

# Slowly Digestible Starch from Debranched Waxy Sorghum Starch: Preparation and Properties

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## ABSTRACT

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Effects of debranching time, storage time, and storage temperature on production and structural properties of slowly digestible starch (SDS) were investigated. Waxy sorghum starch was hydrolyzed by isoamylase for various times (0–24 hr), and the variously debranched products were stored at –30, 1, and 30°C for 1–6 days. Optimal conditions for SDS production were isoamylase treatment for 8 hr and storage at 1°C for three days, resulting in SDS content of 27.0% in the optimum product. Microscopic observation revealed that rapidly digestible starch (RDS) and SDS were removed from the edges and surfaces of the optimum

product by  $\alpha$ -amylase digestion. Digestion conditions that removed RDS and SDS resulted in a residue with a higher transition temperature and enthalpy than raw starch on a differential scanning calorimetric thermogram. Removal of RDS alone did not cause distinct decrements of peak temperature ( $T_p$ ) and enthalpy ( $\Delta H$ ) compared with stored starch. The optimum SDS product showed an amorphous type of X-ray diffractogram. Digestive removal of RDS from the optimum product gave a residue with X-ray peaks similar to B type, which supports that it is partly crystalline. Removal of RDS and SDS gave broader peaks in the X-ray pattern.

Starch is the main source of carbohydrates in the human diet. For nutritional purposes, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), depending on the rate and extent of digestion (Englyst et al 1992).

Nutritional properties of SDS are very important for the treatment and prevention of various diseases. Elevated plasma glucose and insulin levels after a glucose load are associated with noninsulin dependent diabetes (Kraft and Nosal 1975) and cardiovascular diseases (Flodin 1986). Prolonged digestion and absorption of carbohydrates are favorable not only for the dietary management of metabolic disorders such as diabetes and hyperlipidemia (Asp 1994; Würsch 1994), but for healthy subjects due to positive effects on a number of physiological factors (Björck et al 1994). Therefore, much attention is being given to SDS as a new functional material.

RDS and SDS are measured after incubation with both pancreatic amylase and amyloglucosidase at 37°C for 20 and 120 min, respectively. RS is the starch not hydrolyzed after 120 min of incubation (Englyst et al 1992). On the other hand, Guraya et al (2001b) suggested that RDS can be measured after incubation with porcine pancreatic amylase at 37°C for 60 min and SDS can be measured to a certain time interval after which no further increases are noticed. RS is the starch residue after RDS and SDS are removed. Englyst's method, which can be used to determine the portions of starch and starch degradation products, was developed to imitate the physiological conditions of starch digestion (Englyst and Hudson 1996). However, in spite of its advantages, it has not been commonly used for evaluating RS production processes due to the complications of using the mixture of pancreatin, amyloglucosidase, and invertase (Brumovsky and Thompson 2001). In comparison to Englyst's method, Guraya et al (2001b) used only pancreatic amylase and consisted of easier steps.

Wolf et al (1999) and Guraya et al (2001b) attempted the production of SDS based on physical, chemical, and enzymatic treatments. Chemically modified common ( $\approx 27\%$  amylose), waxy, dull waxy (0% amylose), and high-amylose (50% amylose) starches

may be used for the production of SDS. Although the extent of starch digestion was significantly reduced by chemical modification, results revealed the rate of starch digestion was not affected. Therefore, chemical modification may serve as a good source of RS rather than SDS (Wolf et al 1999). Guraya et al (2001b) reported a process for making SDS products using rice starch. Cooked nonwaxy and waxy rice starch suspensions (10%, w/w) were debranched with pullulanase, followed by heating and cooling. High enzyme concentration and less debranching time decreased the amount of SDS, whereas longer times accelerated the production of RS. Furthermore, RDS production decreased inversely with that of SDS and RS.

Most structural properties and production methods for SDS, however, are not yet well understood. Our recent work (*unpublished data*) suggests that amylopectin structure, possessing a relatively high degree of polymerization of short A chains, may influence digestion rate. In addition, the SDS content of waxy rice starch was  $\approx 5\%$  higher than that of nonwaxy rice starch (Guraya et al 2001a). Therefore, waxy sorghum starch may be a good source for SDS production.

