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Functionality of chemically modified wild-type, partial waxy and waxy starches from tetraploid wheats

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ABSTRACT

Partial waxy (reduced-amylose) and fully waxy (amylose-free) tetraploid durum wheats (*Triticum turgidum* L. var. *durum*) were developed by introgression of null alleles at the *Wx-A1* and *Wx-B1* loci from common hexaploid wheat (*Triticum aestivum* L.). Purified starches were obtained from each genotype, and chemically modified by: 1) cross-linking with phosphorus oxychloride, 2) substitution with propylene oxide, and 3) sequential cross-linking with phosphorus oxychloride followed by substitution with propylene oxide. Functional properties were compared to blends of waxy and wild-type durum starches of known amylose contents. Significant differences in functionality were observed amongst the genotypes and blends after each modification. Waxy (0% amylose) and wild-type (30% amylose) typically were at the extremes of the observed ranges of functional properties. In general, the functional properties of the chemically modified starches were dependent upon amylose content. Starches from *Wx-B1* null lines (24% amylose), were an exception. After substitution, such starches had the significantly highest value for RVA final viscosity, and generally performed in a manner similar to starch blends of 12–18% amylose.

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1. Introduction

Wild-type durum wheat (*Triticum turgidum* L. var. *durum*) produces two granule-bound – starch-synthase (GBSS, E.C. 2.4.1.242) isoforms of apparent molecular weight approximating 60 kDa (*Wx-A1* ~ 59.6 and *Wx-B1* ~ 56.9 kDa) (Yamamori et al., 1995). *Wx-A1* and *Wx-B1* null lines each produce only one of the GBSS isoforms, and waxy genotypes produce no GBSS.

Although development of waxy and partial waxy durum wheat (Delwiche et al., 2006; Vignaux et al., 2004) has provided a novel starch for food or industrial use, functionalities may be enhanced by altering the granule characteristics with chemical modifications (BeMiller and Whistler, 1996; Patil and Meschi, 2008; Thomas and Atwell, 1999). In general, waxy starches are not directly used in foods; they are first stabilized via chemical modification (Reddy and Seib, 2000). When waxy starch is cross-linked, the effects include retention of gel clarity during refrigeration, viscosity retention in acidic foods, increased pasting temperature, retention of granule structure, reduced syneresis, reduced crumb staling, resistance to gel separation during freeze–thaw cycling, reduced

gel firmness, increased gel adhesion, and other functional variations. These changes are due to increased water retention and polymer chain mobility restriction which slows the development of retrogradation, and depends upon the degree of cross-linkage (BeMiller and Whistler, 1996; Patil and Meschi, 2008; Thomas and Atwell, 1999). Cross-linked starch applications include gum gel confections, hard confection coating, clear decorative gels, fruit glaze, canned sauces, canned gravies, uniform heating of canned red beans in a retort, refrigerated and frozen desserts, frozen fruit pie, frozen dinner sauces and gravies, meat coatings, retarded staling of baked products, retained integrity of frozen bread products, pharmaceutical and edible films (BeMiller and Whistler, 1996; Patil and Meschi, 2008; Thomas and Atwell, 1999). Digestion resistant starch (RS4), also produced by chemical cross-linking (Shin et al., 2004; Woo and Seib, 2002), may have application as a form of dietary fiber.

Previous investigations have examined the characteristics of chemically modified waxy or partial waxy hexaploid wheat (*Triticum aestivum* L.) starches. Reddy and Seib (1999) compared the response of starches from two partial waxy wheat cultivars to wild-type wheats. The range of amylose content variation in the starches used, however, was slight. Reddy and Seib (2000) compared waxy hexaploid wheat to waxy maize (*Zea mays* L.) starch after chemical modification. Kiribuchi-Otobe et al. (2006) cross-linked two waxy

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hexaploid wheat lines carrying different mutant alleles at the *Wx-D1* locus. However, no systematic comparison of the effects of durum wheat *Wx* genotypes on modified starch properties has been reported. The intent of the present investigation, therefore, was to determine whether different *Wx* genotypes of durum (tetraploid) wheat respond differently to chemical modification, and, if so, whether they may be used to produce starches with different functional properties. Additionally, the effects of both intra-granular amylose content variation, determined by genotype, and inter-granular amylose variation, created by blending of waxy and wild-type starches, were investigated.

