, had a HMW-GS composition of 1, 7+8, and 2+12.

The elution sequence of HMW-GS in the present sample fractionation on the chip was 12, 8, 18, 10, 17, 7, 1, 5, and 2, which was exactly as reported by Uthayakumaran et al (2006). However, the apparent sizes of corresponding subunits may not match exactly the previous results because the protein chip used in the present study had a higher molecular range (230+ kit) than the 210+ kit used by Uthayakumaran et al (2006). Apparent molecular weights of samples used in this study are shown in Table III.

Protein Composition and Physical Dough Tests

SE-HPLC results are presented in Table IV. Significant differences were observed for UPP in which complete waxy had either lower (Svevo waxy) or higher (N11 waxy) values than other lines. Albumin and globulin composition was relatively lower than the regular wheats. All Svevo lines had gliadin-to-glutenin ratios >1.

Physical dough test results are shown in Table V. No large variations were found in MDDT among the waxy lines. In contrast, extensibility parameters R_{max} and extensibility differed significantly. However, there was no specific trend observed for any of the quality parameters studied.

Relatively lower mixograph peak times and tolerance scores were reported for waxy lines (Graybosch et al 2003). In contrast, moderately high peak development times with high peak width at

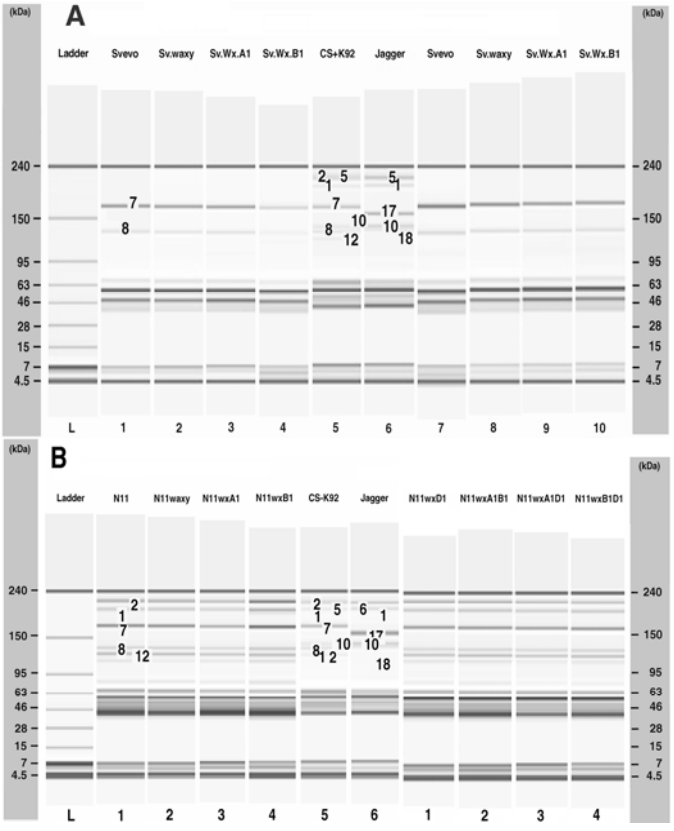

Fig. 1. HMW-GS composition identified by the Lab-on-a-Chip method. **A,** Svevo set. **B,** N11 set. All waxy lines have the same subunit composition as the parent line.

TABLE III
Apparent Sizes (kDa) of HMW-GS Determined Using the Lab-on-a-Chip Method

	Glu-A1			*Glu-B1*			*Glu-D1*		
Subunit	1	7	8	17	18	2	5	10	12
Size (kDa)	202.0	165.6	130.6	152.6	133.1	214.4	214.0	135.7	121.6

8 min were observed with waxy wheat lines used in this study. High mixograph water absorption levels coupled with high protein content in complete waxy lines of both sets suggests that lack of amylose in these lines may cause a corresponding increase of endosperm protein content and other nonpolysaccharides in the flour. Significantly higher water absorption levels with waxy wheat doughs were observed in this study, which may be attributed to high protein and dietary fiber content (Morita et al 2002). High starch damage levels (Bettge et al 2000; Guo et al 2003) or higher arabinoxylan contents (Michniewicz et al 1991) could also cause the increase in water absorption levels of waxy wheat.