The purpose of this study was to establish optimum conditions for SDS production and to investigate structural properties of SDS using waxy sorghum starch.

## MATERIALS AND METHODS

### Materials

Korean native Suwon 5, a waxy sorghum cultivar, was obtained from the National Crop Experiment Station, Rural Development Administration, Republic of Korea. Isoamylase (cat. no. I2758) and porcine pancreatic  $\alpha$ -amylase (cat. no. A3176) were purchased from Sigma Chemical Co. (St. Louis, MO).

### Isolation of Waxy Sorghum Starch

Starch was isolated from waxy sorghum using the alkaline steeping method (Chung et al 2000). Waxy sorghum was decorticated for 4 min with a rice and barley laboratory mill (Sang Yong Machinery Co., Inchon, Korea) and soaked in water for 12 hr, then blended (1:4) with 0.02N NaOH solution for 1 min using a Waring blender. The slurry was filtered through a series of sieves (100, 200, and 300 mesh), and the starch suspensions were stored at room temperature overnight. The supernatant and the residue above the starch layer were discarded. The starch slurry was washed with distilled water until no alkali was detected by pH measurement, then dried at 40°C in a drying oven, and passed through a 100-mesh sieve.

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### Preparation of SDS and RS from Waxy Sorghum Starch

Six sample tubes with 10% waxy sorghum starch slurry (2 mL) were prepared and cooked for 1 hr in an autoclave at 100°C. The temperature of the samples was adjusted to 40°C and pH 3.8 with 6 mL of 0.1M sodium acetate buffer (pH 3.8). The cooked samples were debranched using 48 µL of isoamylase solution (1,250,000 units/mL) for various reaction times (0, 4, 8, 12, and 24 hr), autoclaved at 121°C for 30 min to stop the reaction, and stored for three days at 1°C. Cooked 10% waxy sorghum starch slurry (2 mL) was debranched for 8 hr, reheated (121°C for 30 min), and stored at -30, 1, and 30°C for three days, or at 1°C for 1, 2, 3, 4, 5, and 6 days.

To assess the structures of RDS, SDS, and RS, waxy sorghum starch was stored at 1°C for three days after cooking and debranching for 24 hr at 40°C (referred to as stored starch hereafter). This stored starch was hydrolyzed by α-amylase (10 mL, 290 units/mL [1 unit liberates 1 mg of maltose from soluble starch in 3 min]) for 1 hr at 37°C to remove RDS, and absolute alcohol was added to the sample solution to a final concentration of 80% to stop the reaction. Residue 1 was isolated by centrifugation (3,000 × g, 10 min). Residue 2 was obtained by hydrolyzing the stored starch for 10 hr to remove RDS and SDS, followed by addition of alcohol and centrifugation as described for Residue 1. The residues were freeze-dried.

### Measurement of SDS Content

SDS content was measured according to the method of Guraya et al (2001b) with a slight modification. Phosphate buffer (0.5M, pH 6.9, 15 mL) and porcine α-amylase solution (10 mL, 290 units/mL) were added to the freeze-dried sample (200 mg, wet basis) and incubated with shaking (150 rpm) for 10 hr at 37°C. The hydrolyzed sugar was measured using dinitrosalicylic acid (DNS) method with a minor modification (Kim et al 1992). Maltose concentration was determined using a standard curve of maltose content versus absorbance and %RDS =  $(A - B/C) \times 100$ ; %SDS =  $(D - A/C) \times 100$ ; %RS =  $(C - D/C) \times 100$ ; where *A* is mg of maltose produced on digestion of starch for 1 hr, *B* is mg of maltose produced on digestion of starch at time 0, *C* is total starch (in mg of maltose), and *D* is maximum mg of maltose produced on digestion of starch for 10 hr.

