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Structure and Functional Properties of Sorghum Starches Differing in Amylose Content

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Starches were isolated from grains of waxy, heterowaxy, and normal sorghum. To study the relationship between starch structure and functionality and guide applications of these starches, amylose content, amylopectin chain-length distributions, gelatinization and retrogradation, pasting properties, dynamic rheological properties, and in vitro enzyme digestion of raw starches were analyzed. Heterowaxy sorghum starch had intermediate amylose content, pasting properties, and dynamic rheological properties. Stress relaxation was a useful indicator of cooked starch cohesiveness. Cooked heterowaxy sorghum starch (10% solids) had a viscoelastic-solid type of character, whereas cooked waxy sorghum starch behaved like a viscoelastic liquid. Amylopectin of normal sorghum starch had a slightly higher proportion of chains with degree of polymerization (DP) of 6−15 (45.5%) compared with amylopectin of heterowaxy starch (44.1%), which had a gelatinization peak temperature 2 °C higher than normal sorghum starch. Heterowaxy sorghum starch contained significantly lower rapidly digestible starch (RDS) and higher resistant starch (RS) than waxy sorghum starch.

KEYWORDS: Sorghum; starch; structure; rheological properties; digestion

INTRODUCTION

Sorghum is an important cereal grain due to its drought resistance and relatively low input costs. Worldwide, sorghum is ranked fifth among cereal grains in terms of quantity and importance (*1*). Starch is the major component of grain sorghum, constituting ~70% of dry grain weight (*2*). Many important physicochemical, thermal, and rheological properties of starch are influenced by the ratio of amylose and amylopectin, the two major polymers in the starch granule, and by the structure of amylopectin. Amylose content strongly affects starch gelatinization and retrogradation (*3, 4*), paste viscosity (*5, 6*), gelation (*7, 8*), and α-amylase digestibility (*9*). The fine structure of amylopectin (chain-length distribution) also was found to influence starch gelatinization and retrogradation properties (*10, 11*).

Amylose content of sorghum grain depends on the dose of a recessive gene (*wx*). The endosperm of waxy sorghum contains three recessive waxy genes (*wxwxwx*), that of heterowaxy sorghum contains at least one recessive gene (*WxWxwx* or *Wxwxwx*), and that of normal sorghum contains no recessive gene (*WxWxWx*). Waxy sorghum shows excellent expansion during extrusion and a high feeding value (*1*). However, waxy sorghum hybrids can have inferior agronomic characteristics, such as low yield, poor seedling vigor, and slightly low grain density, compared with heterowaxy and normal sorghum hybrids (*12*). Some studies have been conducted on applications and properties of heterowaxy sorghum, such as production of brewing adjuncts (*13*), steam flaking (*14*), thermal properties (*15*), digestibility (*16*), and water solubility (*17*). However, the fine molecular structure of heterowaxy sorghum starch and its physicochemical properties have not been fully investigated. In this study, amylose content, gelatinization, retrogradation, pasting properties, gelling properties, in vitro enzyme digestion, and amylopectin chain-length distribution of starches from waxy, heterowaxy, and normal sorghum were analyzed. Such information can help identify uses for these starches in food and other industrial applications.

MATERIALS AND METHODS

Materials and General Methods. Grains of nonisogenic waxy (ATxArg1×RTx2907), heterowaxy (ATx631×RTx2907), and normal (A0K11×RTx2741) sorghum hybrids were produced at the University of Nebraska Field Laboratory, Ithaca, under irrigated conditions in 2004. Moisture content and ash were determined by AACC Approved Methods 44−15A and 08−01, respectively (*18*). Protein content was assayed by a protein-nitrogen analyzer (Leco Corporation, St. Joseph, MI). Assays of total starch and amylose/amylopectin ratio were done with commercial kits from Megazyme (Wicklow, Ireland). Starches were isolated from sorghum kernels according to the method described by Xie and Seib (*19*). The in vitro enzyme digestion profile was determined by a modified Englyst's method (*20*).

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Table 1. Starch and Amylose Levels in Grain Sorghum and Starch Recovery, Protein Content, and Ash Content[a]

	grain		starch		
grain sorghum	starch (%)	amylose (%, sb)	recovery (%)	protein (%)	ash (%)
waxy	68.4 ± 0.4	0 ± 0	81 ± 1.3	0.25 ± 0.01	0.10 ± 0.01
heterowaxy	71.2 ± 0.3	14.0 ± 0.6	81 ± 1.2	0.28 ± 0.005	0.12 ± 0.01
normal	71.4 ± 0.3	23.7 ± 0.1	85 ± 0.1	0.28 ± 0.005	0.14 ± 0.01

[a] Values are average of two measurements.

