Starch Solubilization and Retrogradation During Preparation of *Tô* (a Food Gel) from Different Sorghum Cultivars

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ABSTRACT

Starch solubilization and retrogradation were studied during the preparation of a food gel called *tô*. *Tô* was prepared from flours of four sorghum cultivars with different endosperm textures. Flour and water slurry (17% solids) at pH 8.6 was cooked for 5 min to yield a hot paste (fresh *tô*) or allowed to cool for 1 hr at 25°C to yield a stiff gel, (aged *tô*). Fresh and aged *tô* were dispersed in water at 25°C by blending and centrifuging. Soluble starch was extracted at 35°C, and the apparent molecular weight was quantified by high-performance, size-exclusion chromatography. Amylose (AMY) and amylopectin (AMP) starch was only partially solubilized in fresh *tô*. Aged *tô* contained even less soluble starch. Although more soluble starch was observed in fresh *tô* prepared from sorghums with more corneous (hard) endosperm than floury (soft) endosperm, the resulting aged *tô* contained significantly less soluble starch. Increased firmness of aged *tô* prepared from the more corneous sorghums corresponded to more starch retrogradation as measured by loss of soluble starch. Higher molecular weight soluble AMY and AMP were observed in fresh *tô* compared to aged *tô*. Retrogradation of soluble AMY and AMP in fresh *tô* appears to be faster for higher molecular weight than for lower molecular weight polymers.

The gel-forming characteristic of starch in heat-processed foods (puddings, polenta, and porridges) depends primarily on the ability of gelatinized starch to retrograde upon cooling. Partial solubilization of starch molecules, which occurs during gelatinization (Atwell et al 1988, Waniska and Gomez 1992), appears to play a major role in the formation and textural characteristics of starch-based food gels. When soluble amylose (AMY) was added and cooked with native or cross-linked waxy corn starches, gels with different degrees of firmness were formed upon cooling (Ott and Hester 1965). Increased firmness of waxy corn starch gels were observed as levels of soluble AMY increased. However, more soluble AMY was added to native starch compared to cross-linked starch to form gels of equal breaking strengths. Apparently, this was due to more solubilization of amylopectin (AMP). Similarly, solubilized AMP in cooked rice provided a more sticky, less rigid texture compared to that of parboiled rice (Priestley 1977). Thus, functionalities of soluble AMY and AMP affect the formation and textural characteristics of starch-based food gels.

Tô with firmer, nonsticky characteristics is preferred (Rooney et al 1986). A firmer gel was prepared with starch from the corneous versus the floury endosperm fraction of sorghum (Cagampang and Kirleis 1985). Increased soluble starch and firmer *tô* texture was also associated with a more corneous endosperm texture of sorghum (Akingbala and Rooney 1987). However, less soluble starch was observed in firmer *tô* samples prepared from sorghum with a more corneous endosperm texture (Bello et al 1990). Starch of high molecular weight (HMW) was observed in the corneous endosperm (Cagampang and Kirleis 1985). The HMW polymers (AMP and AMY) reportedly contribute to more solubilization (Jackson et al 1989) and retrogradation of starch (Cagampang and Kirleis 1985).

Many factors affect the dispersion of starch in foods: proportion of AMY and AMP, food processing conditions, and conditions of starch extraction (Jackson et al 1989, 1990; Waniska and Gomez 1992). In this study, the solubilization and retrogradation of starch in a processed food were determined during the preparation of *tô* from sorghums with different endosperm textures. Amounts and weight-average molecular weight (M_W) of soluble AMY and AMP in sorghum flour immediately after cooking and after cooling of *tô* were determined and related to *tô* quality.

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MATERIALS AND METHODS

Preparation of Flour and *Tô*

Flours and *tô* were prepared from four sorghums (SC283, SC265, BTx3197, and NSA740) with significantly different endosperm textures and *tô* properties (Bello et al 1990). The proportion of corneous endosperm (compared to floury) decreased in the order of SC283 > SC265 > BTx3197 > NSA740. The endosperm texture of the four sorghum cultivars and the firmness of *tô* prepared from these cultivars were representative of West African sorghums.

Tô was prepared by cooking 56 g of sorghum flour slurry (17% solids, w/w) at pH 8.6 for 5 min with continuous stirring followed by cooling for 1 hr at 25°C (Bello et al 1990). Fresh *tô* is the hot paste (95°C) immediately after cooking. Aged *tô* refers to the gel formed by placing fresh *tô* in a beaker (50 ml) and allowing it to cool for 1 hr at 25°C. The firmness of aged *tô* decreased in the order of SC265 > SC283 > BTx3197 > NSA740.

