Wet-milling properties of waxy wheat flours by two laboratory methods

Abdulvahit Sayaslan a,*, Paul A. Seib b, Okkyung K. Chung c

a Department of Food Engineering, Gaziosmanpaşa University, 60240 Tokat, Turkey
b Department of Grain Science and Industry, Kansas State University, Manhattan, 66506 KS, USA
c USDA/ARS Grain Marketing and Production Research Center, Manhattan, 66502 KS, USA

Received 7 July 2004; accepted 18 November 2004
Available online 18 January 2005

Abstract

Straight-grade flours roller-milled from seven waxy and two control (nonwaxy and partial waxy) wheats (Triticum aestivum) were wet-milled by dough-washing (DW) and dough-dispersion and centrifugation (DD&C) methods to give starch, gluten, tailings, and water-solubles fractions. Compared to the controls, flour yields of the waxy wheats were lower. The waxy flours contained more crude fat and pentosans and less total starch. Gluten proteins of the waxy flours appeared to be weaker. By the DW method, both of the controls but only two of the seven waxy flours could be wet-milled. The remaining five waxy flours failed in the DW method as their doughs lost cohesiveness and elasticity during kneading and washing under water. Those waxy flours were instead wet-milled by the modified DW method, where the doughs were gently hand-rubbed on a 125-μm opening screen to facilitate the passage of the starch and water-solubles through while retaining the gluten agglomerates on the screen. The prime starch and tailings of the waxy flours were not separated as visibly as those of the controls during their centrifugal purification, which in turn led to a reduction in yields and purities of their prime starch and gluten fractions. On the other hand, the wet-milling characteristics of the waxy flours by the DD&C method were comparable to those of the controls because the DD&C method is apparently less dependent than the DW method on the gluten aggregation traits of flours.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Waxy wheat flour; Wet-milling; Starch and Gluten separation

1. Introduction

Wheat is among the leading cereal grains produced in the world, and is used for food (67%), feed (20%), and seed (7%). The industrial uses of wheat, which also includes its wet-milling to produce starch and vital gluten as the major co-products, account for ~6% of total production. Common or bread wheats (Triticum aestivum) with hard or soft endosperm constitute about 95% of total production, whereas durum wheat (Triticum durum) accounts for ~5%. Durum and hard wheats contain more protein (12–16%) than do soft wheats (8–10%) (Oleson, 1994; Seib, 1997; USDA/NASS, 2001). Hard and soft wheats are roller-milled to flour and commonly used for producing yeast-leavened breads and rolls as they form strong viscoelastic doughs when mixed with water. Soft wheat flours, however, give weak doughs and are thus preferred for cakes, cookies and crackers. Durum wheat with the hardest endosperm among wheats is milled to semolina and used in pasta and couscous production due to its strong gluten elasticity and desirable yellow color. Hard or soft wheat flours with

* Mention of firm names or trade products does not imply that they are endorsed or recommended by the USDA, Kansas State University, or Gaziosmanpaşa University over other firms or products not mentioned.

* Corresponding author. Tel.: +90 3562521616x3262; fax: +90 356 2521488.
E-mail address: sayaslan@gop.edu.tr (A. Sayaslan).
weak- to medium-strength gluten are suitable for the Asian noodles, steam breads, and flat breads (Oleson, 1994; Posner, 2000; Seib, 1997). For wet-milling to produce wheat starch and vital wheat gluten, straight-grade or high-yield flours from hard or soft wheats with high-protein content (>11%), good mixing quality (strong gluten aggregation), and low starch damage, ash, and α-amylase activity are preferred (Maningat & Bassi, 1999). Wheat starch is used in a vast variety of food and nonfood applications in unmodified or modified forms; such as acid-thinned, bleached, oxidized, cross-linked, substituted, cross-linked/substituted, and other doubly modified starches (Maningat & Seib, 1997).

Wheat starch is also converted to starch hydrolysis products, such as sweeteners (Schenck & Hebeda, 1992). Vital wheat gluten is a valuable co-product of wheat wet-milling process that yields wheat starch (Bergthaller, 1997; Cornell & Hoveling, 1998; Grace, 1988; Maningat & Bassi, 1999; Maningat, Bassi, & Hesser, 1994). Vital wheat gluten is used in breakfast cereals and snacks, meat and cheese analogs, breading and batter mixes, pizza, and in meat, fish, and poultry products (Bergthaller, 1997; IWGA, 1989; Maningat & Bassi, 1999). Wheat gluten also is of great importance as an industrial protein, and is converted to wheat protein isolate, hydrolyzed wheat protein, and texturized or deamidated wheat gluten (Bergthaller, 1997; Maningat et al., 1994; Maningat, DeMeritt, Chinnaswamy, & Bassi, 1999).

