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Dough Rheology and Wet Milling of Hard Waxy Wheat Flours

LAN GUAN,[†] PAUL A. SEIB,[†] ROBERT A. GRAYBOSCH,[‡] SCOTT BEAN,[#] AND YONG-CHENG SHI*,[†]

[†]Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, [‡]USDA-ARS, University of Nebraska, Lincoln, Nebraska 68583, and [#]USDA-ARS, GMPRC, Manhattan, Kansas 66502

To realize the full potential of waxy wheat (*Triticum aestivum L.*), the wet milling properties of waxy wheat flours including their dough-mixing properties were investigated. Flours of six waxy hard wheats, one normal hard wheat ('Karl 92'), and one partial waxy hard wheat ('Trego') were fractionated by the dough-washing (Martin) process, and the yields and recoveries of starch and gluten were compared. When waxy and normal wheat starches each were blended with a wheat gluten to give a mixture containing 14.5% protein, they gave very different mixograms even though the protein was the same in those blends. Waxy wheat starch absorbed more water than normal wheat starch, which apparently retarded hydration of gluten and dough development. Higher water content had to be used for some waxy wheat flours to develop optimum dough. Washing waxy wheat flour dough under a stream of water caused dough to become slack, spread out more on the sieve, and break apart into several pieces, which when thoroughly washed, coalesced into an elastic dough like the controls. By mixing a weak dough with 2% NaCl solution or by adding hemicellulase, stickiness of the dough subsided during the washing step and thereby improved the recovery of the gluten and starch fractions.

KEYWORDS: waxy wheat; wheat flour; dough rheology; mixography; wet milling; starch

INTRODUCTION

Waxy wheat (*Triticum aestivum L.*) is a potentially valuable specialty wheat (*1*−*3*). The term "waxy wheat" signifies that a wheat lacks amylose and consists of essentially all amylopectin in its endosperm starch. The amylose level in wheat is independent of grain hardness, so soft and hard waxy wheats have been produced (*4*, *5*). A soft white waxy wheat named Leona was released in Idaho, and another named Waxy-Pen was released at the ARS Western Wheat Quality Laboratory in Pullman, WA. Advanced lines of hard winter waxy wheats also are being developed at ARS/USDA in Lincoln, NE, and Farmer Direct Foods, Inc., Atchison, KS.

Waxy wheat can be dry-milled to flours and used directly in food applications. In addition, waxy wheat flour has the potential to be wet-processed to yield two valuable coproducts, waxy wheat starch and vital wheat gluten.

It has been suggested that waxy wheat flour could be substituted for 10−25% normal wheat flour in pan breads, tortillas, pancakes, cakes, sweet goods, and white salted noodles to give increased softness of bite, reduced rate of firming, and increased shelf life (*6*−*8*). Because of its softening effect, waxy wheat flour may be used to replace some of the fat or oil ingredient in a food, thereby reducing calories. Grains with waxy starch promote puffing and friability in expanded cereal snacks and breakfast foods, whereas grains with normal starch promote crunchiness (*8*).

Interest in waxy wheat is high and expected to grow in the future. Cereal food companies are actively evaluating waxy flours, and major food ingredient suppliers are promoting functional wheat flours for food applications. In November 2006, Limagrain Céréales Ingrédients, the largest seed breeder in the European Union, launched what it claimed was the world's first instant waxy wheat flour in Europe (http://www.foodnavigator.com/Science-Nutrition/Limagrain-launches-world-s-first-instant-waxy-wheat-flour).

The wet-milling process fractionates flours into vital wheat gluten, prime starch, tailings (B-grade starch), and water-solubles (*9*−*12*). Previous studies (*13*, *14*) in our laboratory have shown that dough mixing properties are related to wet milling performance, and not all hard waxy wheats can be readily fractionated into starch and gluten by wet-milling. Examination of wet-milling of 15 waxy wheat lines (*13*, *14*) found that all 15 waxy wheat flours were mixed to stiff doughs, but only two of the doughs remained cohesive during washing under a stream of water to remove starch. Most of the dough balls lost their cohesiveness during washing and became soft and runny, making it impossible to remove the waxy wheat starch from a dough mass.

Seven waxy wheat flours and two nonwaxy control flours also were processed by a flour-dispersion process (*13*, *14*). Except for one soft waxy flour, recoveries (79−87%) of gluten protein and their purities (82−88% protein) were comparable to the control flours in the dispersion process. However, purification of prime starch was more difficult and required more processing. Even with added washing, the prime waxy wheat starch still contained 0.4−0.5% protein, compared with 0.3% for normal wheat starch.

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*Corresponding author: phone 785-532-6771; fax 785-532-7010; e-mail: ycshi@ksu.edu.

It was hypothesized that elevated pentosans in the waxy flours interfered with separation and purification of waxy wheat starch (*13*, *14*). Others (*15*) reported that waxy wheat starch granules are easily damaged by physical forces, which also may explain some of the difficulty in purifying waxy wheat starch granules.

After waxy wheat starch is isolated from wet milling, it can be modified to produce thickeners for food use (*16*). Modified waxy wheat starch increased viscosity more than modified waxy corn starch, which makes it more economical to use to thicken foods. In addition, pastes of modified waxy wheat starch showed improved freezer stability compared with similarly modified waxy corn starch. Freezer stability of starch-based thickeners is required for frozen foods.

The objectives of this work were to expand on previous research and evaluate hard waxy wheat from advanced breeding lines, and specifically were to (i) examine factors such as water content, pH, salt, and hemicellulase affecting dough-mixing and rheology of waxy wheat flours; (ii) investigate whether differences in waxy and normal wheat starch affect flour dough rheology; and (iii) identify advanced hard waxy wheat breeding lines that can be fractionated readily into starch and gluten, and develop an improved wet-milling process as needed.

MATERIALS AND METHODS

Materials. Six advanced breeding lines of waxy hard wheats (NX03Y2114, NX03Y2115, NX03Y2205, NX03Y2315, NWX03Y2459, and NX03Y2489), one partial waxy ('Trego') and one normal hard wheat ('Karl 92') were used in this study. Pedigrees for the six waxy wheat samples are listed in **Table 1**.