Fractionation of *Tô*

Fresh or aged *tô* (10 g) was dispersed in 50 ml of deionized distilled water by blending (Bello et al 1990). The slurry was centrifuged to yield soluble and residual solids.

Chemical Analyses

Aged *tô* and residual solids were lyophilized and reground in a cyclone mill to pass through a 400 μm round-hole screen (Udy, Fort Collins, CO). The soluble solids were also lyophilized and ground in a coffee mill (model MC-170, Miracle Mill, Salt Lake City, UT).

Moisture content of *tô* was determined by drying at 130°C for 4 hr. Ash and crude fat contents were determined (AACC 1983). Protein (% N × 6.25) was determined using the micro-Kjeldahl digestion procedure (AACC 1983) and analysis of ammonia nitrogen (Technicon 1976). Starch was determined in autoclaved samples by digestion with amyloglucosidase (Khan et al 1980) and analysis of glucose (Technicon 1978).

Apparent AMY was determined by a colorimetric procedure (Juliano 1971). Potato amylose (type III, Sigma Chemical Co., St. Louis, MO) and a 25% amylose corn starch (Amaizo, American Maize Products Co., Hammond, IN) were used as standards.

Starch Characterization Using High-Performance, Size-Exclusion Chromatography (HPSEC)

Aliquots (40 ml) of soluble solids from fresh and aged *tô* were diluted to 100 ml with distilled, deionized water (1 ml of methanol was added before dilution). Portions (10 ml) of the diluted samples were placed in 20-ml centrifuge tubes and treated at 35°C for 10 min by heating in a water bath followed by centrifuging at 3,400 × g for 10 min (Jackson et al 1990). Starch in the extract was prepared for HPSEC by sonicating for 18 sec to ensure complete dispersion of any polymer aggregates that may have formed after the extraction at 35°C. The sonicated sample was filtered through a 5-μm nylon filter and the filterate was equilibrated at 55°C oven before injection of a 25-μl portion into the SEC columns (Jackson et al 1989). The amounts of soluble AMY and AMP were determined based on eluted peak areas. The apparent molecular weights of each peak maxima in the elution regions of AMY and AMP were calculated based on pullulan standards (Jackson et al 1988). The weight-average molecular weight (M_W) of each peak maxima was calculated as the apparent molecular weight multiplied by the ratio between the peak area and the total peak area of AMY or AMP. The M_W of AMY and AMP was based on the sum of the M_W of all peak fractions in the elution regions of AMY and AMP, respectively.

Starch retrogradation (reduction of soluble starch during aging of a product) was calculated (Whistler and Johnson 1948). The difference in soluble starch in fresh and aged *tô* was expressed as a percent of the soluble starch in the fresh *tô* .

Lyophilized samples (aged tô and aged *tô* residual solids) and flour (0.5%, w/v) were boiled, autoclaved, and sonicated before being extracted at 35°C and analyzed for soluble starch (Jackson et al 1990). The amount of soluble starch was calculated based on the sum of HPSEC detectable AMY and AMP in the extract expressed as a percentage of starch in sorghum flour.