### Determination of Debranching Degree

Debranching degree (%) of debranched starch was determined by the equation

$$[(R_{\text{sample}} - R_{\text{waxy starch}})/(R_{\text{debranched}} - R_{\text{waxy starch}})] \times 100$$

where,  $R_{\text{sample}}$  is reducing sugar in sample debranched for a specific time,  $R_{\text{debranched}}$  is reducing sugar in waxy starch debranched for 24 hr, and  $R_{\text{waxy starch}}$  is reducing sugar in raw waxy starch.

Reducing sugar content was measured using the DNS method. Briefly, an aliquot of 0.5 mL of 1% (w/v) sample was pipetted into a test tube, and 0.5 mL of DNS solution was added. The solution was boiled for 5 min and cooled immediately under running tap water. The absorbance was measured at 575 nm. Maltose was used as a standard in both analyses, and all analyses were performed in triplicate.

### Microscopic Observations

Structural properties of the SDS sample were studied using a scanning electron microscope (JSM 5410LV, JEOL Ltd., Tokyo, Japan). Dried, finely ground sample was placed on double-stick tape mounted on aluminum specimen holder, coated with a thin film of gold (30 nm), and examined at 20 kV.

### Differential Scanning Calorimetry (DSC)

Thermal properties of the SDS sample were investigated using a DSC 120 (Seiko, Chiba, Japan). Indium was used as a calibration standard. A 5-mg sample was weighed in a high-pressure stainless

steel pan to which 15 µL of distilled water was added. The sample pan was sealed and kept at room temperature for 1 hr and heated from 30 to 140°C at 5°C/min. Al<sub>2</sub>O<sub>3</sub> was used as a reference.

### X-ray Diffraction

X-ray diffraction analysis was performed using a Dip 2030 (Indigo 2, Oxford model HX-200w/c, Mac Science, Tokyo, Japan) X-ray diffraction image processor (50kV and 90mA, CuK radiation  $\lambda = 1.541$ , nickel filter). The sample, freeze-dried and kept at room temperature, was scanned through 2θ ranging from 3 to 30°.

### Statistical Analysis

All samples were analyzed in triplicate, and statistical treatment was done using SAS software (v. 8.1, SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

### Changes in SDS Content Depending on Preparation Conditions

Debranching time dependence of SDS formation was examined, and SDS contents of debranched waxy sorghum starch suspensions are presented in Table I. SDS content increased as the debranching time increased, reaching maximum at 8 hr (62.5% debranching degree), and decreased thereafter, whereas the RS content increased. This trend is similar to the result of waxy rice by Guraya et al (2001b) in which SDS content was the highest in the sample debranched for 4 hr. Samples debranched more than 4 hr in this study displayed decreased SDS and increased RS contents.

Storage at different temperatures resulted in differences in the compositions of the starches. Debranched (8 hr) starch stored for three days at -30°C was composed of 82.7 RDS, 5.4 SDS, and 11.9% RS; results at 30°C were 42.0 RDS, 11.3 SDS, and 46.7% RS. Storage of debranched starches at 1°C for three days resulted in 40.9% RDS, 27.0% SDS, and 32.1% RS, showing the highest amount of SDS. Maximum RDS and RS contents were obtained from the starches held at -30 and 30°C, respectively.

As the storage time at 1°C increased up to three days, the amount of SDS increased (Table II). In samples stored more than three days, no marked change in the amount of SDS was observed, although a decrease in RDS and an increase in RS content were detected with increasing storage time.

TABLE I  
Effect of Debranching Degree on SDS Production

Samples <sup>a</sup>	RDS (%)	SDS (%)	RS (%)
0-DB	81.5 ± 0.7	7.1 ± 1.1	11.2 ± 0.9
4-DB	58.3 ± 2.0	24.1 ± 1.5	17.6 ± 0.4
8-DB	40.9 ± 0.3	27.0 ± 0.1	32.1 ± 1.2
12-DB	14.3 ± 2.3	24.7 ± 0.5	61.0 ± 1.4
24-DB	15.9 ± 0.8	9.7 ± 0.6	74.4 ± 1.7

<sup>a</sup> 0-DB nondebranched starch; 4-DB starch debranched for 4 hr, average degree of debranching 31.4%; 8-DB starch debranched for 8 hr, average degree of debranching 62.5%; 12-DB starch debranched for 12 hr, average degree of debranching 82.7%; 24-DB starch debranched for 24 hr, average degree of debranching 100%. Values are averages of three determinations. All samples were stored at 1°C for three days.