The correlation matrix between protein attributes and various quality parameters and initial loaf volume was reported individually for N11 and Svevo sets (Table VI). Dough strength as measured by MDDT (r = 0.75) and R_{max} (r = 0.70) were significantly correlated with UPP in bread wheats, whereas in durum wheat, the values were MDDT r = 0.73 and R_{max} r = 0.59. Extensibility was negatively correlated with both flour polymeric protein (FPP) and percent polymeric protein (PPP) in both sets of waxy wheats. Initial loaf volume was significantly correlated (r = 0.75) with FPP in bread wheats, whereas in durum wheats, initial loaf volume was well correlated with PPP (r = 0.75). Interestingly, a weak correlation was found between initial loaf volume and UPP in bread wheats, which was negatively correlated in durum wheats.

UPP is a parameter that gives a relative measure of molecular weight distribution of polymeric protein based on solubility (Gupta et al 1993). Dough strength measurements such as R_{max} and MDDT correlate well with UPP, indicating that only a portion of proteins with the highest molecular weight contribute to dough strength (Southan and MacRitchie 1999); thus a greater amount of UPP signifies shifts in molecular weights to higher values. In the present study, two different waxy wheat sets behave differently for these correlations. Although significant correlation existed between UPP, MDDT, and R_{max} in bread wheat lines, these correlations were only moderate for durum waxy wheat lines. This poor correlation of UPP with R_{max} in durum lines may be due to a lack of dough strength contributing subunits such as 5+10, as well as the presence of 2+12. In addition, the presence of 7+8 in the N11 set may contribute strength. HMW-GS 7+8 were given a score of 3 in terms of breadmaking quality (Payne et al 1987). Negative correlation of UPP with extensibility and significant positive correlation of UPP with MDDT and R_{max} in the present study were in agreement with results reported previously (Larroque et al 1999; Zhang et al 2008). Initial loaf volume was correlated significantly with FPP and PPP. FPP depended on flour protein content, which was largely determined by environment, whereas PPP was genetically controlled (Southan and MacRitchie 1999). Because all the waxy wheat NILs were grown under the same conditions, the researchers made the assumption that the waxy wheat was contributing largely to PPP.

The important quality attributes such as MDDT, R_{max}, extensibility, and initial loaf volume were normalized to flour protein content to eliminate the effect of variation in protein amounts. Some of these correlation values are indicated in parentheses in Table VI. However, no differences in correlation trends were observed after normalization.

Test Baking Results

Micro-bread loaves (10 g of flour) were baked with both durum (Svevo) and bread wheat (N11) waxy lines. Test baking was performed with 100% waxy, 50% waxy, and 50% bread wheat flour as a blend. The two different sets of waxy lines responded differently with respect to baking parameters. However, all the waxy lines in both sets had higher or at least equal initial loaf volumes as the parent lines (Fig. 2). Interestingly, except for two null lines (D1 and A1D1) in the N11 set, all other waxy lines showed improved initial loaf volume for waxy flour alone over those blended with bread wheat flour. In contrast, for the Svevo set, which had durum in its background, bread made from the waxy lines with 50% blend showed higher initial loaf volumes (Fig. 2). In both sets, under both the treatments (100% waxy or 50% blend), crumb scores were not influenced by blending (Fig. 3).

All the measurements conducted by the C-cell analysis had positive correlations among each other. As mentioned earlier, the dough strength measurements like MDDT, R_{max}, and UPP were poorly correlated with initial loaf volume, whereas initial loaf volume was highly correlated with PPP and FPP.