Wheat kernels were tempered to 15.5% moisture for 20 h and roller milled into straight-grade flour on a Buhler experimental mill (Buhler Co., Uzwil, Switzerland). The flour was stored in polyolefin bags at room temperature. Starch was isolated from the flour samples by using a dough hand-washing method (AACC Method 38-10) with modification (*13*). The purified starch was oven-dried at 40 °C for 2 days, gently ground with mortar and pestle and stored in Ziploc bags.

Hemicellulase (SEBake-X) was obtained from Specialty Enzymes and Biochemicals Co. (Chino, CA). Commercial vital wheat gluten (>72% protein) was obtained from MGP Ingredients Inc. (Atchison, KS).

General Assays. Moisture, ash, and lipid were determined by AACC Methods 44-15A, 08-01, 30-25, respectively (*17*), and protein contents (N × 5.7) by the combustion method on an FP Protein/Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Total starch was determined by AACC Method 76-13 with an assay kit from Megazyme International Ltd. (Wicklow, Ireland). Single kernel characteristics of wheat samples were determined with the Single Kernel Characterization System (SKCS4100, Perten Instruments North America, Inc., Reno, NV). All general assays were done in duplicate.

Water Absorption Capacity of Starches. Water was put into desiccators at 25 °C to obtain 100% relative humidity atmosphere. Three grams (db) of starch of waxy wheat and normal wheat were spread out in Petri dishes, placed in the desiccators, and the weight gained was measured with time. The water-absorption experiments were terminated when weight gain ceased (~4 days). Each sample was examined in duplicate.

Protein Composition of Wheat Flours. Composition of the wheat proteins was determined using a combination of selective extraction and nitrogen combustion analysis of insoluble proteins (*18*). Wheat proteins were extracted from flours with aqueous 50% 1-propanol and analyzed by size-exclusion chromatography as described in Schober et al. (*19*). The total peak area, soluble polymeric protein (SPP) area, gliadin (Gli) area, and albumin-globulin (AG) area were thus obtained along with the amount of insoluble polymeric proteins (IPP).

Gluten Index. Gluten indices of flour samples were determined in triplicate using the Glutomatic system (Perten Instruments AB, Huddinge, Sweden), which comprises a gluten washer (Glutomatic 2200), centrifuge (Centrifuge 2015), and dryer (Glutork 2020). Flour (10.0 g ± 0.01 g) and 2% aqueous sodium chloride (4.8 mL) were placed into the Glutomatic wash chamber fitted with an 88-μm polyester sieve. After gluten separation, wet gluten pieces were subjected to centrifugation, which forced a fraction of the gluten through the sieve and into a specially designed sieve cassette. Gluten index was calculated as the amount of wet gluten remaining on the centrifuge sieve divided by the total weight of wet gluten. Wet gluten was dried to obtain the dry gluten content and water binding of the wet gluten.

Mixograms. Mixograms of flours were determined by using AACC Method 54-40A with a 10-g scale Mixograph (National Manufacturing Co., Lincoln, NE). Water absorption was initially calculated with the equation: absorption (%) = 1.5 (% protein) + 43.6, where protein is based on 14% mb (AACC Method 54-40A). A model dough formula was designed to produce constant protein content (db) from commercial wheat gluten (1.8 g, db) and starch (7.3 g, db) for each test. Addition of water was adjusted to produce acceptable mixograms. Mixograms of waxy and normal wheat flours mixed with 2% aqueous sodium chloride at optimum absorption level also were recorded.

Isolation of Wheat Starch and Gluten by Dough Washing. Flour (100 g, 14% mb) was put in a 200-g bowl pin mixer (National Manufacture Co., Lincoln, NE) and mixed for 1 min. On the basis of the absorption determined with the mixograph, flour was mixed with an optimal amount of water until it reached peak time. The formed dough was covered by a polyolefin film and allowed to rest for 1 h at room temperature. After resting, the formed dough ball was gently hand-kneaded under a stream of water over a wire-mesh sieve with 125-μm openings. Washing was continued until water squeezed from the gluten ball appeared clear, which indicated the majority of starch and soluble material had been washed away. One drop of the wash water was transferred onto a microscope slide, covered by a glass coverslip and then viewed by an Olympus BX51 microscope (Olympus America Inc., Melville, NY) under a 40× objective. The final wash water showed a small number of B-type granules of starch. The gluten fraction (~40 g) was covered and stored at room temperature for 1 h. Wet gluten was weighed into jar, cooled in a freezer, and the jar then attached to a freeze-dryer. The gluten expanded approximately 2-fold because of the reduced pressure, which created surface area for drying. The throughs from the 125 μm sieve were passed through a 75-μm opening sieve, and the "overs" were washed with 50 mL of water. The material (starch "milk") passing through the 75-μm opening sieve was centrifuged at 2500*g* for 20 min. The supernatant was carefully decanted and saved, and the upper gray layer, which was the tailing fraction, was carefully removed with a spatula. The solids content in the supernatant was determined by drying 100 mL of the supernatant at 105 °C in an oven until no further change in weight. Starch in the bottom layer was resuspended in 200 mL of water, and the mixture was centrifuged at 2500*g* for 20 min. Once again, the tailings layer was carefully removed with a spatula. The fibrous residue on the 75-μm sieve and tailings removed from the first and second centrifugation steps were combined and freeze-dried. Starch was weighed after oven-drying at 40 °C for 2 days. Dried starch was analyzed for moisture and protein after being gently ground with a mortar and pestle. Starch recovery was calculated on the basis of total starch in the flour.

In a separate experiment with NWX02Y2459, hemicellulase (0.5 g) was first added to distilled water (65.8 mL), and the enzyme solution was mixed with flour. The fractionation of flour was then performed as described in the previous paragraph.

Light Microscopy. To determine whether there was contamination of normal wheat in waxy wheat, the waxy wheat starches from the six advanced lines along with the partial waxy and normal wheat starches (Trego and Karl 92) were stained with polyiodide ion and viewed under light and dark field microscopy. Isolated wheat starches (~10.0 mg each) were suspended in 1.0 mL of iodine and potassium iodide solution (0.313 g of iodine and 7.5 g of potassium iodide in 500 mL of 50% glycerol) and stained 15 min at 25 °C. Stained starch was viewed under an Olympus BX 51 microscope (Olympus Optical Co. Ltd., Shinjuku-ku, Tokyo, Japan).

Statistical Analysis. SAS software (Gary, NC) was used to perform ANOVA and least significance difference (LSD) analysis. The level of significance was $P < 0.05$ throughout the paper.

