

Effect of Partial Waxy Wheat on Processing and Quality of Wheat Flour Tortillas

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ABSTRACT

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The processing and quality of wheat flour tortillas prepared with partial waxy and normal flour were evaluated. Control procedures and formula were utilized with water absorption varied to obtain machineable doughs. Amylose content was lower in most partial waxy compared with normal wheats. The type of wheat starch did not affect most dough properties or tortilla diameter. Tortilla height and opacity were adversely affected by the decreased amount of amylose in partial waxy wheats.

Sufficient leavening reactions occurred early in baking (after 10 sec) to yield an opaque disk, but some baked tortillas lost opacity and become partially transparent after baking. Starch gelatinizes, disperses, and retrogrades concurrently with the leavening reaction during the short (<30 sec) baking time. Amylose functionality during baking and cooling appears to be involved in the retention of air bubbles in tortillas.

Wheat flour tortillas are consumed in Mexico and are increasing in popularity in the United States (Waniska 1999) where about \$US 2 billion of flour tortillas were consumed in 1999. Processors in the United States increased production efficiency and improved shelf-stability by changing ingredient levels and processing parameters of flour tortillas. Wheat starch, the primary component of wheat flour and of wheat tortillas, has only been evaluated for milling-induced damaged starch in flour tortillas (Wang and Flores 1999). The effect of reduced amylose on tortilla processing and product properties has not been investigated.

Wild-type bread wheat (*Triticum aestivum* L.) carry three loci (*wx-A1*, *wx-B1*, and *wx-D1*) that condition production of the granule-bound starch synthase (GBSS) (Nakamura et al 1992, 1993). GBSS is responsible for the production of amylose in endosperm starch. Wheat lines carrying one or more null, or nonfunctional, alleles often produce starches with reduced amylose content, and have been designated "partial waxy".

Partial waxy wheats exhibit changes in starch gelatinization, pasting and retrogradation properties (Graybosch 1998; Zhao et al 1998; Demeke et al 1999; Araki et al 2000; Seib 2000; Yamamori et al 2000; Yamamori and Quynh 2000). The consensus of these studies indicates that branching structure of amylopectin appears to be similar while the variable amount of amylose appears to determine functional properties. Products prepared with partial waxy wheat or barley may have improved (noodle) or inferior (bread) qualities. The reduced amount of amylose in wheat starch increases granule swelling but decreases product rigidity during baking and storage. These may be desirable attributes for wheat flour tortillas. Hence, partial waxy wheats were evaluated during the production and storage of flour tortillas.

MATERIALS AND METHODS

Plant Materials

Wheat lines used in the study were grown in 1999 at McCook, NE, in a randomized complete block design with three replicates. Allelic composition at the three *wx* loci was determined by gel electrophoresis (Graybosch et al 1998). To obtain sufficient quantities of seed for quality analyses, and to study the effects of partial waxy starch on tortilla properties, a form of bulked segregant analysis

(Michelmore et al 1991) was used. Lines from two breeding populations were bulked by *wx* allelic composition. Five lines of each genotype were combined to form bulked segregants. From a population derived from NE90616/Ike, two genotypes were identified: lines carrying single null alleles at the *wx-A1* locus, and lines carrying null alleles at both *wx-A1* and *wx-B1*. These bulks were designated NE90616(-a) and NE90616(-a-b), respectively. The population derived from MT8713/NE87612/Ike produced MT87612(-a), MT87612(-b), MT87612(-a-b), and MT87612(wild) type. These bulks were formed from lines carrying, respectively, null alleles at *wx-A1*, *wx-B1*, both *wx-A1* and *wx-B1*, and no null alleles (wild-type). The bulked segregants were compared with a wild-type bulk (wild-mix; Siouland, N86L177, Arapahoe and Vista), Ike(-a-b), NE90476(-a-b), and TAM202(-b), a cultivar that carries a null allele at the *wx-B1* locus. The bulked segregant analysis allows the identification of the true effects of genetic loci, while diminishing confounding effects of genetic backgrounds.