As shown in bread loaves made from control (Svevo wheat) and waxy wheat, bread made with waxy wheat had a more porous, open crumb grain with large gas cells in the crumb (Fig. 4). A similar trend was observed with the N11 waxy wheat set (Fig. 5). However, blending with 50% bread wheat flour improved the crumb structure, resulted in less porosity and smaller cells inside the grain (C-Cell images of micro-bread slices in Figs. 4 and 5). Nevertheless, breads made with 100% waxy flour appeared darker in color and with an overall dull appearance that was unacceptable to consumers. Acceptable change in color with improved appearance such as crumb grain may be achieved by blending up to 50% with any bread wheat flour. Test baking results were in agreement with previous studies. Higher loaf volume with im-

TABLE IV
Results from SE-HPLC for Near-isogenic Lines of Svevo and N11 Waxy Wheats[a,b]

	GGR	%UPP	PPP	%Gliadin	%Alb+Glob
Svevo set					
Wheat	1.2	41.9a	42.7a	49.6ab	5.2b
Waxy	1.3	38.6b	42.7a	50.6a	5.0b
Waxy A1$^-$	1.1	41.5a	44.0a	47.0c	6.6a
Waxy B1$^-$	1.2	41.4ab	43.3a	49.2b	6.1ab
N11 set					
Wheat	0.9	49.9bc	41.4abc	47.9ab	9.0b
Waxy	0.9	52.0a	42.4ab	47.5b	8.9b
Waxy A1	1.0	47.3de	40.6c	48.9a	8.6b
Waxy B1	1.1	46.4e	41.0bc	49.0a	8.5b
Waxy D1	0.9	51.7ab	42.9a	47.0b	8.5b
Waxy A1/B1	0.9	49.2cd	42.2ab	47.5b	8.5b
Waxy A1/D1	0.9	51.4ab	42.8a	45.5c	9.7a
Waxy B1/D1	0.9	52.4a	42.5ab	47.2b	8.9b

[a] UPP, unextractable polymeric protein; PPP, % polymeric protein; %Alb +%Glob, %albumin+%globulin; GGR, gliadin/glutenin ratio.
[b] Values in each set followed by the same letters in the same column are not significantly different at $P < 0.05$.

TABLE V
Results from Quality Tests for Near-Isogenic Lines of Svevo and N11 Waxy Wheats[a]

	Mixograph		Extensibility	
	MDDT[b] (min)	Width (at 8 min)	R_{max} (mN)	Extensibility (mm)
Svevo set				
Wheat	4.0ab	26.2a	486a	32.0c
Waxy	4.7b	19.6b	342c	40.0b
Waxy A1	3.6b	23.7ab	317d	43.4a
Waxy B1	3.9ab	23.5ab	459b	30.1c
N11 set				
Wheat	3.7c	21.0bcd	368e	51.4c
Waxy	3.6c	21.8bc	463bc	61.3b
Waxy A1	3.5c	18.9cd	437d	44.0d
Waxy B1	2.9d	18.1d	326f	68.5a
Waxy D1	4.8a	27.3a	554a	35.6f
Waxy A1/B1	3.3c	21.7bc	390e	54.2c
Waxy A1/D1	4.2b	27.4a	442cd	39.0ef
Waxy B1/D1	4.1b	23.2b	469b	41.0de

[a] Values in each set followed by the same letters in the same column are not significantly different at $P < 0.05$.
[b] MDDT, mixograph peak dough development time.

proved dough structure was observed with substitution of 40% waxy wheat flour (Morita et al 2002), whereas 50% substitution resulted in slightly decreased loaf volume with more porous crumb structure and higher retrogradation (Lee et al 2001; Haykawa et al 2004). Crumb grain of the 50% waxy loaves was unacceptable (Barsby et al 2001). Though there was significant difference in initial loaf volume between samples, highest volume was with the waxy line in the N11 set, whereas single nulls had the highest volume in the Svevo set.

Though shelf-life studies have not been conducted on these waxy samples, waxy lines were expected to increase the storage period as reported earlier (Lee et al 2001; Morita et al 2002; Haykawa et al 2004). Breads made from whole waxy wheat flour and 50% substituted flours had inferior color with increment of dark brown color of bread crumb with increased waxy flour content in bread based on the method of Van Hung et al (2007). This was attributed to the high amounts of phenolic compounds present in bran derived from whole waxy flour. Significantly higher amounts of waxy wheat flour polyphenols and arabinoxylans were reported among NILs of waxy wheat (Takata et al 2007). Although the polyphenol content of waxy wheat flour was not analyzed in the present study, the dark brown color of waxy wheat breads might possibly be attributed to the presence of these compounds in flour as well as the Maillard reaction. However, detailed studies are required to confirm the role of polyphenols in color of breads made from waxy wheat flour.