RESULTS AND DISCUSSION

Wheat Kernel Properties. Single kernel characteristics of wheat kernels are listed in **Table 2**. Weights of individual waxy wheat

Table 1. Pedigrees of Waxy Wheat Lines

sample	pedigree
NX03Y2114	Cimarron/RioBlanco//BaiHuo4/L910145/3/Colt/Cody//Stozher/NE86582
NX03Y2115	Cimarron/RioBlanco//BaiHuo4/L910145/3/Colt/Cody//Stozher/NE86582
NX03Y2205	BaiHuo/Kanto107,F2-1//Ike/4/KS831672/3/Rannaya 2/Bez.4/2/Lancota/f9-67
NX03Y2315	BaiHuoMai/Ike//KSSB-369-7/NE88584
NWX02Y2459	BaiHuoMai/Ike//KS91H184/3*RBL//N87V106
NX03Y2489	BaiHuo/Kanto107//Ike/3/KS91H184/3*RBL//N87V106
Karl 92	Plainsman V/3/Kaw/Atlas 50//Parker*5/Agent
Trego	RL6005/RL6008//Larned/3/Cheney/Larned/4/Bennet sib/5/TAM107/6/ Rio Blanco

kernels ranged from 25.6–30.3 mg, compared with 28.7–31.6 mg for normal and partial waxy wheat kernels. Diameters of waxy wheats kernels were close to those of normal wheats (~2.3 mm). The hardness indices (62.8–76.2) indicate that all eight wheat samples would be classified as hard wheats (*20*).

Flour yields of the six waxy hard wheats ranged from 68.8–73.3%, compared with 70.8–72.5% for the partial waxy and the normal hard wheat (S. K. Garimella Purna, personal communication, 2007). The same flour yield was obtained from waxy hard wheat as from normal hard wheat on a Buhler experimental mill but the feed rate of waxy wheat kernels had to be reduced. Otherwise, the waxy flour clogged the sieves during dry milling. Yasui et al. (*21*) reported that waxy wheat from two Japanese mutant lines had reduced flour flowability and gave about 20% lower flour yield than the nonwaxy parents, which was attributed to 20% higher grain fat and β-glucan contents. Lower flour yield also has been reported for waxy soft wheat and waxy spring wheat compared with their wild types (*5*, *22*).

Composition and Gluten Indexes of Wheat Flours. Flour composition is listed in **Table 3**. Protein contents of the waxy flours ranged from 12.00–15.10% (db), compared with 14.04–15.47% for the normal flours. Starch in the flours ranged from 75.0–81.7%, and ash from 0.49–0.67%. Free lipids, extracted from flour with hexane or ethyl ether, were elevated more than 40% in two waxy flours (NWX02Y2459 and NX03Y2489) (**Table 3**). Pentosan content of the six waxy wheat flours varied from 1.4 to 2.1%, while Karl 92 and Trego flours had 1.7% and 1.3%, respectively (S. K. Garimella Purna, personal communication, 2008). Starches in all waxy wheat flours contained less than 3% normal wheat starch as determined by the iodine-staining technique. Amylose content in control wheat flours was 27.4% for Karl 92 (*23*) and 23.0% for Trego (*24*) as determined by a Concanavalin A method.

The level of gliadins (Gli) in the flours, based on total protein content, ranged between 31 and 44%, and insoluble polymeric protein (IPP) between 36 and 55% (**Table 4**). Three of the waxy wheat flours (NX03Y2315, NWX02Y2459, NX03Y2489), contained 41–46% IPP, which indicates weaker gluten mixing properties, but the three other waxy wheat flours contained 49–55% IPP, indicating stronger gluten mixing properties. The differences in IPP content among the waxy wheat lines were probably related to their pedigrees. The first three crosses listed in **Table 1** (NX03Y2114, NX03Y2115, and NX03Y2005) involved more strong gluten parents and as a result, had higher IPP contents. It is interesting to note that the waxy wheat flours examined in this work have higher IPP contents than earlier generations of hard waxy wheat flours (*14*).

Table 2. Single Kernel Characteristics of Waxy and Control Wheats

wheat kernel	weight (mg)	diameter (mm)	hardness Index	moisture (%)
NX03Y2114	27.6 ± 6.7	2.29 ± 0.37	66.7 ± 16.1	9.5 ± 0.6
NX03Y2115	29.0 ± 6.8	2.37 ± 0.36	62.8 ± 13.9	9.4 ± 0.6
NX03Y2205	25.6 ± 6.4	2.22 ± 0.35	73.0 ± 15.9	9.1 ± 0.6
NX03Y2315	30.3 ± 9.2	2.38 ± 0.46	72.5 ± 18.3	9.6 ± 0.7
NWX02Y2459	28.5 ± 7.3	2.35 ± 0.37	67.5 ± 15.4	9.8 ± 0.5
NX03Y2489	27.5 ± 7.2	2.27 ± 0.39	76.2 ± 16.4	9.5 ± 0.6
Karl 92	31.6 ± 7.3	2.43 ± 0.35	64.8 ± 15.8	8.3 ± 1.0
Trego	28.7 ± 8.0	2.31 ± 0.45	75.9 ± 17.5	9.5 ± 0.8

Table 3. Composition of Waxy and Normal (Karl 92 and Trego) Wheat Flours

	level[a], %				
wheat flour	moisture	protein	starch	ash	free-lipid
NX03Y2114	12.4	13.88	75.0	0.50	0.34
NX03Y2115	12.0	13.78	81.7	0.52	0.49
NX03Y2205	11.8	15.10	78.3	0.49	0.48
NX03Y2315	11.6	12.00	81.7	0.58	0.41
NWX02Y2459	12.0	13.33	78.3	0.57	0.66
NX03Y2489	11.7	12.82	80.0	0.64	0.68
Karl 92	13.2	15.47	76.7	0.67	0.40
Trego	12.5	14.04	76.7	0.54	0.49

[a] Moisture level on wet basis, other levels on dry-weight basis.