Five industrial wet-milling processes that start with flour rather than wheat kernel have been employed to isolate wheat starch and vital wheat gluten (Maningat & Bassi, 1999; Seib, 1994). Until the mid 1970s, wheat starch had been produced primarily by the Martin process that was invented in 1835 (Anderson, 1967; Barr, 1989; Fellers, 1973; Zwitserloot, 1989a) and rarely by the Batter process developed in 1944 (Anderson, 1967, 1974; Fellers, 1973). Then, the Alfa-Laval/Raisio (Dahlberg, 1978; Kerkkonen, Laine, Alanen, & Renner, 1976; Maijala, 1976) and Hydrocyclone (Verberne & Zwitserloot, 1978; Verberne, Zwitserloot, & Nauta, 1979) processes were introduced. The most recent wet-milling process commercialized in 1984 is the High-Pressure Disintegration (HD) process (Bergthaller, Lindhauer, & Zwingelberg, 1998; Meuser, Althoff, & Huster, 1989; Witt, 1997; Zwitserloot, 1989a, 1989b). The Martin and Batter processes are considered the traditional processes, whereas the Alfa-Laval/Raisio, Hydrocyclone, and HD processes are deemed the modern-day aqueous dispersion processes. Currently, the Martin (modified modern versions), Hydrocyclone, and Alfa-Laval/Raisio processes are commonly used in North America, and the HD process is popular in Europe (Maningat & Bassi, 1999). The Batter process was mostly abandoned. The advantages of the aqueous dispersion processes include a reduction in fresh water consumption, an increase in prime starch yield, and less sensitivity to gluten protein aggregation characteristics during wet processing (Zwitserloot, 1989a, 1989b). For details on the industrial wet-milling processes for wheat flour, the reader is referred to a recently published review by Sayaslan (2004).

Common wheats are hexaploid with three sets of chromosomes, but they differ in the translation of granule-bound starch synthase isozymes in their kernels (Yamamori & Quynh, 2000). Wild-type (nonwaxy or typical) wheats contain all three possible waxy proteins (Wx-A1, Wx-B1, and Wx-D1) and thus possess normal level (~28%) of amylose in the starch. Partial waxy wheats are null for one or two of the three waxy proteins, which results in reduced level of amylose (~20–26%). Waxy wheats are, however, triple-null for all the three waxy proteins and their starch contains <1% amylose (Kim, Johnson, Graybosch, & Gaines, 2003; Seib, 2000). Besides commonly grown nonwaxy and partial waxy wheats, waxy wheats with hard or soft endosperm have been developed recently and may find uses in wet-milling for waxy starch production, extruded products for volume expansion, frozen foods and doughs for cold-temperature stability, bakery products for shelf-life extension and fat replacement, instant food products for easy-to-cook trait (Bhattacharya, Erazo-Castrejon, Doehlert, & McMullen, 2002; Graybosch, 1998), and for other purposes yet to be discovered. The low gelatinization temperature of waxy wheat starch compared to waxy corn starch may be important in microwaved foods. Hydroxypropylated and cross-linked waxy wheat starch gave a thick paste with high-cold temperature stability. Cross-linking waxy wheat starch, akin to cross-linked waxy corn starch, imparts high and stable viscosity to processed foods (Reddy & Seib, 2000). Maltodextrins with dextrose equivalents of ~1 and ~10 were successfully produced from waxy wheat starches because of their lower levels (<0.1%) of lipids (Lumduabwong & Seib, 2001).

Waxy wheats were first developed in 1994 through conventional plant breeding in Japan (Nakamura, Yamamori, Hirano, Hidaka, & Nagamine, 1995), and later in Canada (Chibbar, Baga, Demeke, & Huel, 1997) and in the USA (Graybosch, 1998; Morris & Konzak, 2001; Souza, 2001—personal communication). Waxy wheats also have been produced through mutagenesis of partial waxy wheats Kanto 107 or Ike (Kirimbuchi-Otobe, Nagamine, Yanagisawa, Ohnishi, & Yamaguchi, 1997; Yasui, Sasaki, Matsuki, & Yamamori, 1997; Konzak, 1998—personal communication). Physical/chemical properties of waxy wheat starches have been thoroughly studied. Hexaploid waxy wheat starch was found to contain 9.0 to 3.2% apparent amylose and 0.12–0.29% lipids (Abdel-Aal, Hucl, Chibbar, Han, & Demeke, 2002; Chakraborty et al., 2004; Kim et al., 2003; Yasui, Matsuki, Sasaki, & Yamamori, 1996), in contrast with 22–25% amylose and 0.8–1.2% lipids in nonwaxy wheat starch.
(Morrison, Milligan, & Azudin, 1984). As expected, waxy wheat starch showed the A-type X-ray pattern and had an increased level of crystallinity compared to wild types, thereby elevating the enthalpy of gelatinization (Chakraborty et al., 2004; Fujita, Yamamoto, Sugimoto, Morita, & Yamamori, 1998; Hayakawa, Tanaka, Nakamura, Endo, & Hoshino, 1997; Kim et al., 2003; Sasaki, Yasui, & Matsuki, 2000). Tetraploid (durum) waxy wheat starches also showed similar gelatinization and pasting properties (Chakraborty et al., 2004; Grant et al., 2001). Although physical and/or chemical properties of waxy wheat starches have been the focus of much research, wet-milling properties of waxy wheat flours and their gluten fractions are yet to be studied. The objective of this study was therefore to determine the wet-milling properties of waxy wheat flours by two laboratory methods that simulate the traditional Martin and aqueous dispersion processes.