Collapse of bread loaves after 24 hr was not observed with waxy wheat flour blends (0, 25, 50, and 100%) tested previously (Barsby et al 2001). Presence of very low or no amylose content of waxy wheat flours significantly affects the retrogradation behavior of breads during storage that might influence the prevention of bread loaf collapse. However, in the present study, initial

TABLE VI
Correlation Matrix for Various Quality Parameters and Protein Attributes of Svevo and N11 Sets[a,b,c]

	R_{max}	Ext	ILV	PPP	FPP	UPP	FP
Svevo set (n = 4)							
MDDT	0.648	–0.671	0.395	–0.305	0.731	0.731	–0.503
R_{max}		–0.436	0.495	0.074	0.993*** (0.992)	0.598 (0.692)	0.333
Ext			–0.906	–0.451	–0.498	0.001	0.316
ILV				0.754	0.511	–0.232 (–0.232)	0.095
PPP					0.028	–0.750	0.483
FPP						0.640	0.222
UPP							–0.243
N11 set (n = 8)							
MDDT	0.869***	–0.861	–0.230	0.482	0.145	0.754**	–0.639
R_{max}		–0.726	0.197	0.704*	0.514 (0.388)	0.700* (0.748)	–0.280
Ext			0.436	–0.356	–0.022	–0.504	0.639*
ILV				0.494	0.753**	0.159 (0.159)	0.638*
PPP					0.887***	0.609	–0.048
FPP						0.323	0.417
UPP							–0.536

[a] MDDT, mixograph dough development time; R_{max}, maximum resistance in microextensibility test; Ext, extensibility; ILV, initial loaf volume; PPP, % polymeric protein; FPP, flour polymeric protein; UPP, unextractable polymeric protein; FP, flour protein.
[b] Values in parentheses normalized to flour protein content.
[c] *, **, ***, Significant at $P > 0.1$, 0.05, 0.01, respectively.

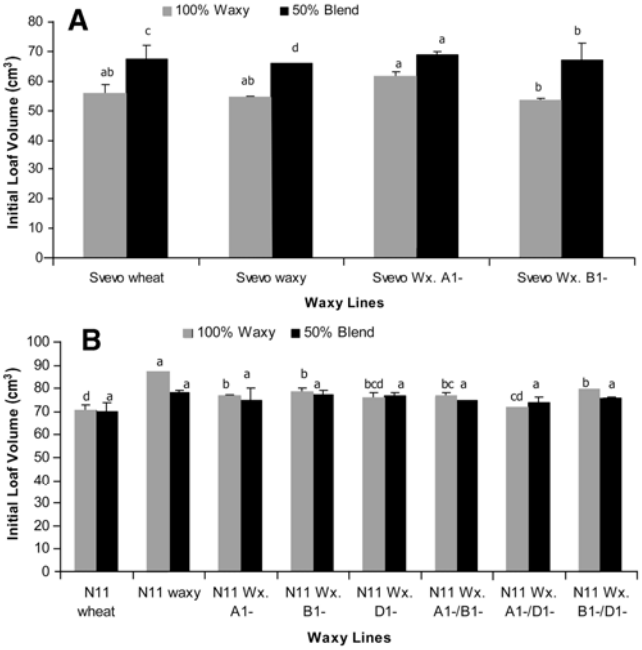

Fig. 2. Initial loaf volume scores from micro-breads made with 100% waxy wheat and 50% blend with commercial wheat for Svevo (**A**) and N11 (**B**) waxy wheats.