Table 4. Protein Composition in Waxy and Normal Wheat Flours[a]

	protein fraction[b], % of protein			
wheat flour	Gli	Alb/Glob	SPP	IPP
NX03Y2114	32.1f ± 0.6	4.0d ± 0.2	12.3b ± 0.1	52.6b ± 0.9
NX03Y2115	34.5e ± 0.1	4.5 c ± 0.1	12.5b ± 0.3	48.1c ± 0.6
NX03Y2205	31.3 g ± 0.1	3.5 e ± 0.1	11.7c ± 0.0	54.8a ± 0.3
NX03Y2315	41.8b ± 0.2	4.8 b ± 0.1	9.2d ± 0.0	43.3e ± 0.3
NWX02Y2459	38.7c ± 0.0	4.9ab ± 0.0	13.1a ± 0.0	40.9f ± 0.4
NX03Y2489	37.1d ± 0.5	4.7 b ± 0.0	12.4b ± 0.2	45.6d ± 0.5
Karl 92	36.4d ± 0.3	3.8 d ± 0.1	11.6c ± 0.1	47.2c ± 0.4
Trego	43.6a ± 0.3	5.0 a ± 0.0	11.7c ± 0.0	35.7 g ± 0.3

[a] Average of two measurements ± standard deviation. Values in the same column followed by the same letter are not significantly different in statistics ($P < 0.05$). [b] Gli — gliadin, Alb/Glob — albumin and globulin, SPP — soluble polymeric protein, IPP — insoluble polymeric protein.

The hard-winter waxy wheat flours generally had lower gluten indices (**Table 5**) than normal hard-winter wheat flours because their doughs lacked elasticity. Waxy wheat flours gave less reproducible indices, especially NWX02Y2459, because their doughs were sticky. Although waxy wheat flour NWX02Y2459 had high protein content, its proteins appeared to have poor aggregation properties because it contained the lowest IPP level and a high free-lipid content (**Tables 3** and **4**).

Two of the waxy wheat flours (NX03Y2315, NWX02Y2459) had gluten indices (~46 and 54) that were 41–49 units lower than the average (~95) of the normal and partial waxy wheats (**Table 5**). Three other waxy wheat flours (NX03Y2114, NX03Y2115, NX03Y2205) were 11–18 units lower, and flour of NX03Y2489 was intermediately lower by ~24 units. The low values of the gluten index of the two waxy wheat flours (NX03Y2315, NWX02Y2459) corresponded to their low levels of IPP. However, an overall correlation between gluten index and IPP in wheat flours was not observed. It should be emphasized that the IPP values reported in this and previous work (*14*) were from wheat flours, not from doughs formed. Control flours (Karl 92 and Trego) had intermediate levels of IPP content but the highest gluten indices, indicating formation of more insoluble

Table 5. Gluten Indeces of Waxy Hard and Normal Wheat Flours Determined on the Glutomatic 2200 System[a]

Flour		NX03Y 2114	NX03Y 2115	NX03Y 2205	NX03Y 2315	NWX02Y 2459	NX03Y 2489	Karl 92	Trego
gluten index (%)	avg	77.1c	83.9b	78.8bc	45.8	53.9e	71.0d	94.4a	95.6a
	st dev	2.55	3.38	0.3	[b]	7.62	1.3	0.17	0.35

[a] Values in the same line followed by the same letter are not significantly different in statistics ($P < 0.05$). [b] Sample failed to have triplicate tests.

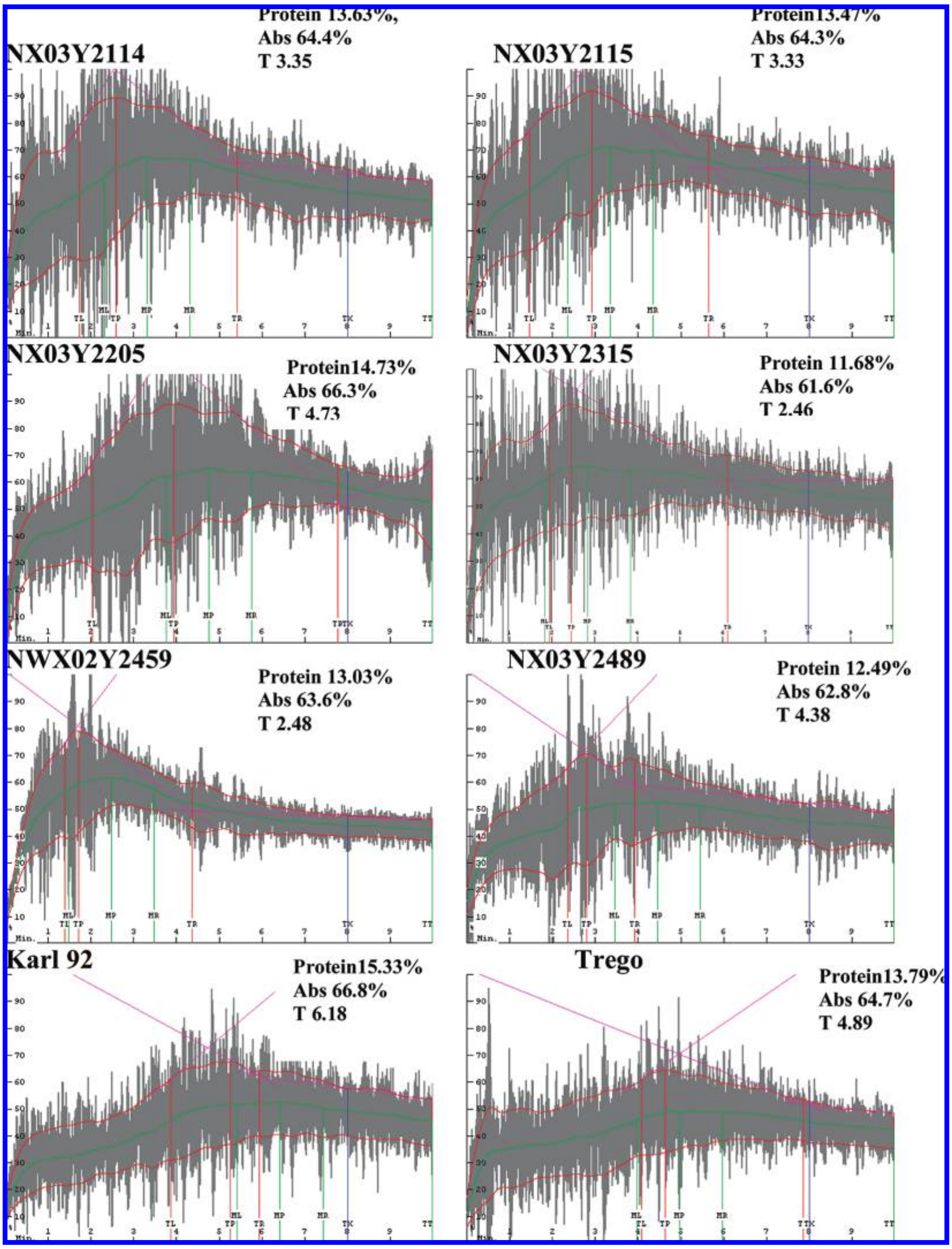
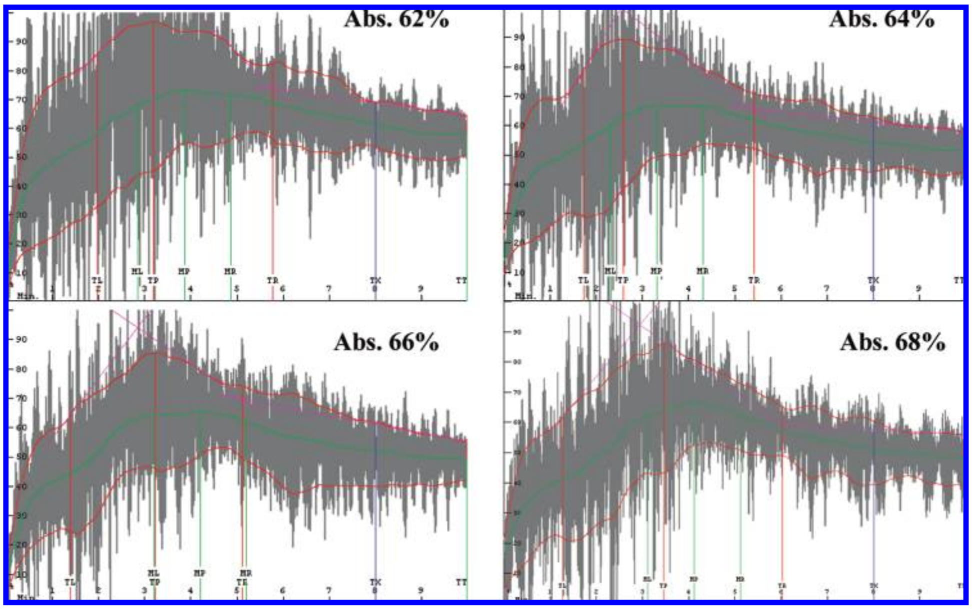