Flour Quality

Wheat was roller-milled (Quadramat Senior, Brabender Instruments, S. Hackensack, NJ). Flour protein and moisture were determined using near-infrared analysis (model DA7000, Perten, Springfield IL). Dough mixing time, resistance, and water absorption were determined according to Approved Method 54-40 (AACC 2000). Amylose was extracted from wheat flour using 1.0N NaOH for 18 hr; the absorbance of the iodide complex was determined according to Approved Method 61-03. Native cornstarch (25.0% amylose, dwb, Argo, Best Food, CPC Int., NJ) was extracted using the same procedure as wheat samples and utilized as the reference.

The control wheat tortilla flour had 10.95% protein content requiring 62.6% water absorption to center the farinograph curve at 500 BU (0.45% ash; 13.2% moisture content; ADM Milling Co., Enid, OK).

Dough Preparation and Tortilla Processing

Dough and tortillas were prepared by methods delineated by Bello and coworkers (1991) with the following modifications. The temperature of the dough was maintained at 31°C during mixing with water (65°C) circulating in copper tubing around the bowl. Dough balls were rested for 10 min in a warm (38°C), moist (70% rh) chamber and baked. Hot-press conditions were 1100 psi, 197°C top platen, 194°C bottom platen, and 1.35 sec dwell time (model 0P01004-02, Lawrence Equipment Co., El Monte, CA). Tortilla pH was maintained at 5.80 ± 0.02. Doughs were prepared at least twice.

The basic formula included 1,000 g of flour (ADM Milling), 60 g of vegetable shortening (all purpose, Anderson Clayton Foods, Dallas TX), 15 g of salt (United Salt Corp., Houston TX), 2 g of sodium-2-stearoyl lactylate (SSL, American Ingredients Co., Kansas City, MO), 2 g of succinylated monoglycerides (Do Control,

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Eastman Chemical Co, Kingsport, TN), 5 g of sodium propionate (American Ingredients), 4 g of potassium sorbate (American Ingredients), 6 g of sodium bicarbonate (commercial baking soda, Arm and Hammer, Church and Dwight Co.), 5.8 g of sodium aluminum sulfate (Ejisa-Budenheim, Santa Catarina, Nuevo Leon, Mexico), 3.3 g of fumaric acid (Bartek Ingredients Inc., Stoney Creek, Ontario, Canada), and 0.03 g of cysteine (Sigma Chemical Co., St. Louis, MO). Water (distilled, deionized) content was 50.5% (baker's percent) for control and was varied to attain uniform dough properties.

Evaluation of Dough Properties

Dough was subjectively evaluated for smoothness, softness, and toughness immediately after mixing. Smoothness refers to the appearance and texture of the dough surface; softness refers to the viscosity or firmness of the dough when compressed; and toughness refers to elasticity of the dough when pulled apart. Each property was rated using a continuous scale of 1–5 where 1 = very smooth, very soft or least tough; 2 = smooth, soft, or slightly tough; 3 = slightly smooth, slightly hard, or tough; 4 = rough, hard, or very tough; and 5 = very rough, very hard, or extremely tough, respectively.

A dough ball was objectively evaluated by rapidly compressing to 0.7 cm and holding for 20 sec according to the stress relaxation method. A dough ball (height 2.1 ± 0.12 cm, diameter 5.2 ± 0.15 cm; weight 45.0 ± 0.8 g) was placed on a flat stationary aluminum platform of a texture analyzer (model TA.XT2, Texture Technology

Corp., Scarsdale, NY). The dough ball was compressed at 10 mm/sec by a flat aluminum probe (10 cm diameter) to 0.7 cm above the platform. The dough ball had an initial surface area of 20 cm² and a final surface area of 55 cm². Measurements of hardness (maximum compression force), force at 20 sec (equilibrium force), adhesive force, and initial modulus of relaxation (slope of $\delta\text{force}/\delta\text{sec}$) were measured or calculated.

Evaluation of Tortilla Properties

Tortillas were analyzed for moisture content and pH level. Tortilla weight, height, and diameter were determined using 10 tortillas selected at random (Friend et al 1995). The specific volume was calculated using measured values of radius, height, and weight. Opacity, the opposite of translucency, was measured subjectively using a continuous scale of 1–100% opaque.