Table 2. Chain-Length Distribution (Area %) of Amylopectin[a]

	degree of polymerization (DP)		
starch	6−15	16−36	≥37
waxy	43.6 a	50.5 a	5.8 a
heterowaxy	44.1 a	50.2 a	5.8 a
normal	45.5 b	49.6 a	4.9 a

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

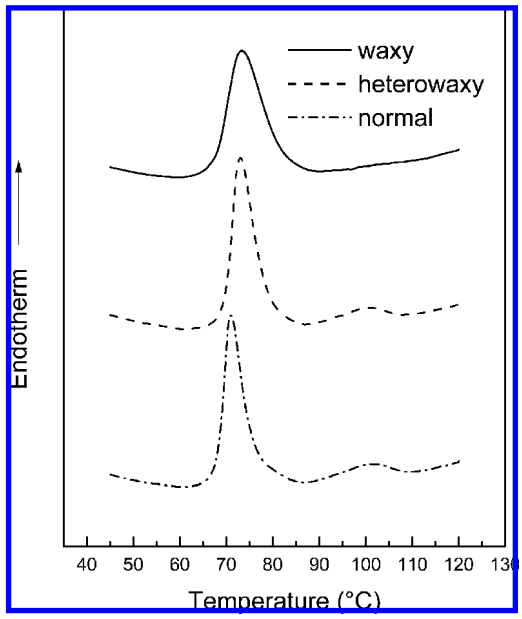

Figure 1. DSC thermograms of heterowaxy, waxy, and normal sorghum starches (starch/water = 1:2, w/w).

Scanning Electron Microscopy and Light Microscopy. Starch was sprinkled onto double-sided adhesive tape and coated with gold−palladium before being viewed with a Hitachi S-3500N scanning electron microscope (SEM) (Tokyo, Japan). For light microscopy work, sorghum starches were stained 15 min at 25 °C by suspending ~10.0 mg of starch 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). Stained starch was viewed under an Olympus BX 51 microscope using a 40X objective (Olympus Optical Co. Ltd., Shinjuku-ku, Tokyo, Japan).

Branch Chain-Length Distribution of Amylopectin. Chain-length distribution was determined by HPAEC-PAD (*10*). Starch (~8 mg) was dispersed in a 0.05 M sodium acetate buffer (2.9 mL, pH 4.2) and then heated in boiling water for 10 min. After the solution was cooled to room temperature, 2 μL of isoamylase (1U/μL, where 1 unit is the amount of enzyme required to release 1 μmol of reducing sugar equivalent from the defined substrate per min at pH 4.0 and 40 °C.) from Megazyme (Wicklow, Ireland) was added, and the mixture was incubated at 37 °C for 24 h. The debranched starch was boiled for 10 min, adjusted to 1.0 mg/mL carbohydrate and 0.15 M sodium hydroxide, and then injected to the HPAEC-PAD system.

Thermal Properties. Gelatinization and retrogradation of starches were measured by differential scanning calorimetry (DSC) as described by Shi and Seib (*10*). Starch and water at a ratio of 1:2 (w/w) was heated at 10 °C/min from 25 to 130 °C. Gelatinized starch was stored at 4 °C for 7 days and then rescanned.

Pasting Properties. Pasting properties were measured on a Rapid Visco Analyzer (RVA) (Newport Scientific, Sydney, Australia) according to the method described by Song and Jane (*21*). Starch suspension (7%, w/w; 28.0 g of total weight) was heated and cooled through a programmed cycle, where the sample was held at 50 °C for 1 min, heated to 95 °C in 7.5 min, held at 95 °C for 5 min, cooled to 50 °C in 7.5 min, and held at 50 °C for 3 min. The rotating speed of the paddle was 160 rpm. The RVA test also was measured at pH 3.0 using a sodium citrate buffer.