Figure 1. Mixograms of waxy and normal wheat flours determined at the calculated optimum water absorption levels. Protein content of flours was given on 14% moisture basis. *T* = peak mixing time.

polymeric protein during dough mixing. The fact that three waxy wheat lines (NX03Y2114, NX03Y2115, and NX03Y2205) had higher IPP levels but lower dough strength suggested inherent genetic differences in the gluten proteins or in the interaction between the gluten proteins and the waxy starch, which is discussed in the next section, where mixing of gluten was altered when waxy starch was added. Continued research is being conducted to determine IPP in flour doughs and changes in protein structure of these wheat flours during dough formation. Taylor and Randall (*25*) investigated the relationship between gluten index and a wide range of commonly used direct and indirect measures of bread making quality on South African wheats. They suggested that gluten index is a measure of gluten elasticity and that total wet gluten yield is an indication of gluten viscosity or cohesiveness.



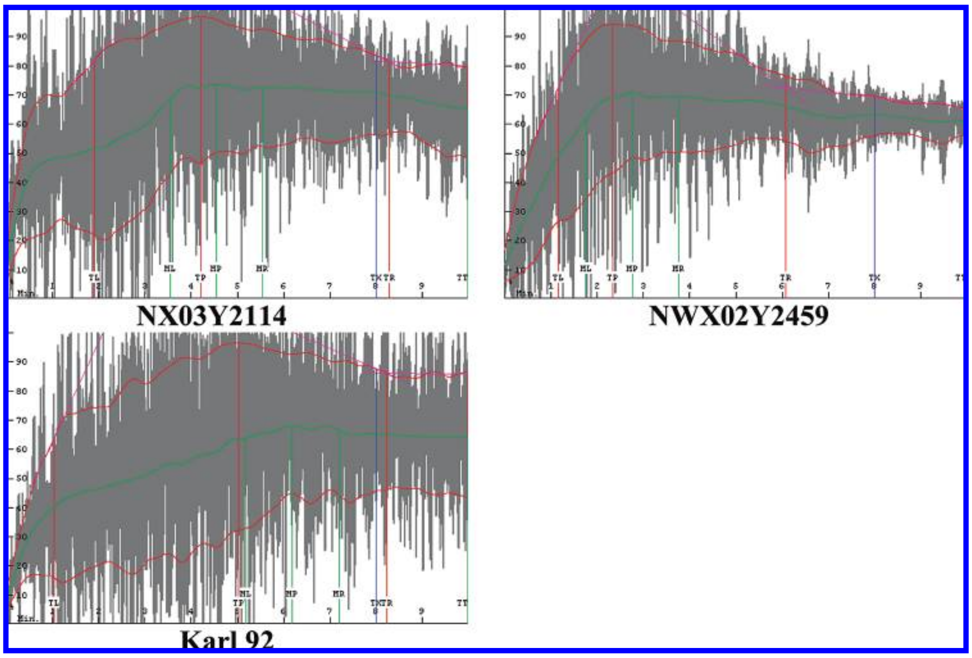
Abs. 62%

Abs. 64%

Abs. 66%

Abs. 68%

Figure 2. Mixograms of waxy wheat flour (NX03Y2114) at different water absorption levels.



NX03Y2114

NWX02Y2459

Karl 92

Figure 3. Mixograms of waxy and normal wheat flours mixed with 2% sodium chloride solution at optimum absorption levels.

Mixograms. Figure 1 shows mixograms of the six hard waxy flours, partial waxy and normal hard wheat flours with calculated water absorptions (AACC Method 54-40A). The mixogram peaks of the waxy wheat flours were generally higher than the control wheat flours. The mixogram of waxy wheat NWX02Y2459 showed weak gluten and less resistant to mixing as indicated by a short mixing time (2.48 min), and a narrow mixing curve with a large breakdown. This mixograph data agreed with the low gluten index and low IPP content of that waxy wheat. On the other hand, the mixograms of NX03Y2114, NX03Y2115, and NX03Y2205 showed an increased mixing time (3.33−4.22 min), broad mixing curve and limited dough breakdown, similar to mixograms of the control flours of Karl and Trego (mixing time 4.7−4.8 min), which were resistant to mixing. The mixogram of waxy flour NX03Y 2315 showed good strength of its gluten during early mixing, but considerable weakening occurred upon overmixing. The IPP content and the gluten index of NX03Y 2315 flour were both low.