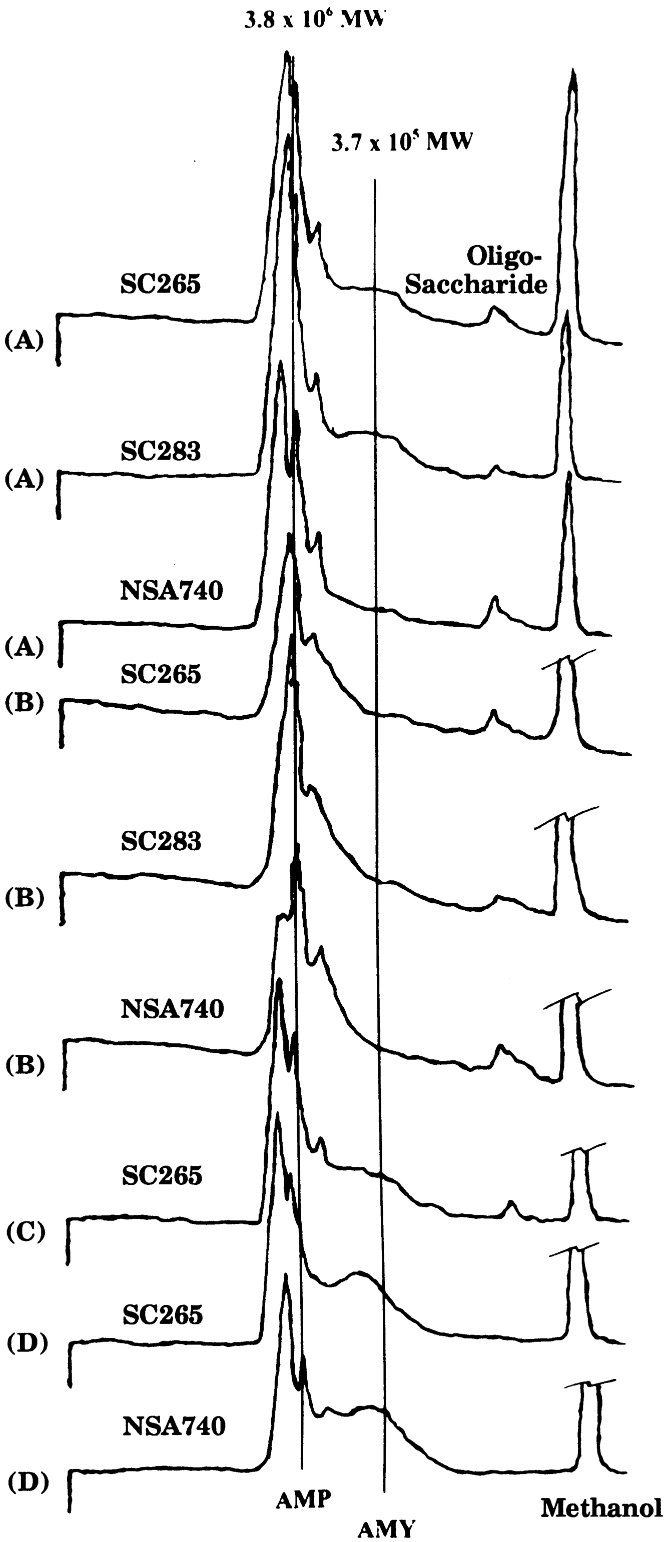

Fig. 1. High-performance size-exclusion chromatograms of starch extracted at 35°C from the soluble solids of fresh *tô* (A) and aged *tô* (B), and from lyophilized samples of aged *tô* (C) and aged *tô* residual solids (D). The lyophilized samples (0.5%, w/v) were boiled (100°C), autoclaved (120°C), and sonicated (18 sec) before being extracted at 35°C.

Statistical Analyses

Duplicate samples of *tô* were prepared, and each sample was analyzed for chemical composition and soluble starch. The experiment was replicated on at least two separate occassions. The mean values were expressed on dry weight basis. Analysis of variance and mean separation using the least significant difference (LSD) procedure were computed using $P > 0.95\%$ (SAS 1987).

RESULTS

Starch Solubilization During Tô Preparation

Processing of sorghum flour into *tô* at 100°C caused the partial dispersion of both AMY and AMP at 35°C (Figs. 1 and 2). Starch solubility ranged from 33–46% in fresh *tô* to 22–32% in aged *tô*. More soluble starch was present in fresh than in aged *tô*, except in *tô* prepared from NSA740, where similar amounts were observed.

In addition to starch, a carbohydrate (oligosaccharide) of low molecular weight (LMW) was also dispersed during processing

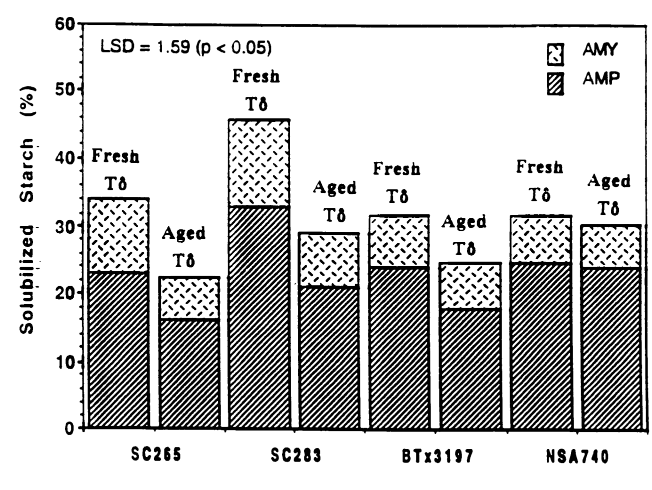

Fig. 2. Soluble starch from fresh and aged *tô* extracted at 35°C. Values are means of three separate determinations. The proportion of corneous to floury endosperm fraction of the sorghum cultivars decreased in the order of SC283 > SC265 > BTx3197 > NSA 740.

of sorghum flour into *tô* (Fig. 1). The oligosaccharide was also observed in lyophilized *tô* after processing at 120°C before extraction at 35°C. However, the oligosaccharide was absent in *tô* residual solids that were similarly processed (Fig. 1). This oligosaccharide was previously observed in both raw and extruded sorghum flours (Jackson et al 1990) and probably resulted from the action of amylases (e.g., α-amylase) on damaged starch in sorghum flours.