Rheological Properties. Small deformation oscillatory measurements were performed using a Bohlin Rheometer System CVOR 150 (Bohlin Rheology Inc., Crambury, NJ) with a cone-plate geometry having a degree of 4. A starch dispersion was prepared by heating the starch slurry (10%, w/w) in a boiling water bath for 5 min with low-speed stirring to prevent sedimentation of starch. The sample was then continuously heated for 10 min without stirring. The dispersion was cooled by holding at room temperature for 5 min and transferred to the cone-plate system before it was gelled. The sample at the edge of the cone plate was covered by silicone oil to prevent moisture loss. The sample was allowed to rest for 40 min before the measurement was started. Strain sweep tests at a temperature of 25 °C and a frequency of 1 rad/s was carried out from strain of 0.001−1 to determine the linear viscoelastic region of the samples. Frequency sweep was measured at 25 °C and a strain of 0.02, which was found within the linear viscoelastic region, over a frequency range 1−100 rad/s. Results were expressed as the storage modulus (G'), loss modulus (G'', and loss tangent (tan δ). Stress relaxation behavior also was measured at a constant strain 0.02 (2%). Rise time for the applied strain was 0.02 s, and the relaxation modulus (G) was obtained as the material was allowed to relax for 1000 s.

RESULTS AND DISCUSSION

Sorghum Starch and Isolation. Heterowaxy sorghum grain had a starch content of 71.2% (**Table 1**), which is similar to that of normal sorghum grain (71.4%). The waxy sorghum grain used in this experiment had a lower starch content of 68.4%, similar to results reported for other waxy sorghums by Hoseney et al. (*2*). Amylose content determined by Megazyme kit using the Concanavalin A method is shown in **Table 1**. Heterowaxy sorghum starch had an intermediate amylose content (14.0%) compared with waxy (0%) and normal (23.7%) sorghum starches. Isolating starch form the three sorghum varieties gave greater than 80% starch recovery, and all three starches contained less than 0.3% protein and 0.15% ash.

SEM and Light Microscopy. Heterowaxy sorghum starch appeared to have granule shape and size similar to normal and waxy sorghum starches. Granules of the three starches were polygonal or spherical in shape, and some granules had dents at the surface. Granule sizes were 5−25 μm by microscopy measurement. The color of iodine-stained waxy sorghum starch was light brown, and normal sorghum starch was dark blue. Three-fourths of the heterowaxy sorghum starch granules were stained dark purple, and one-fourth was light brown. The heterowaxy hybrid was produced by crossing a normal female (ATx631) with a waxy male (RTx2907). The resulting F1 hybrid seed has a normal appearance but is a heterozygous genotype. Grain produced from the heterozygous seed would have a 3:1 phenotypic ratio of normal to waxy granules (*16*). The 3:1 ratio of normal/waxy phenotype in the heterowaxy sorghum grain observed in this study is consistent with the theoretical phenotypic ratio.

Table 3. Gelatinization and Retrogradation of Starch in Water (1/2, w/w) after Heating to 130 °C and Storing at 4 °C for 7 Days[a]

	gelatinization endotherm				retrogradation endotherm				
sorghum starch	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g of starch)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g of AP)	ΔH (J/g of starch)
waxy	67.7 a	73.0 b	82.1 a	14.7 a	41.4 a	50.7 a	66.6 b	5.4 a	5.4 b
heterowaxy	69.6 b	72.8 b	78.6 b	13.7 b	43.9 a	53.4 a	68.0 b	5.9 a	5.1 b
normal	67.9 a	70.7 a	75.7 a	13.2 a	41.8 a	50.5 a	62.7 a	5.6 a	4.3 a

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

Branch Chain-Length Distributions. Three sorghum starches were debranched by isoamylase, and the unit chains were separated and quantified by HPAEC-PAD. The PAD detector has a different molar response with different unit chains (*10, 22*), and the molar responses with unit chains above the degree of polymerization (DP) 17 are not known. In this study, the area percent of each peak was used to compare the chain-length distribution. The most abundant chain of the three sorghum starch amylopectins appeared at DP 12 with a shoulder peak at DP 18−20. Heterowaxy and waxy sorghum starch amylopectin had similar chain-length distributions. Normal sorghum starch amylopectin had a slightly higher proportion of chains with DP 6−15 (45.5%) compared with heterowaxy starch amylopectin (44.1%) at a LSD of 1.1% (**Table 2**).