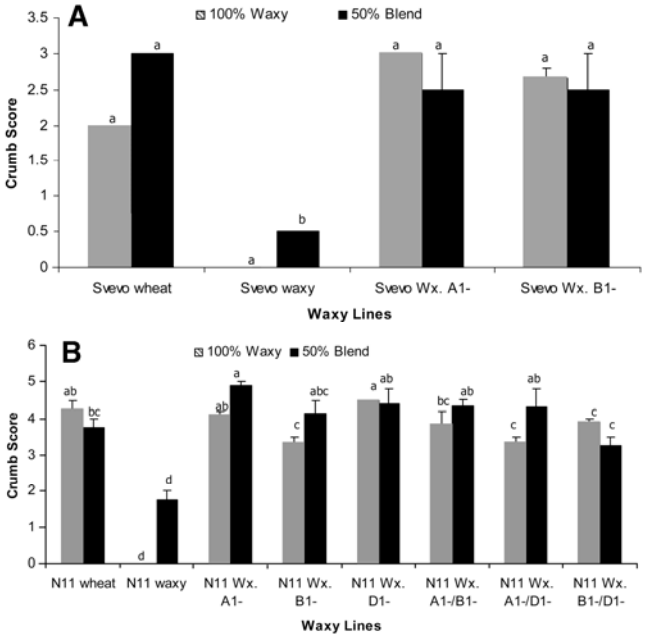

Fig. 3. Crumb scores from micro-breads made with 100% waxy wheat and 50% blend with commercial wheat for Svevo (**A**) and N11 (**B**) waxy wheats.

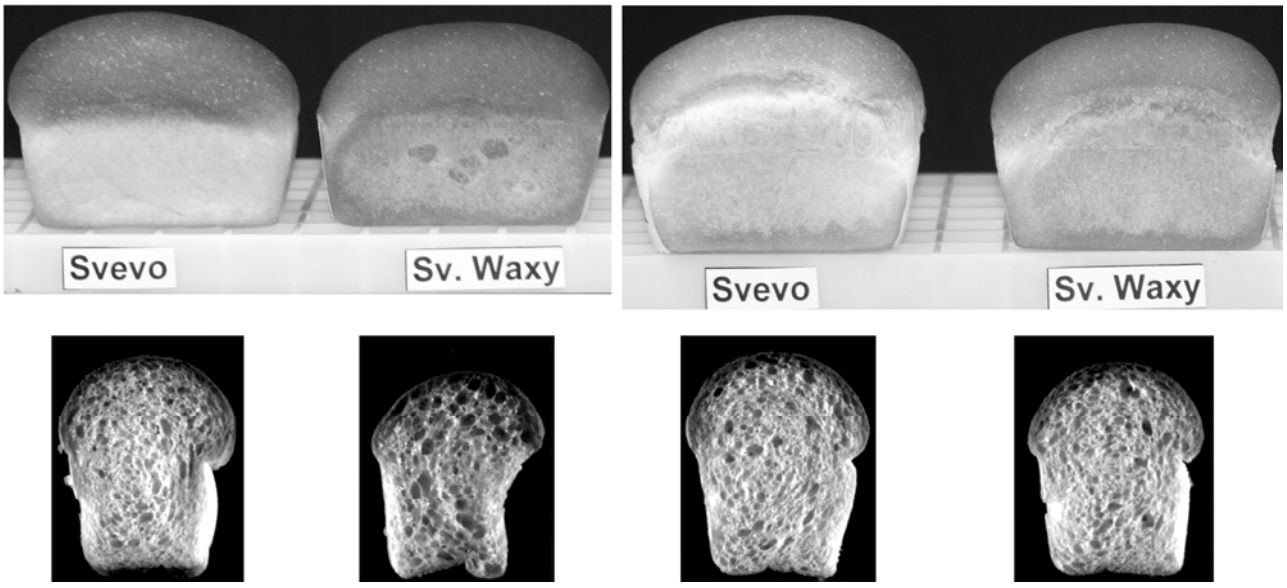

Fig. 4. Comparison of whole breads made from waxy and regular wheats of Svevo set. Breads on left were made with 100% waxy flour; breads on right were made with 50% waxy blended with commercial wheat flour. C-Cell images of corresponding bread slices in bottom row.