2. Experimental

2.1. Wheat and starch samples

Sets of wild-type, partial waxy (*Wx-A1* null or *Wx-B1* null), and waxy (*Wx-A1* and *Wx-B1* double-null) durum wheat lines were developed in 4 genetic backgrounds (Delwiche et al., 2006). Bulk segregant analysis (Quarrie et al., 1999) was used to evaluate the effects of the tetraploid *Wx* loci genotypes. Samples were composited by genotype across genetic backgrounds. Separate composite samples were produced for each crop year. Additional details on plant culture, milling and starch extraction have been described previously (Hansen et al., in press). Percent amylose content of the isolated starches was determined by the dual wavelength amylose assay (Zhu et al., 2007). Waxy and wild-type starches were blended by weight to approximately 30% (wild-type), 24%, 18%, 12%, 6%, and 0% (waxy) amylose retaining crop year identity.

2.2. Chemical modifications

All native starches were modified by cross-linking with phosphorus oxychloride (Reddy and Seib, 2000; Woo and Seib, 2002) at an addition amount of 0.016% phosphorus oxychloride/starch (by weight). Samples were randomized, and reactions conducted in triplicate using 10 g dwb, starch samples. Starch P content of both native and cross-linked samples was determined by an independent commercial laboratory (Medallion Labs, Minneapolis, MN) and reported as ppm (dwb). Substitution was accomplished using 4.15% (of starch weight) propylene oxide (Johnson, 1969; Kesler and Hjermstad, 1964; Leegwater and Luten, 1971). The degree of substitution for hydroxypropylation was determined by colorimetry (Johnson, 1969). Finally, all native starches were each modified sequentially by phosphorus oxychloride cross-linking followed by propylene oxide substitution on three randomly selected samples (Reddy and Seib, 2000; Woo and Seib, 2002). All reactions again were conducted in triplicate.

2.3. Functional and physical properties

Rapid Visco Analysis (RVA) utilized the Series 4 Rapid Visco Analyzer (Newport Scientific Pty. Ltd, Warriewood, NSW, Australia) with viscosity expressed in centiPoise (cP), time in minutes (min) and temperature in degrees Celsius (°C). Standard Protocol 1 found in Applications: 6.1 "General pasting method using the Rapid Visco Analyser", and modified in 7.9 "RVA Cross-linked and Substituted Starch Method" was used on all cross-linked, substituted and sequentially modified starches (Newport Scientific, 1995). Each product from each of the three reactions for each of the chemical modifications of the native starch (genotypes and % amylose blends) was pasted separately, again independently for each crop year.

Modified starch gel textural responses were observed with a TA.Xt2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). The force \times time curves were integrated for gel strength = Area1 and adhesion = Area2.

The temperatures and heat flow into the starch and water system during loss of crystallinity for all cross-linked, substituted and sequentially modified starches were performed using Differential Scanning Calorimetry (DSC) (Pedersen et al., 2007; Ratnayake and Jackson, 2006).

2.4. Statistical analyses

Statistical analyses were performed using PC-SAS (SAS Institute, Cary, NC) Analysis of variance was used to evaluate variation due to the main effects crop year, type (defined as genotypes, or inter-granular blends of waxy with wild-type) and year by type interactions for each physical property within each chemical modification treatment. Mean responses were compared by computation of least significant differences ($p = 0.05$).

3. Results and discussion

3.1. Chemical properties and modifications

Mean amylose contents (Hansen et al., in press) of the four genotypes were: 27.90% (*Wx-A1* null), 24.25% (*Wx-B1* null), 29.85% (wild-type) and 0.00% (waxy). The dual wavelength method recorded no measurable amylose content in the waxy starch. There was a significant difference observed for mean amylose content of the three remaining genotypes, with that of the *Wx-B1* null samples being significantly lower than that of the *Wx-A1* null and wild-type samples. The latter two samples did not differ significantly from each other.

Respective mean P content of the waxy, wild-type *Wx-A1* and *Wx-B1* null lines native starches was 350, 647, 673 and 767 ppm (dwb). Waxy, wild-type, *Wx-A1* and *Wx-B1* cross-linked starches respectively averaged 84, 301, 261 and 268 ppm P. The sequentially modified waxy, wild-type, *Wx-A1* null and *Wx-B1* null starches respectively averaged 99, 110, 100 and 297 ppm P. Thus, P content of all starches fell well within the legal limits of 0.5% (Stephen et al., 2006). As most native starch P content is in the form of phospholipid (Soulaka and Morrison, 2006), the lower P content of the modified starches likely arose from phospholipid leaching during reactions. Hexaploid wheat starch generally is reported to have a P content in the range of 500–600 ppm (Swinkels, 1985; Kasemsuwan and Jane, 1996; Raeker et al., 1998; Franco et al., 2002). Average durum wheat cultivar P contents were reported as 633 ppm (Soulaka and Morrison, 2006). Waxy and partial waxy hexaploid wheat starch P content generally is lower, and with reported values of less than 200 ppm for waxy, and less than 500 ppm for partial waxy samples (Bertolini et al., 2003; Yasui et al., 1996). The partial waxy samples in the present study showed no reduction in P content relative to wild-type.