Gelatinization and Retrogradation Properties. DSC thermograms of three sorghum starches gelatinized at a starch to water ratio of 1:2 (w/w) are shown in **Figure 1**. Heterowaxy and normal sorghum starch displayed a major peak of starch gelatinization and a minor peak due to the melting of an amylose−lipid complex. Gelatinization properties of starches are summarized in **Table 3**. The gelatinization peak temperature of heterowaxy and waxy sorghum starches was about 2 °C higher than that of normal sorghum starch. The peak gelatinization temperature is believed to be an indicator of crystallite quality, which is related to double helix length, whereas the gelatinization enthalpy (ΔH) is a measure of the loss of molecular order (*23, 24*).

Retrogradation of amylopectin in these three starches after heating in two parts of water to 130 °C and storing at 4 °C for 7 days was analyzed by DSC (**Table 3**). On the basis of the total weight of starch, normal sorghum starch showed a lower retrogradation extent than heterowaxy and waxy sorghum starch. However, when enthalpy (H) values were calculated based on the weight of the amylopectin fraction, the extent of retrogradation among the three starches was not significantly different (**Table 3**).

Retrogradation properties (*10, 11*) and digestion (*25*) of cooked starch depend on the fine structure of amylopectin. To have a starch with improved cold storage stability, a sorghum starch with a high level of short chains needs to be developed. In contrast, a sorghum starch with a high level of long chains would result in increased retrogradation and increased resistance to enzyme digestion. Further work is needed to select or develop grain sorghum with different fine structures and retrogradation properties of cooked starches.

Pasting Properties. RVA pasting curves of waxy, heterowaxy, and normal sorghum starch at neutral pH are shown in **Figure 2A**. Like waxy sorghum starch, heterowaxy starch displayed a lower pasting temperature and higher peak viscosity than normal sorghum starch. Amylose inhibits swelling of starch granules by forming complexes with lipid, which results in a lower peak viscosity at a higher pasting temperature. On the other hand, this amylose−lipid complex increases the rigidity of granules by limiting swelling, which could explain why heterowaxy starch had a smaller breakdown than waxy starch.

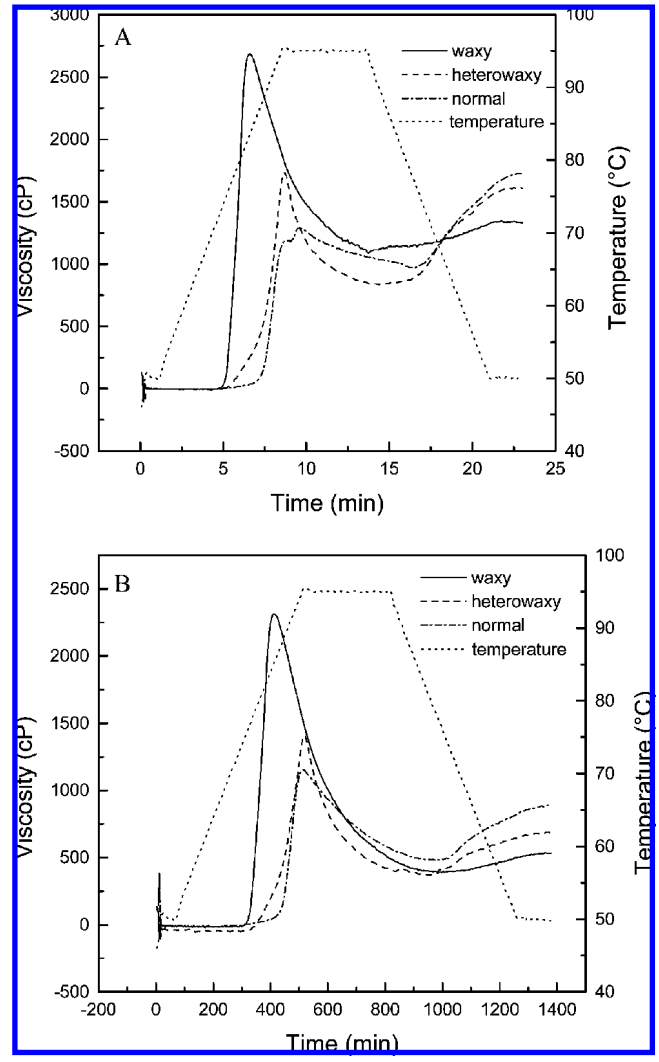

Figure 2. Pasting curves of sorghum starches (7%) measured by Rapid Visco-Analyzer at neutral pH (**A**) and pH 3.0 (**B**).