To determine optimal water absorption, we varied mixograph absorption for each flour. **Figure 2** shows mixograms of waxy wheat flour (NX03Y2114) at different water absorption levels. Doughs made by using 62 and 64% absorption were dry and the mixograms were over scale. At 66% water absorption, the dough showed good strength and was more resistant to breakdown and considered optimum. It should be noted that the calculated water absorption was 64% based on protein content by using AACC Method 54-40A. These results suggest that optimum dough development was not achieved if the water absorption of waxy wheat flour was based on the equation derived from normal wheat flours and their protein contents. Water content should be adjusted for waxy and some normal wheat flours to develop optimum dough. The importance of water absorption on recording a

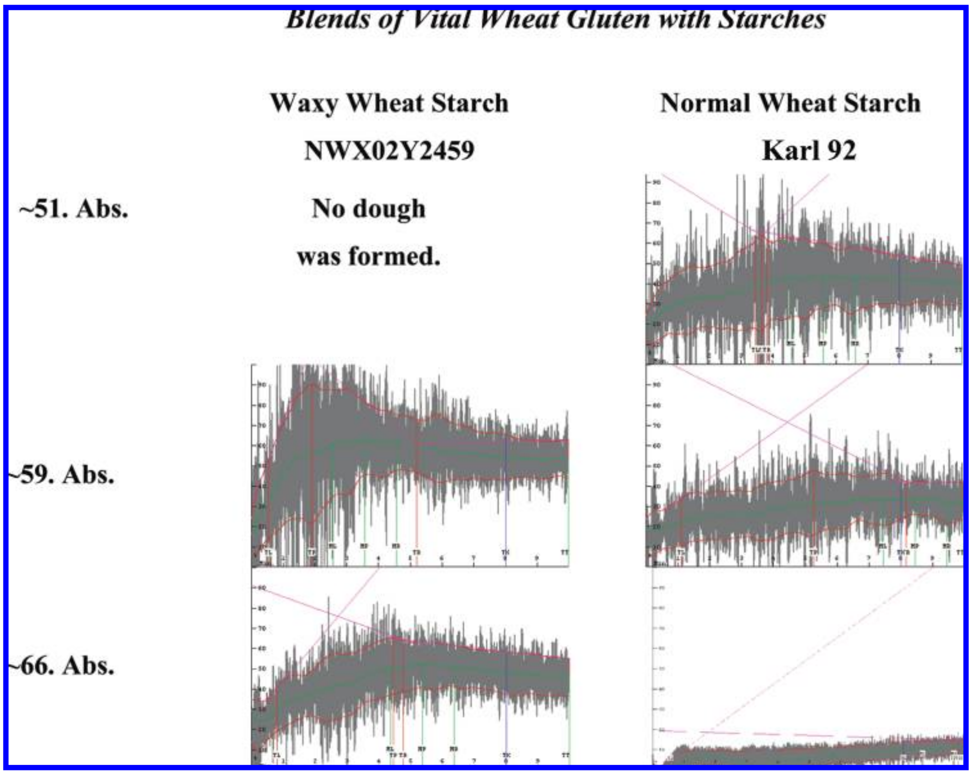


Figure 4. Mixograms of blends of waxy or normal starch (7.3 g, db) with commercial vital wheat gluten (1.8 g).

mixogram has been pointed out for normal wheat flour (*26*, *27*). The mixograms of waxy and normal wheat flours mixed with 2% sodium chloride solution showed longer mixing times, broader mixing curves and significantly reduced breakdown compared with those of flours mixed with water (**Figure 3**). Danno and Hoseney (*28*) reported similar findings on normal wheat flour.

To examine whether differences between waxy and normal wheat starch affect dough rheology, starches were blended with the same gluten to produce blends with 14.5% protein, then mixograms of the blends were determined. At ~66% absorption the blend of Karl wheat starch and commercial gluten failed to give a developed curve. In contrast, the blend of waxy wheat starch (NWX02Y2459) and gluten at the same absorption (+7.5 mL water) gave a developed curve (**Figure 4**). At a lower water content of ~51%, the blend of normal wheat starch and wheat gluten gave a developed dough and mixogram curve, whereas the blend of waxy wheat starch and gluten did not form a cohesive dough but remained mostly a moistened flour. These results may be explained by an increased water absorption capacity of waxy wheat starch, which competes with wheat gluten for the water added. There was too little water added for wheat gluten to hydrate and form a protein network when the blend having waxy wheat flour was mixed at ~ 51% absorption.

At 100% relative humidity at room temperature, waxy wheat starches NX03Y2114 and NWX02Y2459 absorbed 106 and 119% water (w/w), respectively, whereas a normal wheat starch (Karl 92) gained 98%, indicating that waxy starch absorbs more water than normal starch (**Table 6**). Baik and Lee (*29*) reported that the water retention capacity of waxy wheat starch (80−81%), determined after soaking in water at 25 °C, was much higher than that of regular wheat starch (55−62). Wheat flour NWX02Y2459 had more starch damage (S. K. Garimella Purna, personal communication, 2008) and the isolated starch had higher water absorption (**Table 6**). It has been suggested that waxy wheat starch granules are easily damaged by physical forces (*15*). However, flours of waxy wheat NX03Y2114 and Karl 92 had similar levels of damaged starch (data not shown), but waxy starch isolated from NX03Y2114 had higher water absorption. We conclude that the difference in water absorption between waxy and normal wheat starch probably affects the distribution of water between starch and wheat gluten, which in turn affects hydration and gluten development in mixograms of the flours. In addition, the difficulty of removing protein from waxy vs normal wheat starch may be caused by the greater buoyancy of the waxy starch granules because of their increased water absorption.