2. Materials and methods

2.1. Materials

Six experimental waxy wheat lines were obtained from Dr. C.F. Konzak (Northwest Plant Breeding Co., Pullman, WA), Dr. R.A. Graybosch (USDA/ARS, University of Nebraska, Lincoln, NE), Dr. C.F. Morris (USDA/ARS-WWQL, Washington State University, Pullman, WA), and Dr. R.N. Chibbar (National Research Council, Plant Biotechnology Institute, Saskatoon, SK, Canada). One of the samples was received as flour. The waxy wheat samples were coded as follows: Wx-A, Wx-B, Wx-C, Wx-1, Wx-2, and Wx-3. All of the samples were experimental waxy wheat lines, but the Wx-1, Wx-2, and Wx-3 wheat lines were reportedly in their advanced stages of field trials and selection for agronomic traits. Leona waxy wheat sample, a waxy wheat variety, was provided by Dr. E.J. Souza (University of Idaho, Aberdeen, ID). Control nonwaxy (Kar1 92) and partial waxy (Ike) wheats were from Dr. J. Martin (Hays Branch of State Agricultural Experiment Station, Kansas State University, Manhattan, KS). The waxy wheats used in the study were reportedly 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.

2.2. Methods

2.2.1. General methods

Wheat samples were cleaned on a Carter-Day dockage tester (Carter-Day Co., Minneapolis, MN) and their test weights measured with a test weight apparatus from Burrows Equipment Co., Evanston, IL. Single kernel characteristics of wheat samples were determined on the Single Kernel Characterization System (SKCS 4100, Perten Instruments North America, Inc., Reno, NV). All wheat samples were tempered at 15.5% moisture for 20 h and roller-milled to straight-grade flours on a Buhler experimental mill (Buhler Co., Uzwil, Switzerland). Lightness values (L*) of flours were measured using a Minolta Chroma Meter (Minolta Co., Osaka, Japan).

Moisture, ash, crude fat, and crude fiber contents were determined by the American Association of Cereal Chemists (AACC, 2000) methods 44-15A, 08-01, 30-25, and 32-10, respectively. Protein contents (N × 5.7) were measured by combustion method on an FP Protein/Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Total starch and starch damage were determined by the AACC methods 76-13 and 76-31, respectively, 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/amyllopectin and Ceralpha α-amylase assay kits from Megazyme International Ireland Ltd., Wicklow, Ireland.

Mixograms of flours were obtained by the AACC method 54-40A using a 10-g mixograph (National Manufacturing Co., Lincoln, NE). The mixograms were interpreted as described by Finney and Shogren (1972). Gluten index (GI) and falling number of 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.

Insoluble polymeric protein (IPP) contents of flours were determined essentially by the method of Bean, Lyne, Tilley, Chung, and Lookhart (1998). Flour (1.0 g, db) was mixed with 4 ml of 50% (v/v) aqueous 1-propanol and the mixture dispersed by hand with a spatula. The sample was mixed continually for 5 min on a vortex mixer, centrifuged at 8160g for 5 min, and the supernatant discarded. This extraction procedure was done a total of three times. After the extractions, the pellet was freeze-dried and analyzed for protein (N × 5.7). The IPP content was calculated as a percentage of total protein in a flour sample.

Sodium dodecyl sulfate (SDS) sedimentation volumes of flours were determined by the AACC method 56–70 with modifications. Flour (2.0 g, 14% mb) was weighed into a 100-ml graduated cylinder and 20.0 ml of water containing 0.0004% bromophenol blue (w/v) was added. The cylinder was capped and hand-shaken for 15 s, then placed on a specially designed (USDA/ARS Grain Marketing and Production Research Center, Manhattan, KS) table-top wrist-action shaker for 225 s at room temperature. Next, 20.0 ml of 2.5% (w/v) aqueous SDS solution was added. After gently shaking, the cylinders were placed on the shaker for another 6 min. Finally, 10.0 ml
of 0.47% (v/v) lactic acid solution was added and the cylinder placed on the shaker for 6 min. The cylinder was removed and placed on a rack with a back lighting for 20 min prior to reading sedimentation volume.