The mean molar substitution value for the hydroxypropylated starches was 0.040, with a mean standard deviation of 0.01, and no significant differences.

3.2. Functionality

ANOVA (not shown) detected no significant effects due to crop year or crop year \times type interaction for RVA properties of cross-linked, substituted, or sequentially modified starches. The degree of cross-linking achieved in this study drastically diminished RVA viscosities of wild-type and partial waxy samples (Fig. 1, Table 1). Cross-linking of waxy starch, however, produced a product with

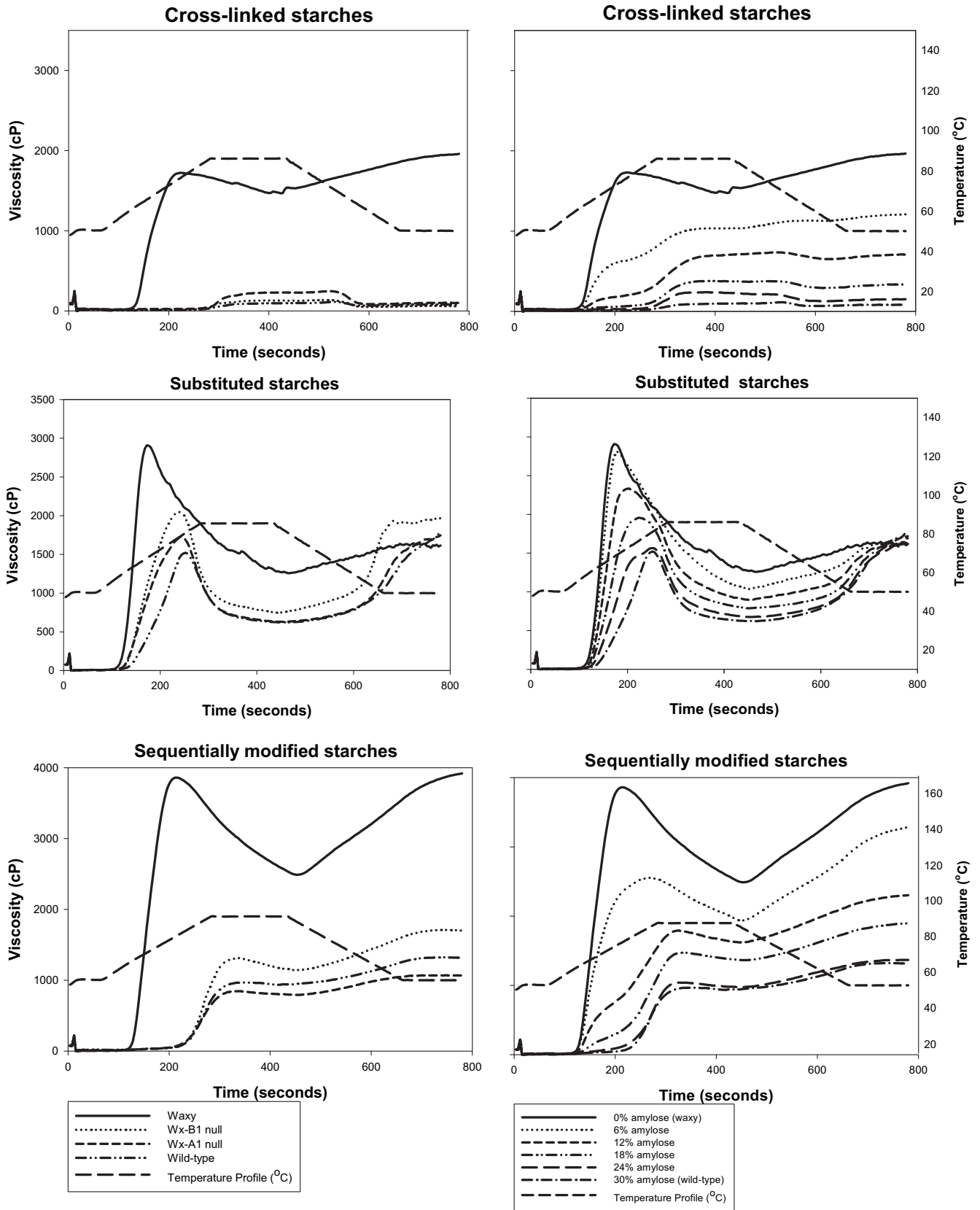


Fig. 1. RVA pasting curves for cross-linked, substituted and sequentially modified starches and starch blends from durum wheats. Curves represent mean responses of samples from 2003 to 2004 harvest years.