TABLE II  
Effect of Storage Time on SDS Production

Samples <sup>a</sup>	RDS (%)	SDS (%)	RS (%)
1d-Starch	64.0 ± 1.0	15.0 ± 1.9	21.0 ± 0.6
2d-Starch	46.0 ± 2.3	24.0 ± 1.4	30.0 ± 1.7
3d-Starch	43.0 ± 1.1	27.4 ± 0.8	29.6 ± 1.5
4d-Starch	43.0 ± 1.4	27.0 ± 2.0	30.0 ± 1.2
5d-Starch	41.9 ± 0.2	26.7 ± 0.8	31.4 ± 1.0
6d-Starch	40.0 ± 1.5	26.0 ± 1.3	34.0 ± 2.4

<sup>a</sup> 1d-6d, Starch stored for 1-6 days. All samples were debranched for 8 hr and stored at 1°C. Values are averages of three determinations

These results may be explained through three sequential steps for the crystallization of crystallizable substances. Crystallization stages can be classified into nucleation, propagation, and maturation (Morris 1990). The rate of crystal nucleation approaches 0 at  $T_m$  (temperature of melting) and is maximum at  $T_g$  (glass transition

temperature), while the rate of crystal growth approaches 0 at  $T_g$  and is maximum at  $T_m$ . The net rate of crystallization is obtained at  $T = (0.5)(T_g + T_m)$ , generally close to room temperature (Morris 1990; Eerlingen et al 1993). Our results showed that storage of debranched starch at 30°C formed maximum RS as expected. Storage at 1°C promotes nucleation, but not propagation and maturation. Because propagation is a diffusion-controlled phenomenon, it would be close to zero at refrigeration temperature (Guraya et al 2001b). Slade and Levine (1987) suggested that low-temperature storage results in recrystallization to lower  $T_m$  in retrograded wheat starch gels, less symmetrically perfect polymorphs than those produced by storage at room temperature. Therefore, the SDS fraction could be imperfect crystallites with lower density as suggested by Guraya et al (2001b).

### Microscopic Observations

Cooked and cooked-debranched samples showed that the granular structure disappeared, and bigger, irregularly shaped particles with a continuous spongy-like porous network were observed, which was probably due to freeze drying (data not shown). Stored starch showed plate-like and smooth surfaces different from cooked and cooked-debranched samples, which is probably due to the method of drying and storing the sample (Fig. 1A). Residue 1 showed that edge of the stored starch structure was broken off, revealing small pores on the surface, presumably due to the enzymatic digestion (Fig. 1B). Residue 2 appeared to have somewhat more angular and pitted surfaces than Residue 1. Edges of the residues were digested away and fissures could be observed. In addition, the surface was extensively pitted by enzymatic digestion (Fig. 1C). It is generally acknowledged that  $\alpha$ -amylase preferentially attacks amorphous regions of the starch, and solid regions are less accessible and are hydrolyzed at a slower rate (Vasanthan and Bhatta 1996). Therefore, RDS is mostly amorphous and SDS has a more ordered structure than RDS.

### Differential Scanning Calorimetry

Thermal properties of raw waxy starch and cooked-debranched starch fractions are summarized in Table III. The gelatinization endotherm of the raw waxy starch consisted of two peaks. The first peak was the main peak, while the second peak appeared as a shoulder of the first peak. Similar patterns were also observed in raw rice starch, waxy maize, and waxy barley (Jacobs et al 1995). The  $T_p$ ,  $T_c$ ,  $T_c - T_o$ , and  $\Delta H$  values of stored starch were greater than those of raw starch due to the production of linear chains during isoamylase digestion. In general, the  $T_m$  of amylopectin crystallites is lower than that of amylose crystallites (Karim et al 2000). Thus, branched crystallites are less stable than linear crystallites. In addition, unlike raw starch,  $T_p$  of cooked starch fractions showed a single endothermic peak, which implies that crystalline structure of the raw starch granule was disrupted during cooking then recrystallized into a single crystalline during cooling.