Other flour components (ash, fat, and protein) were also partially solubilized during the preparation of *tô* (Table I). *Tô* soluble solids were primarily starch with much reduced levels of protein, ash, and fat. Apparent AMY content was significantly less in *tô* soluble solids than residual solids (Table I). Ash levels in *tô* soluble and residual solids were higher for NSA740 than they were for the other cultivars. Flour prepared from NSA740, the cultivar with a softer endosperm texture contained more ash, fat, and protein and less starch and apparent AMY than the other cultivars. Moisture content of fresh or aged *tô* was 80–82%.

The higher processing-extraction temperature (120°C compared to 100°C) caused more dispersion of starch in flour and in lyophilized *tô* (Fig. 3). More starch dispersed from flour than from lyophilized aged *tô*. This indicates that aged *tô* contains retrograded starch. More starch dispersed from flour prepared from sorghum with a corneous endosperm texture compared to a soft endosperm texture, suchs as NSA740.

TABLE I
Composition (%) of *Tô* Soluble Solids, *Tô* Residual Solids, *Tô*, and Flour Prepared from Sorghums with Different Endosperm Textures[a,b]

Sample/Cultivar	Ash	Fat	Protein[c]	Starch	Amylose[d]
Tô soluble solids[e]					
SC265 (39.6)	1.12	0.27	3.28	93.5	24.6
SC283 (43.3)	1.10	0.15	2.51	95.1	24.6
NSA740 (47.0)	1.46	0.07	4.55	89.5	16.8
Tô residual solids[e]					
SC265 (60.4)	1.70	0.17	20.0	72.9	26.6
SC283 (56.7)	1.85	0.21	20.7	68.5	27.8
NSA740 (53.0)	3.25	0.57	22.7	65.9	24.6
Tô					
SC265	1.44	0.78	12.5	83.0	26.8
SC283	1.51	0.75	12.6	82.1	26.3
NSA740	2.45	0.64	13.9	74.8	19.6
Flour					
SC265	0.92	1.78	12.1	83.2	29.0
SC283	1.00	1.60	12.5	82.2	27.9
NSA740	1.90	3.19	13.9	75.6	21.4
$LSD_{0.05}$[f]	0.06	0.28	0.59	1.58	1.72

[a] *Tô* was aged for 1 hr at 25°C after cooking (aged *tô*).
[b] Values are means of three separate determinations.
[c] % N × 6.25.
[d] Apparent amylose content of starch.
[e] Lyophilized and ground before analysis. Amount of solids is expressed as a percent of total solids (in parentheses).
[f] Least significant difference.

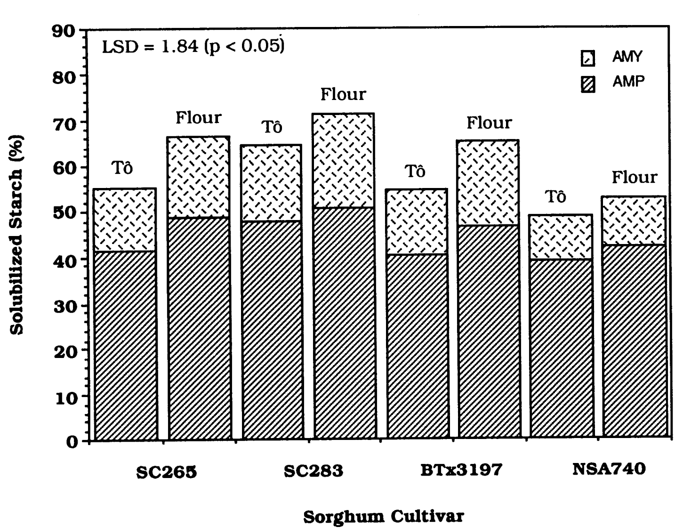

Fig. 3. Soluble starch from lyophilized *tô* and flour (raw) processed at 120°C then extracted at 35°C. Values are means of two separate determinations. The proportion of corneous to floury endosperm fraction of the sorghum cultivars decreased in the order of SC283 > SC265 > BTx3197 > NSA 740.