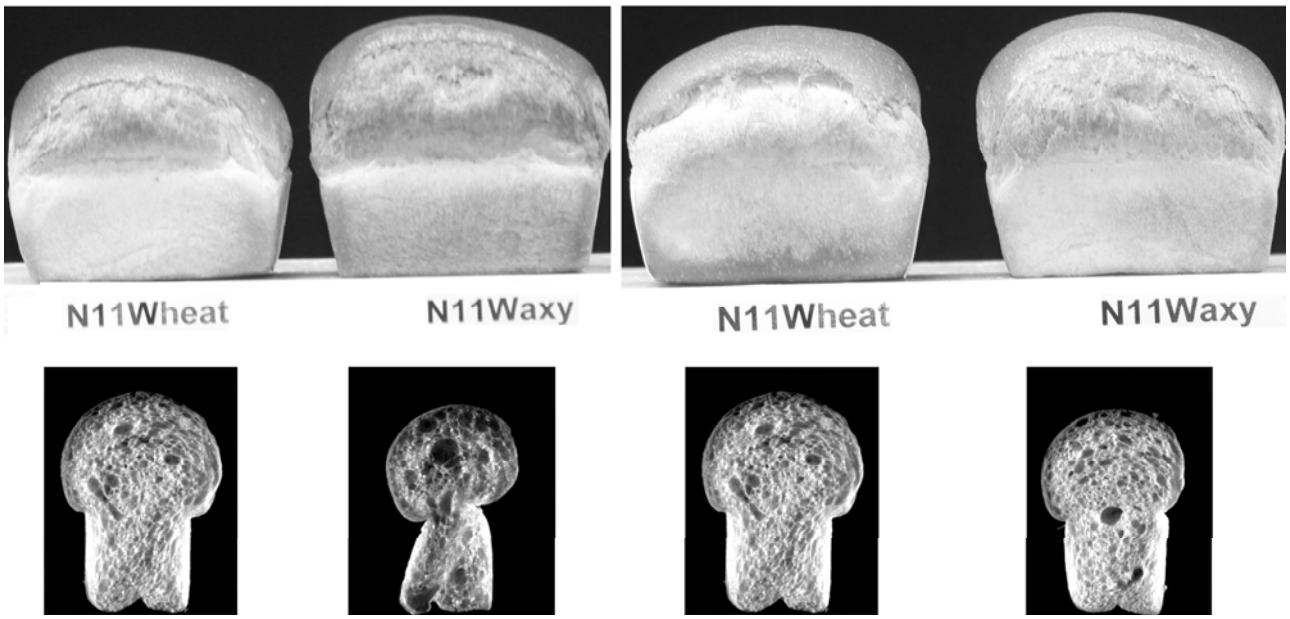

Fig. 5. Comparison of whole breads made from waxy and regular wheats of N11 set. Breads on left were made with 100% waxy flour; breads on right were made with 50% waxy blended with commercial wheat flour. C-Cell images of corresponding bread slices in bottom row.

loaf volume (i.e., within first 24 hr of baking) was increased as the amylopectin content increased, but the crumb grain structure became increasingly unstable and collapsed (Figs. 4 and 5).

CONCLUSIONS

Initial loaf volume, which is an important breadmaking quality, was highest with 100% waxy wheat flour and was not affected by 50% blending with bread wheat flour. However, dark brown color and poor appearance with large gas cells in the internal crumb was observed with 100% waxy flour; this is unacceptable to consumers of traditional pan bread. As the amylopectin content of the starch increases in waxy lines, loaf expansion increases, but the structure become increasingly unstable and collapses even within the first day of baking. In protein composition, waxy wheats have relatively lower albumins and globulins than regular wheat. Bread and durum waxy wheat lines behave differently for correlations made between protein attributes and quality measurements. Dough strength parameters such as MDDT and R_{max} were significantly correlated with UPP in both the waxy wheat lines studied. Extensibility was negatively correlated with FPP and PPP. Initial loaf volume was significantly correlated with FPP in bread wheats, whereas in durum waxy lines, there was good correlation between ILV and PPP.

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