The  $T_p$ ,  $T_c$ ,  $T_c - T_o$ , and  $\Delta H$  values of the stored starch were higher than those of Residues 1 and 2. In Residue 1, no distinct decreases in  $T_p$  and  $\Delta H$  were observed, whereas dramatic decreases

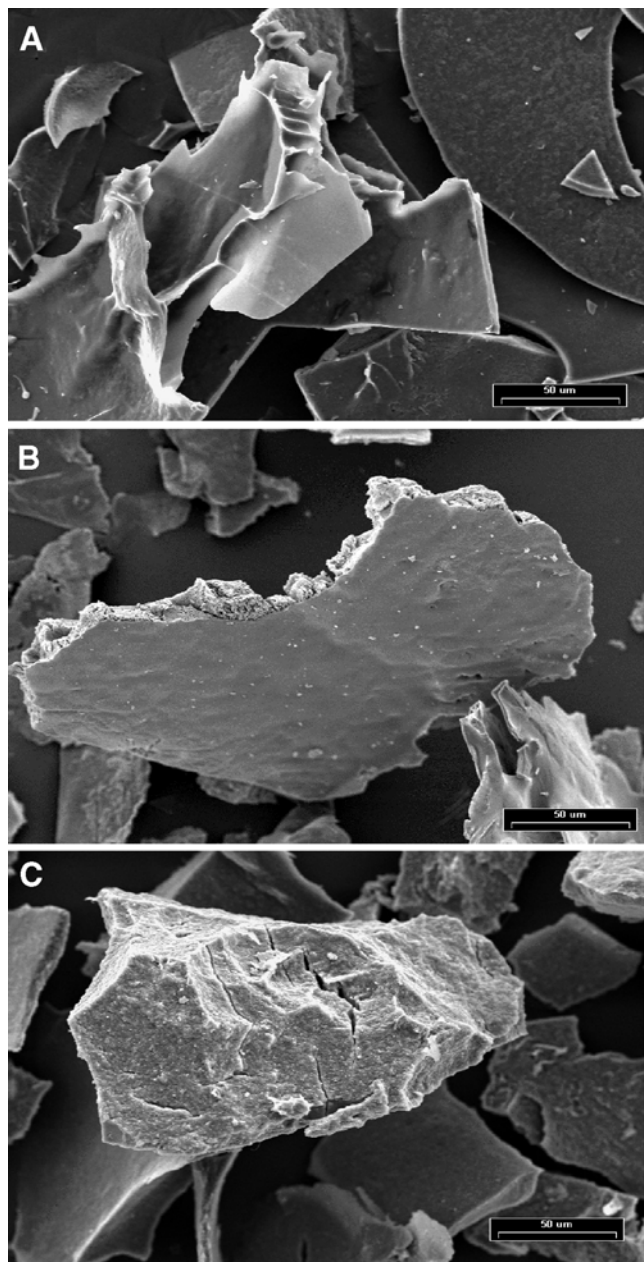


Fig. 1. Scanning electron micrograph of stored starch (A), Residue 1 (B), and Residue 2 (C).

TABLE III  
Thermal Properties of Starch Fractions<sup>a</sup>

Samples	$T_o$ (°C)	$T_p$ (°C)	$T_c$ (°C)	$T_c - T_o$	$\Delta H$ (mJ/mg)	
Raw starch	68.9 ± 0.9	79.0 ± 0.5 <sup>b</sup>	89.3 ± 2.7 <sup>c</sup>	106.6 ± 0.2	37.7 ± 0.8	9.1 ± 0.8
Stored starch <sup>d</sup>	67.1 ± 1.7	94.8 ± 1.5	116.3 ± 2.0	49.2 ± 1.5	13.8 ± 2.1	13.8 ± 2.1
Residue 1 <sup>e</sup>	68.0 ± 0.5	93.9 ± 2.1	116.3 ± 0.3	48.3 ± 1.0	13.0 ± 0.9	13.0 ± 0.9
Residue 2 <sup>f</sup>	68.5 ± 1.2	87.8 ± 1.2	116.2 ± 3.0	47.7 ± 3.8	10.7 ± 0.8	10.7 ± 0.8

<sup>a</sup> Values are averages and standard deviations of three determinations.