Heterowaxy starch showed a higher setback than waxy starch, which reflects the three-dimensional structure formed by amylose. At acid pH (3.0), all three starches displayed reduced peak viscosity and increased breakdown compared with neutral pH (**Figure 2B**). Modifications are needed to improve the acid and shear stability of these sorghum starches.

Rheological Properties. The linear viscoelastic region, at which rheological properties are independent of strain, was determined through strain sweep tests. Storage modulus (G') usually remains independent up to a critical value and then decreases when samples start to move (*26*). For cooked sorghum starches at 10% concentration (w/w), normal starch had a narrower linear viscoelastic region (up to ~0.04 (4%) strain) than heterowaxy starch (up to ~0.5 (50%) strain) (**Figure 3**). The storage modulus (G') of cooked waxy sorghum starch did not change significantly with strain up to 1. The smaller linear viscoelastic region of the cooked normal starch gel was

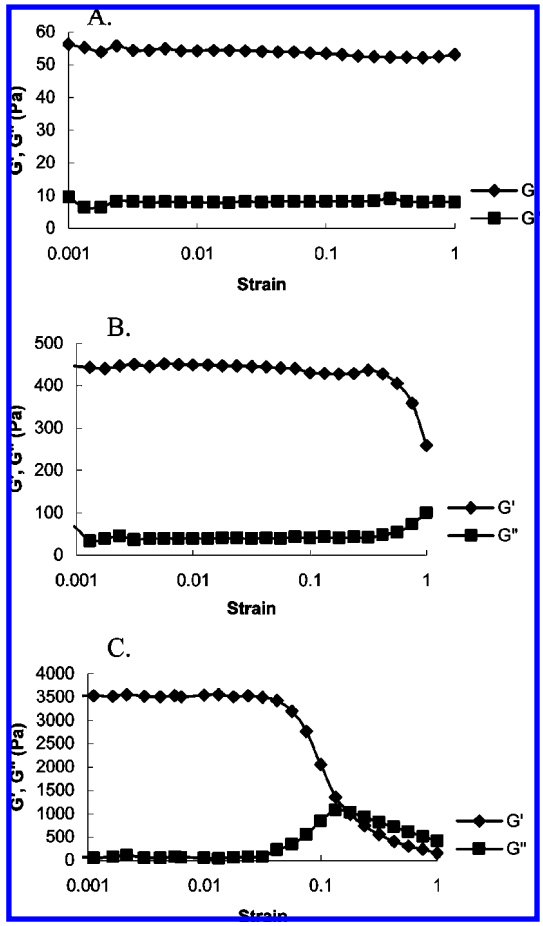

Figure 3. Effect of strain on storage modulus (G') and loss modulus (G'') of a 10% dispersion of waxy (**A**), heterowaxy (**B**), and normal (**C**) sorghum starch. The starch−water mixtures were heated in a boiling water bath, then cooled to 25 °C, and held for 40 min before recording mechanical properties.

consistent with its observed stiffness. A strain of 0.02 (2%), which was within the linear viscoelastic region of all three starches, was chosen for further rheological tests.

The storage modulus (G') within the linear region indicates elastic properties. Cooked normal sorghum starch (10%) had a high G' value of 3500 Pa, while heterowaxy and waxy sorghum starches had low G' values (500 and 50 Pa, respectively). Gelling of starch is also interpreted in terms of tan δ (*27*). In general, starch cooked in water and cooled is found to be a firm, self-standing, true gel when tan $\delta < 0.1$. The mixture remains a paste when tan $\delta > 0.1$ (*27*). Normal sorghum starch at 10% was a strong gel (tan $\delta < 0.1$) after cooking and cooling, and heterowaxy sorghum starch (with tan $\delta \sim 0.1$) was a very weak gel. The waxy sorghum starch dispersion remained a paste (tan $\delta > 0.1$) at a concentration of 10% after cooking and cooling (**Figure 4**). The structure of a starch gel is considered to be a three-dimensional amylose matrix filled by swollen starch granule remnants (*28–32*). Differences in amylose content can affect both granule rigidity and the continuous network.

Frequency sweeps of the three starches are shown in **Figure 5**. The storage modulus (G') of cooked and cooled normal sorghum starch (10%) was almost independent of frequency, indicating a typical "true gel" characteristic. Few molecular rearrangements within the network occurred over the short time scale of the applied strains. However, cooked and cooled heterowaxy and waxy sorghum starches showed G' values increasing with frequency, which is typical of a paste undergoing structural relaxation over the short time scale.