Tortillas were objectively evaluated by extending a strip (35 × 75 mm) according to the extensibility method (Joseph 1999). Tortilla strips were cut from the tortilla avoiding the puffed portions (to maintain sample uniformity) using an acrylic template. The ends of the strip were held by grips that were separated by 22 mm and extended at 1.0 mm/sec using the texture analyzer. The force, the modulus of deformation, and work during extension were measured or calculated.

Tortilla stability was subjectively evaluated using the rollability test (Bello et al 1991). Tortillas were wrapped around a dowel (1 cm

TABLE I
Milling, Flour Quality, and Amylose Content of Partial Waxy Wheats

	Milling Yield (%)	Protein (%) (14% mb)	Mixing Time(min)	Mixograph Resistance (MU)	Amylose (% dwb)
Control	...	10.95	4.0	4.7	17.7
Wild-mix	66.7	9.96	4.5	4.5	17.4
IKE(-a-b)	63.5	10.74	3.5	5.1	14.3
NE90616(-a-b)	69.9	9.74	4.1	4.6	16.6
NE90616(-a)	65.9	9.83	4.9	4.3	...
NE90476(-a-b)	67.7	9.74	3.3	5.0	15.5
MT87612(wild)	64.4	9.72	3.3	4.6	17.5
MT87612(-a-b)	66.1	10.33	3.0	4.9	16.0
MT87612(-a)	65.5	9.74	4.0	4.8	15.9
MT87612(-b)	64.4	9.62	4.4	4.3	16.2
TAM202(-b)	73.8	9.90	3.6	5.3	17.8
LSD ^a	1.3	0.29	0.25	0.15	0.21

^a Least significant difference ($P < 0.05$).

TABLE II
Characteristics of Control Dough, % Change in Dough Properties Due to Partial Waxy Wheats, and Significant Regression Variables

	Water Absorption (%)	Force (N)			Rating ^a		
		Compression	Equilibrium	Adhesive	Softness	Toughness	Press
Control	50.5	64	6.2	-23	2	2	2
LSD ^b	0.95	9.6	1.2	5.1	0.6	0.6	0.7
Change from control							
Wild-mix	-1.0	2	9	-38	-41	-35	-43
IKE(-a-b)	-1.8	28^c	61	-32	-12	-6	-24
NE90616(-a-b)	-1.0	33	52	4	-29	-24	-33
NE90616(-a)	-1.0	11	31	7	-35	-29	-38
NE90476(-a-b)	0.2	38	76	-12	-6	0	-24
MT87612(wild)	-2.2	85	160	-51	18	18	-5
MT87612(-a-b)	-1.8	55	139	16	6	18	48
MT87612(-a)	-1.0	26	60	16	-12	-6	-14
MT87612(-b)	-1.0	90	181	7	-41	-41	-29
TAM202(-b)	-1.0	75	138	15	61	55	57
Multiple linear regression coefficients ^d							
Constant	ns	ns	ns	ns	-0.28	-4.33	-3.87
Amylose	ns	ns	ns	ns	0.012	0.014	ns
Mixograph resistance	ns	ns	ns	ns	0.001	0.001	0.031
Amylose × protein	ns	ns	ns	ns	ns	-0.03	ns
Amylose × resistance	ns	ns	ns	ns	ns	0.14	0.07

^a Subjective rating scale where: 1 = less viscous, less tough to stretch, easier to press, to 5 = most viscous, most tough to stretch, hardest to press.

^b Least significant difference ($P < 0.05$).

^c Bold values (%) are significantly larger or smaller than control.

^d Significant variables $P > 0.95$; ns = not significant; protein content did not contribute to any regression model; most interaction terms were not significant.

diameter) and cracking and breaking of tortillas was subjectively evaluated using a continuous scale from 5 (no cracks or breakage) to 1 (broke immediately, impossible to roll). Shelf-stability was defined as the number of days the tortilla could be rolled without breaking (rollability score 3.0).

Statistical analyses of replicated data were conducted to separate means and determine linear relationships (Essential Regression add-in to MS Excel 97, Version 2.218, 1999).