Molecular Size of Soluble Starch

Starches with broad M_W ranges were revealed in chromatograms of fresh and aged *tô* (Fig. 1). The soluble AMP exhibited multiple elution peaks in both fresh and aged *tô* and in lyophilized *tô* and residual solids. This was observed for all the four sorghums, including cultivar BTx3197 (data not shown). Less defined peak was observed for soluble AMY, except in lyophilized samples that were processed at 120°C before extraction at 35°C.

The AMY and AMP extracted at 35°C were of higher M_W in fresh *tô* compared to aged *tô*, except for NSA740 (Table II). The AMY (5–6 × 10^5 M_W) and AMP (13–17 × 10^6 M_W) extracted from lyophilized *tô* and flour (after a 120°C processing-extraction step) were even higher in M_W (Table II). The M_W of AMY from NSA740 was not affected by aging of *tô* nor processing-extraction temperature.

Starch Retrogradation During *Tô* Preparation

Amounts of soluble starch (both AMY and AMP) decreased during aging of *tô* (Fig. 2). The loss of soluble starch in fresh *tô* was expressed as a percent of soluble starch and termed "starch retrogradation" (Fig. 4). Substantial amounts of AMY and AMP were not able to be solubilized (retrograded) during the cooling of these starch pastes. Starch retrogradation was significant in *tô* prepared from sorghums with intermediate and corneous endosperm textures but not in *tô* prepared from sorghum with a floury texture.

DISCUSSION

Native starch in sorghum flour became partially solubilized during processing into *tô* and during sample preparation. The solubilization of starch was favored by physical (heat), plasticizer (water), mechanical (shear), and chemical (alkali) conditions dur-

TABLE II
Estimated Weight-Average Molecular Weight (M_W) of Soluble Amylose and Amylopectin Extracted from Fresh *Tô*, Aged *Tô*, Lyophilized *Tô*, and Flour from Sorghum with Different Endosperm Textures[a]

Sample/Cultivar	Amylose (× 10^5 Da)	Amylopectin (× 10^6 Da)
Fresh *Tô* extracted at 35°C		
SC265	5.7	3.8
SC283	5.1	3.7
BTx3197	4.0	4.2
NSA740	2.9	3.7
Aged *Tô* extracted at 35°C		
SC265	2.0	2.8
SC283	2.5	2.8
BTx3197	2.3	3.4
NSA740	2.5	3.3
Lyophilized *Tô* processed at 120°C then extracted at 35°C		
SC265	5.6	14.2
SC283	5.0	14.5
BTx3197	5.4	15.8
NSA740	2.5	16.5
Flour processed at 120°C then extracted at 35°C		
SC265	5.7	13.6
SC283	5.6	13.8
BTx3197	5.2	15.4
NSA740	2.5	17.0
$LSD_{0.05}$[b]	0.6	0.8

[a] Values are means of three separate determinations.
[b] Least significant difference.

ing processing of *tô*. The mechanical shearing force during blending of the sample also favored solubilization of starch. Nonstarch flour components (protein, lipid, and ash) were also solubilized during *tô* preparation. The presence of ash and fiber adversely affected the functionality of starch in *tô* (Bello et al 1990).

More soluble AMY and AMP were observed in fresh *tô*, lyophilized *tô*, and flour prepared using sorghums with more corneous endosperm textures compared to those with a floury endosperm texture. More starch solubilization was also reported when flour from a corneous versus floury endosperm sorghum (Akingbala and Rooney 1987) or flour from micronized versus unprocessed sorghum (Craig and Stark 1984) was heated (<100°C) in water with gentle agitation. Similarly, higher starch solubilities were exhibited from corn with a corneous versus floury endosperm texture after heating (<100°C) in water with gentle stirring (Leach et al 1959).

Aggregation of aqueous dispersions of soluble AMY and AMP precedes the development of a gel network and crystallization of starch molecules during storage under appropriate conditions (Miles et al 1985, Ring et al 1987, Gidley 1989). Many associations can occur (AMY-AMY, AMP-AMP, AMY-AMP) between soluble and insoluble polymers under appropriate conditions (Hizukuri et al 1988, Waniska and Gomez 1992). The warm aqueous conditions of fresh *tô* apparently caused considerable starch-starch interactions. Additional starch-lipid interactions (amylose-lipid complex) may have contributed to the decreased dispersion and solubility of AMY and AMP during the aging of *tô*. This was confirmed when 0.5% sodium stearoyl lactylate was added to sorghum flour during *tô* preparation; the *tô* had a softer texture after aging and contained less soluble AMY (Bello 1989).