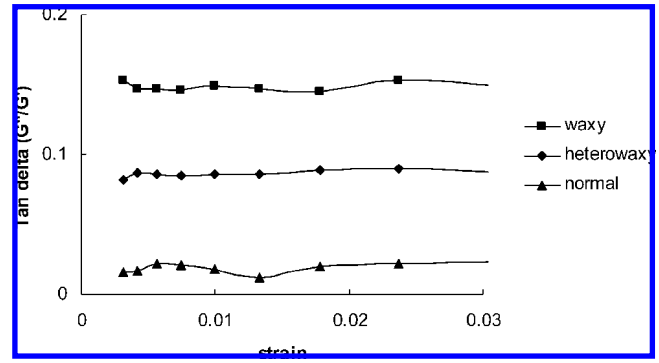

Figure 4. Tan δ of waxy, heterowaxy, and normal sorghum starch at 25 °C and 10% (w/w) concentration within linear viscoelastic region. Starch−water mixture treated as described in caption to **Figure 3**.

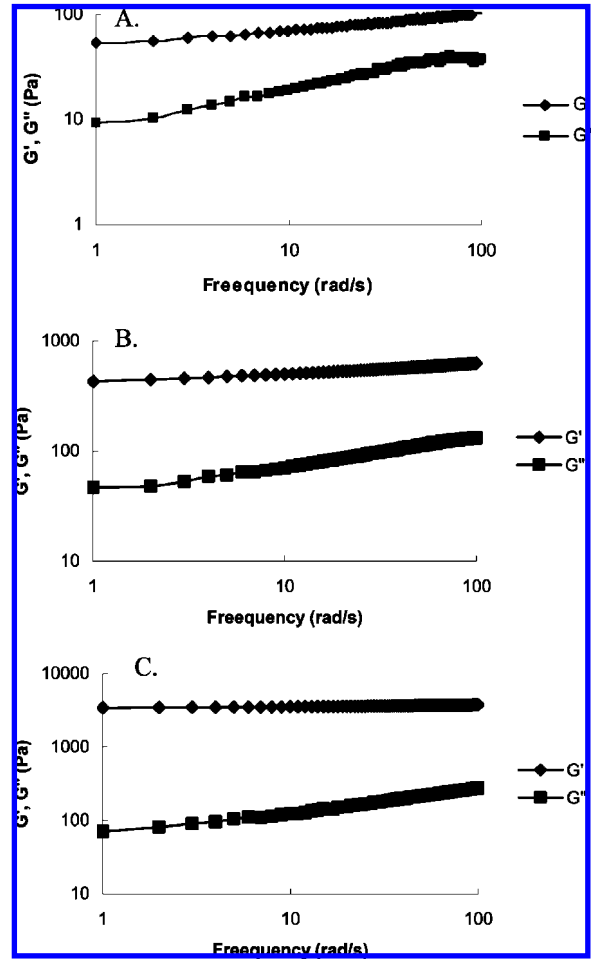

Figure 5. Effect of frequency on storage modulus (G') and loss modulus (G'') at 25 °C of 10% dispersion of waxy (**A**), heterowaxy (**B**), and normal (**C**) sorghum starch.

Stress relaxation results for waxy, heterowaxy, and normal sorghum starch dispersion are shown in **Figure 6**. The value of $G(t)/G(0)$, which is the ratio of the shear modulus at any time to the initial shear modulus, was determined. The rise time for the applied strain was 0.02 s. Stress relaxation data after 10 × rise time were used. The shear modulus at 0.2 s was set as the initial shear modulus value of $G(0)$. Waxy starch had a sharper drop of $G(t)/G(0)$ from 0.2 to 0.8 s. In addition, the value of $G_f/G(0)$, which is the ratio of the shear modulus at 1000 s and at 0.2 s, was small (~0.4) for cooked waxy sorghum, indicating a more cohesive liquidlike material rather than a solid. In contrast, heterowaxy and nonwaxy sorghum starch displayed