Total and soluble pentosans in flours were estimated according to colorimetric method of Douglas (1981). The concentration of pentose sugar D-xylose after acid-catalyzed hydrolysis of pentosans in a flour (for total pentosan assay) or in its water extract (for soluble pentosan assay) was estimated from a D-xylose standard curve. Soluble pentosans were extracted from a flour prior to colorimetric determination according to Hashimoto, Shogren, and Pomeranz (1987). Flour (100 mg, 14% mb) was weighed into a stoppered 15-ml glass tube, 10.0 ml of distilled water added and the tube shaken at 30 °C in a reciprocal water bath (Precision Instruments, Winchester, VA) at a medium speed for 2 h. The sample was then centrifuged at 2000 g for 5 min and the supernatant collected for determination of soluble pentosans. Soluble and total pentosans were presented as % D-xylose (Douglas, 1981).

Granule size distributions of starches were determined on a laser-diffraction particle analyzer (Lecotrac-LTS150, Leco Corp., St. Joseph, MI) equipped with a recirculator, an on-line ultrasonic probe, and an interfaced computer with Lecotrac software. Starch (~0.2 g) was mixed with distilled water (5 ml) and allowed to stand at room temperature for 24 h. After vigorous agitation by hand, the suspension (~1–2 ml) was added to isotonic buffer solution (300 ml) (sodium chloride, 8.6 g/L; potassium chloride, 0.38 g/L; ethylenediamine tetraacetic acid, 0.4 g/L; 2-phenoxyethanol, 0.2 g/L; in deionized water) being circulated at 40 ml/s through a standard cell. The addition of the starch/water mixture was stopped when the particle analyzer produced a signal indicating that a sufficient number of particles were present for analysis. Then the ultrasonic device was energized for 60 s prior to particle size measurement. The software converted the diffracted light data to particle diameters between 0.7 and 700 μm, and the volume percentages of particles were calculated assuming that the particles were spheres.

2.2.2. Wet-milling of flours by dough-washing (DW) method

Wheat flours were fractionated by the AACC gluten hand-washing method 38-10 with modifications, which resembles the traditional Martin (dough-washing) process, to give wet gluten, prime starch, tailings, and water-solubles. Flour (100 g, 14% mb) in a 100-g bowl pin mixer (National Manufacturing Co., Lincoln, NE) was mixed with gradual addition of water (60–70 ml, 25 °C) until the mixture became a cohesive, stiff dough-ball and cleaned itself from the mixing bowl (3–5 min). After resting the dough under a moist cover for 1 h at room temperature, the dough was hand-kneaded gently under a stream of water over a 125-μm opening sieve until almost all starch and soluble materials were removed. However, certain doughs failed to maintain their cohesive and elastic properties during hand-kneading under water. To facilitate the wet-separation of starch and gluten, those doughs were instead gently hand-rubbed on the screen under a spray of water to allow the passage of the starch and water-solubles while retaining the gluten protein agglomerates on the screen. This method of wet-milling was in this work called the modified dough-washing (MDW) method. Washing of the gluten mass by both the methods (DW and MDW) was stopped when 1–2 drops of water squeezed from the gluten into a beaker gave clear water free of starch granules. The gluten fraction was placed in a container, covered, and stored at room temperature for 1 h. The wet gluten was then shaped into a sphere, pressed as dry as possible between a pair of watch glasses, and weighed. Wet gluten was partly frozen in freezer (~1 h), cut into ~1-cm³ pieces, and placed in jars that were attached to a freeze-dryer (Flexi-Dry/MP, TMS Systems, Inc., Stone Ridge, NY). The reduced pressure caused the gluten pieces to expand and created a high-surface area for rapid freeze-drying. The freeze-dried gluten, which typically contained <5% moisture, was ground in a Thomas-Wiley intermediate mill (Thomas Scientific Co., Philadelphia, PA) to pass through a 420-μm opening sieve.

The so-called starch milk, which passed through the 125-μm opening sieve, was resieved through a 75-μm opening sieve, and the mass remaining over the 75-μm opening sieve was washed with water (50 ml). The material that remained atop the screen was collected. The material passing through the 75-μm opening sieve was centrifuged at 2500g for 20 min, and the supernatant was collected. The upper-pigmented layer (tailings) atop the white starch was carefully removed with a spatula. The starch was resuspended by hand-shaking in water (200 ml) and centrifuged at 2500g for 20 min. The supernatant was collected, combined with the supernatant collected in the first centrifugation step, and sampled (100 ml) to determine water-soluble solids. The tailings were carefully removed with a spatula. The remnants of the 75-μm opening sieve and the tailings removed after the first and second centrifugation steps were combined and freeze-dried. The starch on the bottom layer was collected and oven-dried at 40 °C for two days. The dried starch (~8% moisture) was gently ground with a mortar and pestle before being analyzed for moisture and protein.