Some of the starch that was solubilized from sorghums with a more corneous endosperm texture retrograded during aging of fresh *tô*. Apparently, the soluble starch that retrograded contributed to the firmness of aged *tô* ($r = 0.60$, $\alpha < 0.05$) (Bello 1989). Similar results were observed when *tô* was prepared using isolated starch from sorghums with different endosperm textures (Bello et al 1990). Hence, starch solubilization and retrogradation contribute to *tô* texture.

Some intermediate or HMW AMY and AMP appears to have retrograded during the aging of fresh *tô* and probably contributed to increased firmness of aged *tô*. The soluble AMY in fresh *tô* prepared from sorghums with more corneous endosperm texture was similar in M_W to AMY from normal corn and amylomaize starches (Takeda et al 1988, 1989; Bello and Bradbury 1991). The M_W of AMY from *kuzu* and potato (Suzuki et al 1981, Hizukuri and Takagi 1984), water chestnut (Hizukuri et al 1988), and lotus (Suzuki et al 1992) starches were larger than those of the soluble AMY in fresh *tô*. Starch from the corneous endosperm of some sorghums contains AMY and AMP of higher M_W than starch from the floury endosperm (Cagampang and Kirleis 1985). The firmer textures of gels produced from the corneous endosperm starch were attributed to more starch retrogradation (Cagampang and Kirleis 1985).

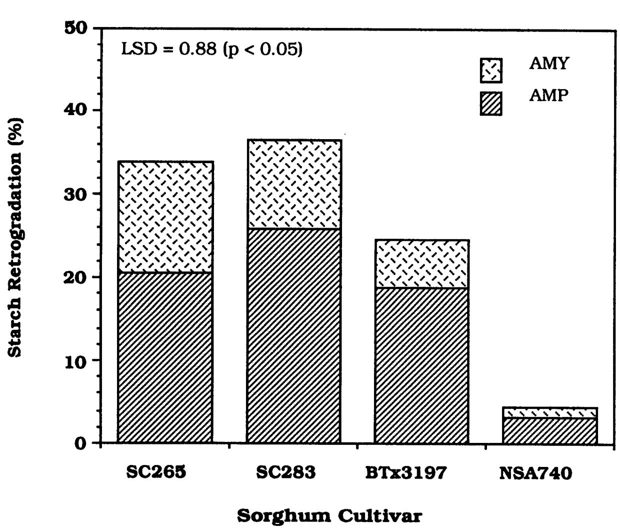

Fig. 4. Extent of retrogradation of soluble starch during aging of *tô* for 1 hr. Values are means of three separate determinations. The proportion of corneous to floury endosperm fraction of the sorghum cultivars decreased in the order of SC283 > SC265 > BTx3197 > NSA 740.

The firmer texture of aged *tô* prepared from more corneous endosperm sorgums compared to more floury sorghums suggests a faster rate of retrogradation of soluble AMY and AMP of larger M_W. Increased rates of retrogradation occurred with increased molar fraction of AMP branch chains with a degree of polymerization of 14–24, while retrogradation was not as rapid with increased molar fraction of branch chains with degree of polymerization of 6–9 (Shi and Seib 1992). Viscosity development and gel-forming properties of reconstituted AMY and AMP were affected by branch chain length (Jane and Chen 1992). Firmer gels were formed when AMP with long branch chains was reconstituted with small, intermediate and HMW AMY. Weaker gels or no gels were formed when AMP with intermediate and short branch chains were reconstituted with those amyloses (Gidley and Bulpin 1989, Jane and Chen 1992).

The results of this investigation suggest some relationship between the extent of starch solubilization and the rate of starch retrogradation during the preparation of *tô*. More starch solubilization corresponded to more starch retrogradation as measured by loss of soluble starch. More starch solubilization, more retrogradation and firmer gels were observed in *tô* prepared from sorghums with a more corneous endosperm texture. Retrogradation of soluble AMY and AMP in fresh *tô* appears to be faster for HMW than for LMW polymers. Further study is needed to better understand the possible relationships between starch molecular size (i.e., AMY and AMP branch chain length distribution) and the extent of starch solubilization and retrogradation during the preparation of *tô* from sorghums with different endosperm textures.

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