Table 1
Means from Rapid Visco Analysis of cross-linked starches purified from tetraploid wheats grown in 2003 and 2004.

Treatment ^a	ViscP ^b (cP)	ViscTr (cP)	Brakdn (cP)	ViscF (cP)	Setbak (cP)	PkTime (min)	TPaste (°C)
6	1030	997	34	1194	198	6.5	52.3
12	690	632	58	693	61	6.8	50.7
18	374	282	93	329	48	6.3	51.5
24	234	113	122	139	27	6.1	52.1
AN	227	71	156	91	20	6.5	51.7
BN	134	48	86	60	13	6.1	53.1
WT	74	39	35	55	17	6.6	52.7
WX	1834	1400	434	1960	560	5.6	50.5
Mean	575	447	127	565	118	6.3	51.8
lsd	571	287	315	405	140	0.7	1.9

^a WX = waxy genotype or 0% amylose, WT = wild-type genotype or 30% amylose, AN = *Wx-A1* null partial waxy genotype, BN = *Wx-B1* null partial waxy genotype, 6 = 6% amylose, 12 = 12% amylose, 18 = 18% amylose, 24 = 24% amylose.

^b ViscP = peak viscosity; ViscTR = trough or hot paste viscosity; Brakdn = breakdown or the difference between peak viscosity and hot paste viscosity; ViscF = final or cold paste viscosity; Setbak = set back or the difference between hot paste viscosity and cold paste viscosity; PkTime = time from start of the run to peak viscosity; TPaste = pasting temperature.

enhanced and stable RVA viscosities. The peak and final viscosities of cross-linked waxy starch were markedly higher than those of the remaining three genotypes. Peak and final viscosities of the blended cross-linked samples fell between the waxy and wild-type samples, and generally were proportional to amylose content. The cross-linking level employed in this study was within the range used by Reddy and Seib (2000) as typical of industrial applications.

Peak viscosity of waxy substituted starch also was statistically greater than that of all other samples (Table 2, Fig. 1), with the exception of the 6% amylose blend. Peak viscosities of substituted starches generally were proportional to amylose content, with one exception. Peak viscosity of the *Wx-B1* null substituted starch was significantly greater than that of the other genotypes, and nearly identical to that of the 18% blend, even though the *Wx-B1* null sample had an amylose content of 24.25%. Final RVA viscosity of the *Wx-B1* null substituted starch was statistically greater than that of all other genotypes and blends. Final viscosities of substituted starches of all other genotypes and blends did not differ from each other. The *Wx-B1* null native starch also displayed functional properties not in proportion to its amylose content (Hansen et al., in press). Peak times of all samples did not vary, with the exception

Table 2
Means from Rapid Visco Analysis of substituted starches from tetraploid wheats grown in 2003 and 2004.

Treatment ^a	ViscP ^b (cP)	ViscTr (cP)	Brakdn (cP)	ViscF (cP)	Setbak (cP)	PkTime (min)	TPaste (°C)
6.0	2836	1023	1813	1614	591	3.0	50.4
12.0	2417	889	1529	1620	732	3.5	52.0
18.0	2179	784	1394	1720	935	3.8	51.5
24.0	1950	671	1278	1692	1020	4.0	51.0
AN	1953	627	1326	1748	1121	3.9	51.3
BN	2128	744	1384	1967	1223	3.9	51.2
WT	1591	616	975	1786	1170	4.1	50.6
WX	3043	1201	1843	1615	414	2.8	51.1
Mean	2262	820	1443	1720	901	3.6	51.1
lsd	254.8	62.2	148.1	132.6	104.7	0.2	0.7

^a WX = waxy genotype or 0% amylose, WT = wild-type genotype or 30% amylose, AN = *Wx-A1* null partial waxy genotype, BN = *Wx-B1* null partial waxy genotype, 6 = 6% amylose, 12 = 12% amylose, 18 = 18% amylose, 24 = 24% amylose.