RESULTS

Flour Quality

The partial waxy wheats had reasonable milling yields (63.5–73.8 ± 0.6 %) using a mill with one break and one roller unit (Table I). Protein content was 9.6–11.0 ± 0.13%. This compares favorably with wheat flours currently being used to manufacture tortillas (9.5–11.5 % protein) (Serna-Saldivar et al 1988; Waniska 1999). The proteins in partial waxy flours exhibited intermediate to good quality. Mixograph resistance, a measure of protein quality, correlated with mixing time ($r = 0.72$, $\alpha = 0.05$) but not signi-

ficantly with protein content. Amylose contents of partial waxy wheats were 1.0–3.3% lower than the amylose content of wild-type wheats, except for TAM202(-b) which had a similar amylose content. Yamamori and Quynh (2000) also observed differential effects of the three waxy alleles on amylose content. Milling yield and flour protein quality did not significantly correlate to protein or amylose contents of the samples analyzed. However, Gaines et al (2000) observed higher milling yield in wheats with less amylose content.

Dough Properties

Dough water absorption tended to be lower for partial waxy flours compared with the control (Table II). Objective dough compression and equilibrium forces were significantly higher for most treatments compared with the control; however, several doughs were more sticky than control: MT87612(wild), NE90616(-a), and Wild-mix. Subjective dough ratings indicated that most treatments were easier or similar to process compared with the control, except for TAM202(-b) and MT87612(-a-b). Multiple regression analysis revealed that protein content was not significantly associated to tortilla dough measurements (Table II). However, the mixograph

TABLE III
Characteristics of Control Tortillas, % Change in Tortilla Properties Due to Partial Waxy Wheats, and Significant Regression Variables

	Diameter (cm)	Height (mm)	Specific Volume (cm ³ /g)	Opacity (%)	Shelf Stability (days)
Control	18.5	2.8	1.84	92	12
LSD ^a	0.6	0.3	0.22	9	1.5
Change from control					
Wild-mix	1	-11	-8	-3	17 ^b
IKE(-a-b)	1	-29	-28	-18	0
NE90616(-a-b)	2	-14	-12	-6	-7
NE90616(-a)	1	-9	-6	-1	-11
NE90476(-a-b)	0	-27	-29	-18	33
MT87612(wild)	-3	-20	-29	-6	0
MT87612(-a-b)	-1	-29	-32	-7	0
MT87612(-a)	0	-11	-16	-5	0
MT87612(-b)	-4	-9	-17	-5	-21
TAM202(-b)	-13	-19	-4	—	—
Multiple linear regression coefficients ^c					
Constant	ns	-0.13	ns	58.96	ns
Amylose	ns	-0.15	ns	3.57	ns
Mixograph resistance	ns	ns	ns	-6.66	ns

^a Least significant difference ($P < 0.05$).

^b Bold values (%) are significantly larger or smaller than control.

^c Significant variables $P > 0.95$; ns = not significant; protein content did not contribute to any regression model; most interaction terms were not significant.

TABLE IV
Objective Rheology of Control Tortillas After 1 and 12 Days of Storage, % Change in Tortillas Properties Due to Partial Waxy Wheats, and Significant Regression Variables

	Rupture Force (N)		Rupture Distance (mm)		Rupture Work (N·mm)		Modulus (N/mm)	
	1 day	12 days	1 day	12 days	1 day	12 days	1 day	12 days
Control	5.6	9.2	4.5	1.9	23.6	13.0	2.3	3.9
LSD ^a	0.95	1.22	0.52	0.15	4.9	2.1	0.25	0.26
Change from control								
Wild-mix	-20^b	8	33	3	5	5	-30	-5
IKE(-a-b)	-19	9	65	17	42	19	-29	-4
NE90616(-a-b)	-19	16	28	-6	5	3	-29	7
NE90616(-a)	-26	-1	12	-10	-16	-15	-29	-4
NE90476(-a-b)	-22	-1	52	24	25	18	-30	-14
MT87612(wild)	-8	25	17	5	5	22	-20	12
MT87612(-a-b)	-11	38	34	4	21	33	-22	21
MT87612(-a)	-14	14	16	3	3	11	-17	4
MT87612(-b)	-21	4	16	16	-14	7	-33	-1
TAM202(-b)	-8	24	26	-1	28	20	-4	5
Multiple linear regression coefficients ^c								
Constant	0.34	ns	8.24	3.55	-329.9	-2.06	-0.55	ns
Amylose	ns	ns	-0.05	-0.10	18.2	ns	ns	ns
Mixograph resistance	ns	ns	1.12	ns	74.94	3.49	ns	ns
Amylose × resistance	0.03	ns	ns	ns	ns	ns	0.03	ns

^a Least significant difference ($P < 0.05$).