2.2.3. Wet-milling of flours by dough-dispersion and centrifugation (DD&C) method

Wheat flours were fractionated into wet gluten, prime starch, tailings, and water-solubles by essentially a dough-dispersion and centrifugation (DD&C) method
reported by Czuchajowska and Pomeranz (1993, 1995), which somewhat simulates the aqueous dispersion processes. Dough preparation step was similar to that in the DW method. The developed stiff dough-ball was covered with water—a total of 200 ml of water including that added to the flour in the dough mixing stage. After resting for 30 min at room temperature, the dough and liquid were transferred to a Waring blender and dispersed at high speed for 1 min. The dry solids concentration of the slurry was adjusted to ~27% with additional water prior to centrifugation at 2500 g for 15 min. Upon centrifugation, the supernatant was collected and the top layer of sediment, which consisted mainly of gluten plus insoluble pentosans, damaged starch, and small granular starch, was carefully removed from the prime starch layer (bottom). The top layer was gently manipulated under a stream of water (3 × 200 ml) to obtain the cohesive gluten fraction. The gluten fraction was treated similarly to those in the DW method to obtain dry gluten. The prime starch layer and the washings (starch milk) of the gluten fraction were combined and purified by washing with water and centrifugation twice as described in the DW method.

2.2.4. Data analysis

Gluten and starch separation and the physical/chemical analyses were carried out at least in two replications, and the means were compared by the Tukey’s HSD test in one-way analysis of variance (ANOVA), using the Statistical Analysis System Software, V. 6.12 (SAS Institute, Inc., Cary, NC).

3. Results and discussion

3.1. Wheat kernel properties

Karl 92 and Ike wheats are both the U.S. hard red winter wheat varieties. Karl 92 is a nonwaxy wheat that contains all three waxy proteins (Wx-A1, Wx-B1, and Wx-D1) and thus possesses normal level (~28%) of amylose in its starch. Ike wheat is a partial waxy wheat, double-null for the Wx-A1 and Wx-B1 proteins, with reduced amylose (~21%). The waxy wheats are, however, triple-null for all the three waxy proteins and their starches contain <1% amylose (Graybosch et al., 1998; Seib, 2000). The waxy wheats often contain Ike partial waxy wheat in their genetic backgrounds.

The waxy wheat lines used in the study were produced by either mutagenesis of Ike, or by crossing Ike or Kanto 107 wheats, both null for the Wx-A1 and Wx-B1 proteins, with a Chinese wheat cultivar Bai Huo null for the Wx-D1 protein. Some of the waxy lines were further backcrossed with Klastic wheat. All wheat samples were sound with no sprout damage; the α-amylose activities ranged from 0.07 to 0.20 Ceralpha units/g kernel and the falling numbers of the control flours were well above 400 s (Table 1). The falling number of only one waxy flour sample (Wx-3) was determined. In accordance with the previous findings (Abdel-Aal et al., 2002; Graybosch, Guo, & Shelton, 2000) that the Wx-3 waxy flour gave an extremely low falling number (75 s) even though it was milled from a sample of sound kernels with a low α-amylase activity (0.12 Ceralpha units/g kernel). In sound waxy and nonwaxy wheat flours, α-amylase activities were reported to range from 0.04 to 0.16 Ceralpha units/g flour (Abdel-Aal et al., 2002; Graybosch et al., 2000; Yasui, Sasaki, & Matsuki, 1999), while a sprout-damaged wheat flour had an α-amylase activity of 0.33 (Graybosch et al., 2000).

The waxy wheats possessed hard, soft, and mixed-hardness endosperms, whereas the control wheats had hard endosperms (Table 1). All waxy wheat lines and the control wheats contained relatively high levels of protein, ranging from 12.8% to 14.9% (14% mb). Leona waxy wheat, however, contained the lowest protein (10.6%). The test weights of the waxy wheats were within the range of the control wheats (76–80 kg/hL) except for the Wx-2 (74 kg/hL) and Wx-3 (73 kg/hL) lines. A large variation was observed in terms of single kernel weights (~26–40 mg). Single kernel diameters of the waxy wheats were in general close to those of the control wheats (~2.5 mm), with a minimum of 2.0 mm to a maximum of 2.8 mm (data not shown).