^b ViscP = peak viscosity; ViscTR = trough or hot paste viscosity; Brakdn = breakdown or the difference between peak and hot paste viscosities; ViscF = final or cold paste viscosity; Setbak = set back or the difference between hot paste viscosity and cold paste viscosity; PkTime = time from start of the run to peak viscosity; TPaste = pasting temperature.

that the waxy sample displayed a significantly shorter value; a similar observation was made for native waxy starch (Hansen et al., in press).

Relative to cross-linking alone, sequential modification increased the viscosity of all samples (Fig. 1, Table 3). Both peak and final viscosities of the waxy sequentially modified starch were markedly elevated and statistically greater than all other samples. Peak time of the waxy sample also was significantly lower than that of all other samples. Again, there was an amylose-dependent trend evident amongst the remaining samples and blends, with the exception once again of the *Wx-B1* null sample. Peak and final viscosities of this sample did not differ from the 12% amylose blend. The observed final viscosities of the sequentially modified starches indicate food processors could achieve any desired response simply via mechanical blending of waxy and wild-type wheats. Reddy and Seib (1999) did not observe amylose-dependent responses when starches from two partial waxy wheats were compared to one wild-type sample. However, they used starches with only a narrow (23%–28%) range of amylose content variation, the samples were not derived from common genetic backgrounds, and were not from wheats grown in the same environment. Thus, many confounding effects, removed in the present study, may have influenced their results.

3.3. Texture

No significant crop year or interactions were observed for gel texture measurements. Cross-linked starches showed few significant differences from each other (Table 4). The *Wx-A1* null sample produced a gel with an Area1 (gel strength) value significantly greater than that of the *Wx-B1* null, but all other cross-linked starches did not differ from each other. Area2 (adhesion) values of cross-linked starches showed no significant differences.

After substitution or sequential modification, starches showed nearly identical responses. Significant differences were observed between waxy and wild-type samples for both Area1 and Area2, with wild-type displaying the greater values (Table 4). Area1 values for both modifications of all other samples showed amylose-dependent absolute values, but rarely were statistically significant differences observed. Area2 values differed only between waxy and all other samples. Wild-type substitution and sequentially modified gels were stronger and displayed less adhesion than waxy samples.

Table 3
Means from Rapid Visco Analysis of sequentially modified starches purified from tetraploid wheats grown in 2003 and 2004.

Treatment ^a	ViscP ^b (cP)	ViscTr (cP)	Brakdn (cP)	ViscF (cP)	Setbak (cP)	PkTime (min)	TPaste (°C)
6	2700	1932	768	3283	1351	4.6	52.3
12	1800	1616	184	2303	687	5.6	51.2
18	1482	1359	123	1898	539	5.6	51.2
24	1039	936	102	1351	414	5.7	51.6
AN	855	785	70	1067	282	6.0	51.1
BN	1765	1474	290	2802	1327	5.9	51.7
WT	975	930	45	1317	387	5.9	52.9
WX	3886	2488	1399	3920	1432	3.6	50.8
Mean	1829	1451	378	2261	812	5.4	51.6
lsd	856	521	494	1517	1023	1.1	n.s.

^a WX = waxy genotype or 0% amylose, WT = wild-type genotype or 30% amylose, AN = *Wx-A1* null partial waxy genotype, BN = *Wx-B1* null partial waxy genotype, 6 = 6% amylose, 12 = 12% amylose, 18 = 18% amylose, 24 = 24% amylose.

^b ViscP = peak viscosity; ViscTR = trough or hot paste viscosity; Brakdn = breakdown or the difference between peak and hot paste viscosities; ViscF = final or cold paste viscosity; Setbak = set back or the difference between hot paste viscosity and cold paste viscosity; PkTime = time from start of the run to peak viscosity; TPaste = pasting temperature.

Table 4

Means from texture evaluation of gels from chemically modified starches purified from tetraploid wheat grown in 2003 and 2004.