^b Bold values (%) are significantly larger or smaller than control.

^c Significant values $P > 0.95$; ns = not significant; protein content and the amylose × protein content did not contribute to any regression model.

resistance and amylose content were associated in some manner with subjective but not objective dough measurements. Higher mixograph resistance of flour also correlated with a less soft and tougher dough among elite and commercial wheats (Sullins 1997, Kelecki 2001). However, it is not clear why lower amylose content flour resulted in a softer and less tough dough.

Tortilla Characteristics

Tortilla moisture content ($31.94 \pm 0.18\%$), pH (5.35 ± 0.12), and weight (39.6 ± 0.29) were similar for all treatments. The adjustments to water absorption yielded similar product characteristics using controlled processing conditions. Tortilla diameter (18.5 ± 0.2) was not affected by treatment (Table III).

Tortilla height ($r = -0.66$, $\alpha = 0.040$) and opacity ($r = -0.83$, $\alpha = 0.006$) (Table III) were negatively correlated with amylose content. Regression analysis supports the involvement of less amylose in the decrease of tortilla height and opacity. Lower mixing resistance also contributed to decreased opacity of tortillas. Specific volume ($r = -0.54$, $\alpha = 0.108$) tended to decrease with less amylose content. The exception was MT87612(wild) where lower height and specific volume were exhibited even though the flour had similar amylose content as the control. Variation in wheat flour protein functionality changes tortilla properties (Sullins 1997; Kelecki 2001).

All tortillas changed during storage such that more force and work were required to extend the tortilla and the tortilla ruptured in less distance compared with fresh (1 day) tortillas (Table IV). Changes in starch and protein functionality are responsible for staling of tortillas (Waniska 1999). Most treatments at 1 day of storage required less force per distance (modulus) to extend the tortilla and the tortillas ruptured in greater distances compared with control. At 12 days of storage, most treatments had similar values for modulus and distance to rupture, except for MT87612(-a-b) which required more force to extend; however, three of four 2-null samples required more force to extend and work to rupture. More work to rupture at 12 days of storage was also required for tortillas prepared from TAM202(-b) and MT87612(wild).

Multiple regression analysis suggested that amylose content of flour and protein quality (mixograph resistance) affected objective measures of tortilla texture (Table IV). Less amylose content of flour contributed to longer distance before rupture (1 and 12 days of storage) and increased work to rupture (1 day of storage) of tortillas. Greater protein quality contributed to longer rupture distance (1 day of storage) and increased work to rupture (1 and 12 days of storage) of tortillas. This is consistent with industry observations that higher protein quality increases the chewiness of tortillas initially and during storage (Waniska 1999).

The shelf-stability of wild-mix and NE90476(-a-b) improved while MT87612(-b) exhibited diminished stability compared with control tortillas (Table IV). Even though shelf-stability of tortillas varied, it was not significantly related to amylose content or protein amount or quality.

DISCUSSION

All tortillas prepared using flour with higher amylose contents were thicker and more opaque, regardless of the variation in protein quality. Decreased tortilla height and opacity, however, were observed using the partial waxy flours with the lowest amylose content. Starch gelation does not occur in the dough before the hot-press (due to low temperature, $<35^{\circ}\text{C}$), is initiated during hot-pressing ($375\text{--}425^{\circ}\text{C}$, <1.5 sec to attain a dough temperature of $55\text{--}70^{\circ}\text{C}$), and continues during baking ($350\text{--}450^{\circ}\text{C}$, $18\text{--}35$ sec). About 95% of the starch loses crystallinity during baking (Seetharaman et al, *in press*). Thus, addition of partial waxy wheat flour probably affected tortilla properties by having a variable amylose-amylopectin dispersion or gel network formation during processing of tortillas.