Table 6. Water Absorption Capacities of Two Waxy Wheat Starches (NX03Y2114 and NWX02Y2459) and Normal Wheat Starch (Karl 92)[a]

wheat starch	weight gained (%)
NX03Y2114	106.2b
NWX02Y2459	119.2a
Karl 92	98.4c

[a] Values in the same column followed by the same letter are not significantly different in statistics ($P < 0.05$).

Wet Milling of Waxy Wheat Flours by Dough Washing. Absorption-optimized doughs were used when evaluating the wet-milling performance of waxy wheat flours by the dough-washing procedure. The fractions of wet gluten, prime starch, tailings, and water-solubles, as well as the protein contents of the gluten and starch fractions, are given in **Table 7** for waxy, partial waxy and normal wheat flours. Compared to the doughs from Karl or Trego wheat, washing waxy wheat doughs under a stream of water caused the dough to become slack, spread out more on the sieve and break apart into several pieces (**Figure 5**). However, the slack dough pieces did not block the screen, and when approximately two-thirds of the starch had been washed away, the small dough pieces coalesced into one elastic doughball that behaved like the controls. Mixing a hard waxy wheat flour with 2% sodium chloride solution did not improve the cohesiveness of the dough during dough washing, but the dough was less sticky on its surface. Our results suggest that these advanced breeding lines of

Table 7. Wet-Milling Data for Wheat Flours by Dough-Washing Method Starting with 100 g (14%mb) of Flour[a]

				dry gluten fraction			starch fraction						
wheat flour	flour protein (g)	flour starch (g)	wet gluten (g)	wt (g)	protein content (%)	recovery (%)	wt (g)	protein content (%)	MC[b] (%)	recovery (%)	soluble solids (g)	tailings (g)	total solids recovery (g) (%)
NX03Y2114	13.6	73.7	44.9	14.1	82.2	85.2bc	51.4	0.16	4.7	66.5b	9.4	11.5	84 (97)
NX03Y2115	13.5	79.9	48.8	15.2	71.3	80.3d	44.3	0.16	11.2	58.2c	12.7	15.2	82 (96)
NX03Y2205	14.7	76.4	43.9	16.3	79.6	88.3ab	46.8	0.21	5.3	58.0c	11.9	12.3	85 (99)
NX03Y2315	11.7	79.5	39.7	13.1	75.8	84.9bc	46.4	0.17	12.3	66.0b	17.0	15.4	86 (101)
NWX02Y2459	13.0	76.5	48.0	15.0	66.8	77.1de	46.1	0.05	9.3	56.4c	10.0	9.4	76 (89)
NX03Y2489	12.5	77.9	37.3	13.3	78.0	83.0c	48.6	0.05	4.6	59.5c	10.9	13.0	83 (97)
Karl 92	15.3	76.0	43.7	13.8	85.8	77.4de	52.4	0.27	4.8	65.6b	6.0	10.9	81 (94)
Trego	13.8	75.4	33.8	10.8	82.3	64.4f	59.0	0.14	6.4	73.2a	8.7	10.6	86 (100)
NWX02Y2459 + 0.5 g hemicellulase	13.0	76.5	50.5	17.2	67.0	88.6abc	45.3	0.04	5.9	55.7c	11.0	10.9	82 (95)
NWX02Y2459 + 2%NaCl	13.0	76.5	49.6	16.3	72.4	90.0a	60.0	0.33	6.5	73.3a	9.57	4.0	86 (100)

[a] Values in the same column followed by the same letter are not significantly different in statistics ($P < 0.05$). [b] MC = moisture content.

Figure 5. Residues from doughs of waxy (NX03Y2114) and normal wheat flours after washing under a stream of water (600 mL).

hard waxy wheats can be wet-processed in commercial operations. Even so, the dough of waxy wheat flour NWX02Y2459 was especially weak and sticky during the early stages of dough washing, and it gave relatively poor recoveries of gluten and starch with low purity (**Table 7**). Adding hemicellulase to the dough of waxy wheat flour NWX02Y2459 improved gluten recovery but not starch recovery. On the other hand, waxy wheat flours NX03Y2114, NX03Y2205, and NX03Y2315 gave 85−88% recovery of protein in the gluten fraction with 76−82% protein content, and 58−67% recovery of starch with 0.16−0.17% protein content (**Table 7**). Total recovery of solids in wet milling of the flours ranged from 94−101%, except for waxy flour NWX02Y2459 with 89% solids recovery (**Table 7**).

Adding hemicellulase to relatively poorly performing waxy wheat flour NWX02Y2459 reduced dough stickiness during the early stage of washing and thereby improved gluten recovery (**Table 7**). It appears that addition of ∼0.6% (db on flour) hemicellulase hydrolyzed some insoluble hemicellulose, which increased the level of soluble hemicellulose and strengthened the dough mixing curve (**Figure 6**). However, adding 1.2−2.4% hemicellulase depolymerized most of the soluble hemicelluloses and the dough became less stiff. Several studies have shown that hydrolases that act on nonstarch polysaccharides can be added as a processing aid in the separation of wheat flour into starch and gluten. Weegels et al. (*30*) found that cellulases and hemicellulases improved gluten yield, protein recovery in gluten, gluten coagulation and starch yield from flour that had intermediate processing properties. Redgwell et al. (*31*) suggested that xylanase has a beneficial effect by decreasing interaction between gluten protein and water-extractable arabinoxylans, which is one component of nonstarch polysaccharides. Courtin and Delcour (*32*) reported that xylanase that effected hydrolysis of the water-soluble arabinoxylans improved separation of vital wheat gluten and starch. However, protease and amylase contaminated in hemicellulases are unfavorable for gluten-starch separation and should be avoided because their actions increase the amount of soluble protein and carbohydrate, which in turn increases viscosity of the starch slurry and results in a less efficient separation of starch, protein and water-solubles.

Contamination of Waxy Wheat with Normal Wheat Starch. **Figure 7** shows photomicrographs of iodine-stained granules of one waxy line (NX03Y2315) and one normal (Karl 92) wheat starch. Both waxy and normal wheat starches showed two types of granules, the large disk-shaped (Type A) and the small sphere-shaped granules (Type B). Under light field illumination, amylose-containing granules of starch gave a blue color. In contrast, granules containing amylopectin granules gave a red-brown color. Waxy wheat starches showed a clear birefringence under crossed-Nicol prisms (dark field), whereas normal wheat starches showed a faint birefringence with bluish surrounding area. By counting we found that waxy wheat starch NX03Y2315 contained less than 3% normal wheat starch granules. The same results were found for other isolated waxy wheat starches. The observed low level of contamination could have arisen from out-crossing or mechanical mixing during harvest or seed cleaning operations.