3.2. Flour properties

Flour composition and properties are listed in Table 1. All waxy wheat samples gave less flour than the control hard wheats by roller-milling. It was noticed that the fractions of shorts from the waxy wheats, specifically from soft waxy wheats, were heavily contaminated with flour. In order to recover the contaminated flour, the shorts were sieved (150 μm opening sieve, 2 min) and the recovered flours were combined with their respective flours. Still, the flour yields from the waxy wheats were ~3–7% lower than the two control wheats. The Wx-2 sample gave even less flour (~15%), which was perhaps caused by its quite small (2.0 mm) kernel size. The reduced flour yields from the waxy wheats with soft endosperm may be explained by the typical inferior bolting quality of soft wheats (Posner & Hibbs, 1997). Also, soft wheats were milled on a Buhler experimental mill that was set for hard wheat milling. Souza (2001—personal communication) reported that soft waxy wheat endosperm tended to flake on the smooth reduction rolls during milling. The test weights of neither the controls nor the waxy wheats correlated with their flour yields. The reduced flour yields from the waxy wheats with hard endosperm, however, might be explained by their elevated crude fat and pentosan contents (Table 1). Fats provide cohesiveness to flours and reduce their
flowability (Neel & Hoseney, 1984). Yasui et al. (1999) reported reduced (20%) flour yields from two waxy wheats produced from Kanto 107 by mutagenesis. They attributed the decrease in waxy flour yield to a decline in their flowability that might be caused by their elevated fat (20%–24%) and β-glucan (30%) levels. Kim et al. (2003) also found that waxy wheats yielded less flour than partial waxy and nonwaxy wheats. However, the elevated crude fat contents determined for waxy wheat flours might be considered an artifact of the lipid extraction. With essentially no amylose present, there is nothing to bind flour lipids, hence rendering the lipids more accessible to the solvent and inflating the values.

The protein contents of the waxy flours ranged from 9.4% to 14.2% compared to 12.1% to 12.9% for the controls (Table 1). The lightness values (L*) of the flours varied from 90.1 to 93.6. As compared to the control flours, the waxy flours contained 20–40% more fat, 10–20% more total pentosans, and 10–30% more crude fiber. Also, waxy wheat flours had 3–7% lower total starch than the controls, perhaps because the flux of carbohydrates into starch was diminished due to the absence of amylose biosynthesis. Amylose levels of the waxy samples were higher than the expected levels, ranging from 0.6% to 2.3%, whereas the controls gave the expected values (Table 1). Other investigators (Abdel-Aal et al., 2002; Chakraborty et al., 2004; Kim et al., 2003) also reported varying amylose levels (0.9–3.2%) for waxy wheats. This discrepancy could be attributed to the variations in samples and methods of analysis used in different studies.

### 3.3. Wet-milling properties of flours by dough-washing (DW) method

All wheat samples were separately roller-milled to straight-grade flours, physically and/or chemically characterized, and wet-milled to give wet gluten, prime starch, tailings, and water solubles fractions. The two control flours and only two of the waxy wheat (Wx-1 and Wx-3) flours could be wet-milled by the original DW method (Table 2). The doughs from those flours maintained their cohesiveness and elasticity during kneading and washing under water, thus enabling washing of the starch from the gluten fraction. The remaining five waxy wheat flour doughs lost much of their cohesive and elastic properties immediately upon starting to knead under the stream of water, despite the fact that cohesive stiff doughs were initially formed from all the flours, including the waxy flour doughs whose gluten agglomerates were broken apart into small pieces and became sticky during washing. In order to wash the starch away and isolate the gluten, those doughs were wet-milled by hand-rubbing them on the 125-μm opening screen (modified DW method, MDW). Gentle rubbing and washing of the small dough pieces and gluten

---

**Table 1**

<table>
<thead>
<tr>
<th>Composition and properties of straight-grade flours roller-milled from control and waxy wheat samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flour sample</strong></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Karl 92 (nonwaxy)</td>
</tr>
<tr>
<td>Lea (partial waxy)</td>
</tr>
<tr>
<td>Wx-A</td>
</tr>
<tr>
<td>Wx-B</td>
</tr>
<tr>
<td>Wx-C</td>
</tr>
<tr>
<td>Wx-1</td>
</tr>
<tr>
<td>Wx-2</td>
</tr>
<tr>
<td>Wx-3</td>
</tr>
<tr>
<td>Leona</td>
</tr>
</tbody>
</table>

* a 14% flour moisture basis (flour moisture levels ranged from 11.5 to 13.5%).

b Not determined due to blinding of Glutomatic washing chamber sieve (88-μm opening) during gluten washing.

c Not measured due to blinding of Glutomatic washing chamber sieve (88-μm opening) during gluten washing.

d Not reported since it was received as flour.
agglomerates on the sieve retained gluten agglomerates on the sieve while allowing the starch granules and the soluble solids to pass through the screen. Because of the stickiness of their gluten, many of the screen openings became plugged during washing, which increased the time and water usage required to purify the gluten fraction. Interestingly, the wet gluten fractions retained on the screen recovered their elasticity and cohesiveness during washing at a point where about two-thirds or more of the starch were washed away from the gluten fractions. This may be explained by the increase in the concentration of the gluten proteins in the gluten fractions and/or removal of an unknown component from the dough mass, which enabled the gluten proteins to interact with each other, agglomerate, and provide cohesiveness. Furthermore, when the starch milk passing through the screen from the waxy flour dough washings were centrifuged to recover the starch fraction, a thick intermediate fraction (tailings) was found between the supernatant and the bottom prime starch layer. The intermediate layer contained gluten protein particles mixed with mostly small starch granules and insoluble pentosans. In contrast, centrifugation of the starch milk from the control flours gave a sharp interface between the supernatant and sedimented starch with only a thin layer of tailings (darkened zone) atop the starch.