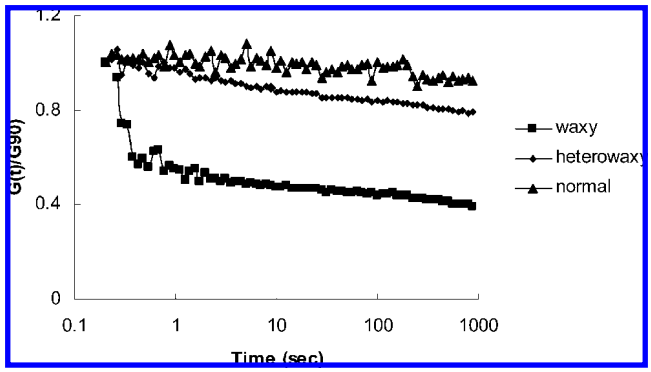

Figure 6. Stress relaxation curves for waxy, heterowaxy, and normal sorghum starch at 25 °C and 10% (w/w) dispersion.

Table 4. Digestion Profiles of Raw Starches[a]

sorghum starches	RDS (%, db)	SDS (%, db)	RS (%, db)
waxy sorghum starch	21.5 a	68.4 a	8.4 c
heterowaxy sorghum starch	12.0 b	61.7 b	23.7 a
normal sorghum starch	12.5 b	68.5 a	17.9 b

[a] Values followed by the same letters in the same column are not significantly different ($p < 0.05$). RDS, rapidly digestible starch; SDS, slowly digestible starch; and RS, resistant starch.

less stress relaxation and had $G_f/G(0)$ values of 0.8 and 0.9, respectively, indicating a more elastic-solid type character. It seems that the amylose content has a great impact on stress relaxation and the texture of cooked starch. The amylose content of heterowaxy and normal sorghum starches was 14.0 and 23.7%, respectively. It would be interesting to identify or develop a sorghum starch with 5−10% amylose and examine its rheological properties.

In Vitro Enzyme Digestion Profiles. Digestion profiles of the three sorghum starches in the raw state were determined by the modified Englyst's method (**Table 4**). Normal and heterowaxy sorghum starch gave significantly less rapidly digestible starch (RDS) and more resistant starch (RS) than waxy starch. In general, digestibility of starch is inversely proportional to amylose content (*33, 34*). One surprising result in this study is that heterowaxy sorghum starch contained a higher level of RS than normal sorghum starch. The low digestibility of heterowaxy sorghum starch compared with normal sorghum starch might be explained by amylopectin chain-length distributions. Amylopectin of heterowaxy sorghum starch had fewer chains of DP >15 than that of normal sorghum starch. Benmoussa et al. reported that variability in rice starch amylopectin fine structure could affect the digestibility of cooked rice starch and RDS was positively correlated with very short linear chains (*35*). A slowly digestible starch was produced by increasing branching density of normal maize starch through enzyme modification (*36*). Sorghum generally has a low starch digestibility among cereals (*37*) and has potentials to be used as an ingredient for low-glycemic food. Further research is needed to manipulate the ratio of amylose to amylopectin and their fine structures in sorghum, thereby developing sorghum with even more RS and SDS. Grain sorghum with amylose content greater than 40% could have a significantly increased RS content. Increasing the level of α-1,6 linkages (*36, 38*) or side chain length (*39*) could elevate the SDS content of a waxy sorghum. When a sorghum flour or starch is used in a food application, processing conditions can change the structure of starch and alter the rate of digestion (*40*). It is a challenge to retain SDS structure of starch in a food application or generate SDS during a food production process (*39, 40*).

Overall, heterowaxy sorghum starch had intermediate amylose content compared with waxy and normal sorghum starch and had an amylopectin chain-length distribution similar to waxy starch. Like waxy starch, heterowaxy starch had a decreased pasting temperature and increased peak viscosity. After cooling, a 10% paste of heterowaxy starch was a soft gel, while a normal starch mixture resulted in a hard gel. Stress relaxation results showed that cooked and cooled heterowaxy sorghum starch (10% solids) had a viscoelastic-gel type of character comparable to cooked normal sorghum starch, whereas cooked waxy sorghum starch behaved like a viscoelastic liquid without extensive intermolecular entanglements or intermolecular cross-links. Heterowaxy starch contained significantly lower RDS and higher RS than waxy starch. The low digestibility of heterowaxy starch might be desirable when included in foods with low- or reduced-glycemic response. Heterowaxy sorghum starch could be chemically modified to give unique rheological properties. Further breeding work is needed to develop sorghum starches with low amylose content (5−10%) and altered amylopectin structure, which could provide unique rheological properties, improved cold-storage stability, or increased RS and SDS contents.

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