Treatment ^a	Cross-linked		Substituted		Sequentially modified	
	Area1 ^b (Ns)	Area2 (Ns)	Area1 (Ns)	Area2 (Ns)	Area1 (Ns)	Area2 (Ns)
6	1.02	-0.49	0.70	-0.31	1.60	-0.66
12	1.32	-0.51	1.06	-0.31	2.72	-0.36
18	1.53	-0.42	2.45	-0.21	4.27	-0.18
24	1.52	-0.39	2.97	0.22	5.46	0.18
AN	2.07	-0.38	2.63	0.33	5.56	-0.06
BN	0.95	-0.37	2.72	-0.11	4.68	0.95
WT	1.81	-0.41	3.45	0.66	9.77	0.49
WX	1.36	-0.41	0.95	-0.17	2.60	-0.60
Mean	1.45	-0.42	2.10	0.01	4.58	-0.03
lsd	0.90	n.s.	1.30	0.31	2.24	0.86

^a WX = waxy genotype or 0% amylose, WT = wild-type genotype or 30% amylose, AN = *Wx-A1* null partial waxy genotype, BN = *Wx-B1* null partial waxy genotype, 6 = 6% amylose, 12 = 12% amylose, 18 = 18% amylose, 24 = 24% amylose.

^b Area1 = gel compression to 4 mm (Newton seconds), Area2 = reverse stroke (Newton seconds).

Wild-type samples were “cleaner releasing”, as indicated by the more negative values for Area2.

3.4. Thermal transition

Across the three types of chemical modification, similar responses were observed. DSC peak temperatures were greatest in the waxy samples, as was ΔH , (enthalpy) (Table 5). The two partial waxy samples were similar to wild-type, and the blended samples displayed amylose-dependent results. This suggests that even after chemical modification, the most important factor governing thermal transitions was the degree of crystallinity of the sample, and was inversely related to amylose content. Waxy starch, composed entirely of 100% amylopectin, required the highest energy input for achievement of the thermal transition.

In general, functional properties of the modified starches behaved in amylose content-dependent fashions. Waxy and wild-type samples generally differed from each other, and blends ranging from 6% to 24% amylose behaved in a concentration predicted manner. End-users could, therefore, adjust the ratio of amylose to obtain desired end-results, especially in terms of starch pasting properties. Altering amylose content, other than at the

Table 5

Means from Differential Scanning Calorimetry evaluation of modified starches purified from tetraploid wheats grown in 2003 and 2004.

Treatment ^a	Cross-linked		Substituted		Sequential cross-linking and substitution	
	Peak ^b (°C)	Enthalpy (J/g)	Peak (°C)	Enthalpy (J/g)	Peak (°C)	Enthalpy (J/g)
6	60.7	15.8	56.3	13.7	58.4	15.3
12	60.6	15.2	56.0	13.7	58.0	15.1
18	59.9	15.2	56.0	13.0	57.1	14.6
24	59.5	14.4	55.4	12.3	58.1	13.6
AN	59.0	13.8	55.4	13.3	56.6	12.5
BN	59.2	13.9	55.3	13.6	58.6	14.6
WT	58.3	12.9	55.2	11.7	56.3	13.4
WX	60.9	16.8	54.9	13.7	58.4	15.3
Mean	59.8	14.7	55.6	13.1	57.7	14.3
lsd	0.7	1.4	0.72	1.60	2.1	1.5

^a WX = waxy genotype or 0% amylose, WT = wild-type genotype or 30% amylose, AN = *Wx-A1* null partial waxy genotype, BN = *Wx-B1* null partial waxy genotype, 6 = 6% amylose, 12 = 12% amylose, 18 = 18% amylose, 24 = 24% amylose.

^b Peak = peak transition temperature, Enthalpy = heat of transition per unit mass.

extremes of waxy and wild-type, did little to alter starch gel textural properties. On the other hand, if very large departures from wild-type are desired, waxy wheat starch offers a new substrate for modified starch production.

Partial waxy (*Wx-A1* and *Wx-B1* nulls) starch functionalities generally performed intermediately between the waxy and wild-type starches, but often with values less than expected by comparison with the inter-granule amylose blends. *Wx-A1* null (intra-granule 26% amylose) was occasionally found to have values not significantly different from the 24% inter-granule amylose blend, and *Wx-B1* null (intra-granule 24% amylose) was found to have values not significantly different from the 12% or 18% inter-granule amylose blends. This indicated amylose content changes within the starch granule had a greater affect on functionality response than the same amylose content changes effected by blending of wild-type and waxy starch granules. Although there may be other factors, amylose reduced within the granule may be less available due to entrapping amylopectin structure than amylose in the wild-type granules diluted with amylose-free waxy granules in the blends.

By reagent selection the functional properties of partial waxy genotypes and % amylose blends of wild-type with waxy wheat starches may be altered for specific end use applications. At the reagent concentrations utilized, waxy and wild-type were very frequently among the extreme significance groups for the functionality properties. The most notable exception was RVA final viscosity for the substituted pasting curves where *Wx-B1* null (intra-granule, 24% amylose) was the only significantly different and high value.

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