Other investigators (Hamer, Weegels, Marselle, & Kelfkens, 1989; Meuser, 1994; Meuser et al., 1989; Weegels, Marselle, & Hamer, 1988; Zwitserloot, 1989a) have reported differences in the rate and extent of gluten agglomeration during wet-processing of wheat flours. The reasons for the reduced rate and strength of gluten agglomeration in flour-water doughs and slurries have not been fully understood. High molecular weight glutenins and glutenin macropolymer formed by the glutenin particles have been shown in the last 10 years to add strength (elasticity) to a wheat flour dough (Antes & Wieser, 2001; Bekkers, Lichtendonk, Graveland, & Plijter, 2000; Don, Lichtendonk, Plijter, & Hamer, 2003a, 2003b; Schropp & Weiser, 1996; Singh & MacRitchie, 2001; Southan & MacRitchie, 1999; Uthayakumar, Stoddard, Gras, & Bekes, 2000; Veraverbeke, Verbruggen, & Delcour, 1998; Wooding, Kavale, MacRitchie, & Stoddard, 1999). However, the wet-processing quality of a wheat flour and its relationship to flour protein quality remain mostly unknown.

Gluten proteins in the waxy wheat flours appeared to be weaker than those of the controls as indicated by their lower SDS sedimentation volumes and IPP contents (Table 1) and shorter mixograph mixing times and lower mixing tolerances (Fig. 1). The IPP contents of flours were reported to correlate strongly with dough mixing time and mixing tolerance (Bean et al., 1998). Also, elevated insoluble pentosans of the waxy flours (Table 1) may be the reason for the loss of their cohesiveness and elasticity during kneading. Pentosans
(2–3%) of wheat flours (Hashimoto et al., 1987; Lineback & Rasper, 1988) were reported to bind or hold 5- to 10-fold water by their mass (Bushuk, 1966; Courtin & Delcour, 2002) and to increase the viscosity of the liquid phase during wet-milling. This in turn caused a reduction in the sedimentation rate of small B-starch granules in a centrifugal field, translating to a reduced starch yield (Witt, 1997). Pentosans also interfered with gluten formation and aggregation during dough mixing through viscosity-related interference of pentosans on gluten aggregation (McCleary, 1986; Wang, Hamer, Vliet, & Oudgenoeg, 2002), steric hindrance of gluten aggregation due to formation of a pentosan network during dough mixing (Rouau, El-Hayek, & Moreau, 1994; Labat, Rouau, & Morel, 2002; Weegels, Marselle, & Voorpostel, 1991), and through direct interaction of pentosans with glutenin particles during dough mixing (Wang et al., 2002; Wang, 2003; Wang, Hamer, et al., 2003a; Wang, Oudgenoeg, Vliet, & Hamer, 2003b), all of which are expected to affect the wet-milling properties of flours. In waxy wheats, genetic backgrounds, not the waxy trait per se, are more likely the cause of weak glutsens.

Other methods of wet-milling were reported to be less dependent on agglomeration traits of gluten in a dough (Sayaslan, 2004; Zwitserlood, 1989a, 1989b), and such methods may work better for the separation of vital gluten and starch from flours with poor gluten strength/elasticity. According to Zwitserlood (1989a), some weak flour doughs that weaken excessively in the Martin process can be wet-milled by the HD process. In that process and possibly in the other dispersion processes (Hydrocyclone and Alfa-Laval/Raisio), the sensitivity to gluten agglomeration quality exhibited in the Martin process may be marginalized because density differences of the flour components appear to be the primary separation factor in those processes. Also, concentrating the gluten proteins and removing the viscosity-building soluble pentosans early in the wet-milling steps through centrifugation, which is practiced in the dispersion processes, may facilitate the wet-milling of waxy wheat flours. In fact, this approach proved to be viable for the wet-milling of the waxy wheat flours that failed by the DW method (see results in the DD&C method below).

As expected, the yields of dry gluten fractions were closely associated with the protein contents of their flours. With the exception of Wx-2 and Leona, the waxy wheat flours mostly yielded more dry glutsens than the control flours, but their protein contents (purity) were ~7–20% lower (Table 2). This particular Leona waxy wheat sample gave the least dry gluten because of its extremely low protein content (9.4%). A high-protein Leona sample is likely to give improved gluten yield and wet-milling quality. The difficulty encountered during washing the starch away from the gluten reduced the purities of the waxy flour gluten fractions. The protein contents of the glutsens isolated in this work were all >73%, meeting the minimum 73% protein (N x 5.7, db) required by the Codex Alimentarius of the Food and Agriculture Organization (FAO, 2002). Commercial glutsens have been found to contain 73–80% protein on dry solids basis (Miller & Hoseney, 1996). Dreese, Faubion, and Hoseney (1988) showed that starch accounted for about 8% of the impurities in the glutsens from different flours.