In conclusion, to realize high yields of roller-milled flour from hard waxy wheat, more sieving capacity appears to be needed. Waxy wheat starch absorbs 10−20% more water than normal wheat starch, which affects dough mixing properties. Higher water content needs to be used for some waxy wheat

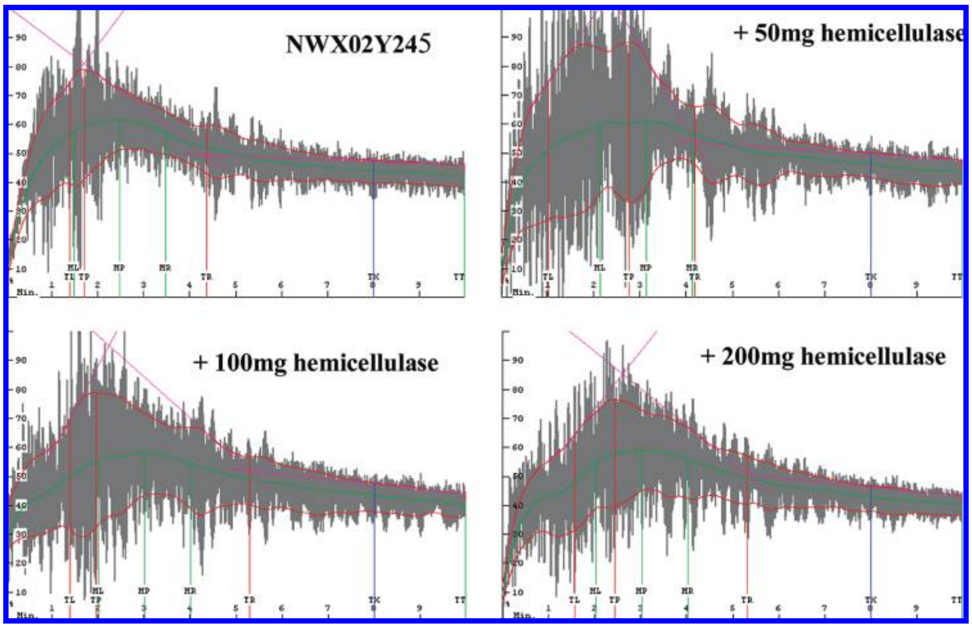


Figure 6. Effect of hemicellulase on the mixogram of waxy flour dough (NWX02Y2459) at optimum absorption of 63.6% and a flour protein of 13.03% (14% mb).

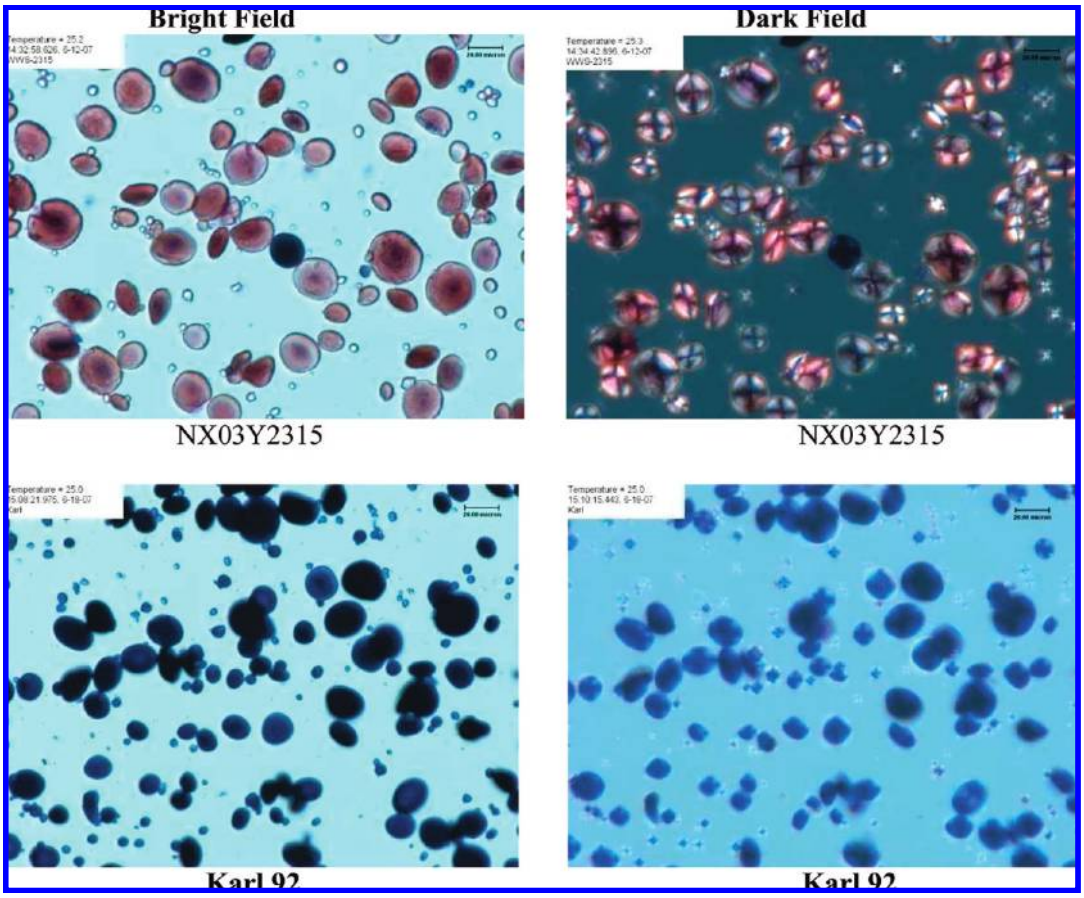


Figure 7. Photomicrographs of iodine-stained starch samples viewed under light (left side) and dark field (right side).

flours to develop optimum doughs. Wet-milling of waxy wheat flours appears feasible. The stickiness of a waxy wheat flour dough may be reduced by adding low levels of hemicellulase, and mixing a weak waxy flour dough with 2% NaCl solution improves the recovery of the gluten and starch fractions. The future work will be to determine the quality and properties of gluten and waxy wheat starch isolated from hard waxy wheat flours.

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