Similar amounts of leavening gases were formed in each treatment because the formula, dough properties, and processing conditions

were similar. All treatments were opaque at the end of the first tier of the three-tier oven; hence, the leavening reaction appears to be sufficient. The lack of retention of the air bubbles appears to be the cause of the thinner, less opaque tortillas. Opacity of tortillas is primarily caused by the diffraction of light from the many dispersed air bubbles (Niranjan 1999; Waniska 1999). Air bubbles are poorly retained in bread dough $>80^{\circ}\text{C}$ after heating for >8 min (Campbell and Mougeot 1999; Fan et al 1999). Tortilla dough attains $55\text{--}70^{\circ}\text{C}$ in <1.5 sec during hot-pressing and in the oven the tortilla surface away from the top platen attains $>90^{\circ}\text{C}$ in 10 sec (Waniska, *unpublished*). The reactions of leavening and starch gelatinization occur simultaneously early during baking while setting of tortilla dough appears to occur during the third tier of the oven (McDonough et al 1996).

Amylose is probably involved in the retention of air bubbles in the hot dough system during and immediately after baking of tortillas. Therefore, a variable amount of amylose may have been dispersed from the starch granules resulting in the formation of an amylose gel with varying strength in the tortilla. Opaque tortillas were produced when wheat flour was replaced with 20% barley flour varying in amylose (0–43%) content (Pascut and Waniska, *unpublished*). The substantial increase in β -glucan (4–8%) levels in barley flour apparently increased the viscosity of the continuous phase to retain the dispersed air bubbles during thermal processing. However, tortilla opacity decreased with higher ($\geq 25\%$) levels of 4% amylose barley flour.

The texture of tortillas is due to protein quality and starch functionality (Wang and Flores 1999; Waniska 1999). Flour with more protein quality corresponds to tortillas requiring more work to rupture and higher modulus. Flour with more amylose content corresponds to tortillas that rupture in a shorter distance but with higher modulus and work to rupture. Increasing protein quality and the amylose gel potential are mechanisms that form stronger structures in tortillas.

The reduction in amylose content of partial waxy wheat (1 and 2 null) may have changed the structural stability of the dough during baking of tortillas. Differences in swelling of the starch granules, rigidity of the gelatinized starch granules, and of amylose functionality could be the cause of these changes. Zeng et al (1997) observed greater shear thinning and lower final viscosity of partial waxy wheat starch during pasting. Increased swelling volume and the less rigid, more deformable gelatinized starch granules of partial waxy wheats may improve softness of Asian noodles (Sasaki and Matsuki 1998; Seib 2000). During the early stages of cooking noodles made from partial waxy flour, the starch swells quickly, deforms and fills the pores near the noodle surface, and the resulting gel resists erosion by the cooking water. The increased amount of amylose leached from partial waxy compared with normal wheat starch during pasting was proposed to contribute to the strength of Asian noodles.

It is clear that 1) partial waxy starch will more quickly bind water, swell and increase the viscosity in a low shear environment; and 2) a variable amount of amylose leaches and forms a gel network in the tortilla. The moisture and a_w of the tortilla dough decreases as a result of baking: $\approx 39\%$ moisture and $0.99 a_w$ in dough and $\approx 32\%$ moisture $0.94 a_w$ in tortillas. The increasing dough temperature increases a_w during baking but starch gelatinization lowers a_w by increasing water-binding sites. Amylose solubility in this higher viscosity, low free-moisture environment is difficult to measure or predict. The amount and strength of the amylose gel network that forms during baking provides the structure of most bakery products (Zobel and Kulp 1996). A lower amount of amylose leaching from the granule during baking is consistent with a decreased height and opacity of tortillas.

Partial waxy wheat could be processed into flour tortillas with generally similar properties as normal, wild-type tortillas. Lower amylose content primarily affected retention of air bubbles as observed by decreased opacity and height of tortillas after accounting for variations in protein quality. A secondary effect of lower amylose content was the modification of the texture of tortillas on the first day.

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