As listed in Table 2, the starch recoveries from the waxy wheat flours (~72–78%) were statistically similar to the recoveries (~76–80%) from the control flours, except for the reduced recovery (~68%) from Leona. However, some of the waxy starches were contaminated with more proteins (0.4–0.5%) than the starches from the controls (<0.3%). Granule size distributions were
determined for selected waxy starches (Fig. 2); as expected they showed a typical bimodal size distribution with ~25–30% by weight of the granules below 10 µm. In other words, granule size distributions of the waxy and control starches isolated by the two methods of wet-milling were comparable, which is consistent with the results of Yoo and Jane (2002) and Abdel-Aal et al. (2002). The soluble flour solids varied from 5.8% to 9.9% in the waxy flours (Table 2), which is within the range of 5–15% water-solubles found in wheat flours (Hamer et al., 1989; Maijala, 1976; Witt, 1997). About one-fifth of the flour weight for all the control and waxy wheat flours was unaccounted for in the gluten, starch, and water solubles fractions (Table 2). The unaccounted solids for Leona flour were even higher (~29%). Those unaccounted solids were mostly in the tailings removed during centrifugal purification of the starch fractions.

3.4. Wet-milling properties of flours by dough-dispersion and centrifugation (DD&C) method

Because of the difficulties encountered during the wet-milling of the waxy wheat flours by the DW method as discussed above, which requires a strongly agglomerated gluten in dough for efficient separation of the starch and gluten fractions, the method described by Czucha-jowska and Pomeranz (1993, 1995) was tested. In that method, a stiff dough was mixed to optimum and rested under ~1 parts of water for 30 min. Then the dough was sheared with additional water in a blender to form a dispersion. The dispersion is centrifuged to give mainly three layers; a top supernatant, a middle protein-rich layer, and a bottom layer of practically pure starch. It appears that the gluten proteins were agglomerated, mostly during dough mixing, and the protein strands in the agglomerates were dislodged from starch granules during high-shear dispersion step. During the centrifugation, the dispersed gluten is thought to form a fine mesh network which allows the passage of the rapidly sedimenting large starch granules. After centrifugation, the viscous supernatant containing the soluble pentosans and the sedimented starch layer (~50% of total starch) was easily removed from the middle protein-rich layer. The middle layer in the centrifuged mixture was sufficiently cohesive even at the beginning of kneading so that the remaining starch was easily removed by kneading and washing under water.

Leona waxy flour gave a similar wet-milling trend by both the DW and DD&C methods; a lower quantity of dry gluten, more protein in starch, and elevated water-solubles (Tables 2 and 3) probably because of its low protein quantity and quality. However, when wet-milled by the DD&C method, Leona waxy wheat gave even lower dry gluten and protein recovery. The DD&C method, as compared to the DW method, employed more shear during the wet-processing, which was likely to damage already weak gluten aggregates in Leona sample. In contrast to the DW method, the separation of starch and gluten fractions from all other waxy wheat flour doughs by the DD&C method was accomplished with similar ease to that of the controls. In other words, waxy flour doughs that failed for wet-milling in the original DW method could be wet-processed by the DD&C method.

The waxy wheat flours wet-milled by the DD&C method gave similar or elevated yields of gluten with reduced purities as compared to the control flours (Table 3). Thus, both wet-milling methods gave reduced protein levels in the gluten fractions from the waxy wheats. However, the protein contamination in the waxy starch fractions was almost cut in half to <0.3% by the DD&C method of wet-milling (Table 3) compared to the DW method (Table 2).
4. Conclusions

Waxy wheats gave reduced flour yields, and the flours contained less starch but more crude fat and pentosans. The gluten proteins of waxy wheats were weaker than those of the nonwaxy and partial waxy control wheats as indicated by their poor mixograms, decreased IPP contents, and lower SDS sedimentation volumes. It is more likely the genetic backgrounds of the waxy wheats, not the waxy trait per se, are the causes of their weak gluts. Some of the waxy flour doughs failed in the wet-milling by the DW method as the DW method relies heavily on the gluten aggregation traits of flours. It is therefore recommended that wet-milling of the waxy wheat flours with weak gluten be done by an aqueous dispersion process that is less dependent on gluten strength and less affected from the deleterious interference of pentosans on gluten formation and aggregation.

In this regard, the DD&C aqueous dispersion method of wet-milling used in this study facilitated the wet-milling of the waxy flour doughs and gave ~80% recovery of flour protein in the gluten fraction with ~80% protein, and ~75% waxy starch recovery with <0.3% protein.

References


international workshop on carbohydrates as organic raw materials (pp. 163–176). Vienna: WUV-Universitätsgeslag.