<sup>b</sup> Main peak.

<sup>c</sup> Shoulder peak.

<sup>d</sup> Waxy sorghum starch stored at 1°C for three days after cooking-debranching.

<sup>e</sup> Stored starch hydrolyzed by  $\alpha$ -amylase for 1 hr.

<sup>f</sup> Residue 1 hydrolyzed by  $\alpha$ -amylase for 9 hr.

were observed for Residue 2. Barichello et al (1990) suggested that  $T_p$  is an indication of structural stability and resistance to gelatinization. The enthalpy of gelatinization reflects the loss of double helical order rather than crystalline order (Cooke and Gidley 1992).

The differences in  $T_c - T_o$  may be due to the presence of crystallines, which are composed of small crystallites, each possessing slightly different crystal strength (Vansanathan and Bhatta 1996). Therefore, cooked-debranched starch probably produced several different types of crystallites during storage. Some of these crystallites were successively hydrolyzed from the amorphous region by  $\alpha$ -amylase.

Based on the results of DSC, the first hydrolyzed fraction was inferred to be RDS, which is mostly amorphous. Following the amorphous region, a less perfect crystallite was hydrolyzed through enzyme digestion, as supported by a significant difference in enthalpy between Residue 2 and Residue 1. This indicates that double helices of Residue 1 were removed during the enzyme digestion, and this residue had a less ordered structure than Residue 2. Residue 1 included SDS, which possibly consisted of less perfect crystallites and amorphous components.

Residue 2 could be RS; however, this result does not agree with those of previous reports (Sievert and Pomeranz 1990; Szczodrak and Pomeranz 1991; Sievert and Wüsch 1993). In general, endothermic transition temperature of retrograded RS is  $>140^\circ\text{C}$  (Sievert and Pomeranz 1990; Szczodrak and Pomeranz 1991; Sievert and Wüsch 1993). However, in our study the transition temperature was determined to be  $\approx 88^\circ\text{C}$ . This difference is presumably due to the differences in the levels of retrograded amylose. For retrograded RS production, amylo maize containing  $\approx 70\%$  amylose is generally the starting material (Sievert and Pomeranz 1989, 1990; Czuchajowska et al 1991; Guchala and Pomeranz 1993). Also, it could be due to the differences in the analytical method, the conditions of RS formation, and the starch source. For example, RS isolated by the TDF method showed a higher thermal stability than that isolated by the Englyst method (Brumovsky and Thompson 2001). Sievert and Pomeranz (1990) reported that repeated autoclaving-cooling of starch produced thermally very stable RS. This treatment, performed under more drastic conditions than ours, might have led to a higher transition temperature.

### X-ray Diffraction

X-ray diffraction patterns of raw starch, cooked starch, cooked-debranched starch, stored starch, and Residues 1 and 2 are displayed in Fig. 2. Raw starch showed an A-type pattern as indicated by typical peaks at  $11.5^\circ$ ,  $15.3^\circ$ ,  $17.5^\circ$ , and  $23^\circ$  (Planchot et al 1997), whereas cooked and cooked-debranched starches did not show any peaks, mostly due to the fact that they are mainly composed of amorphous region. Furthermore, the X-ray diffraction pattern of stored starch was similar to those of cooked and cooked-debranched starches. These results suggest that stored starch contained amorphous regions to a significant extent, which is reflected as background on the X-ray diffractogram of stored starch (Nara and Komiya 1983). Also, the enthalpy of Residue 1 showed no significant change in DSC thermogram compared with stored starch (Table III), which indicated that enzyme hydrolysis removed mainly amorphous region rather than crystalline region. X-ray diffraction and DSC results implied that RDS consisted of mostly amorphous components and existed as an exposed part of the optimum SDS product.

Compared with Residue 1, X-ray diffractogram of Residue 2 showed two broad peaks, one ranging from  $\approx 13^\circ$  to  $18^\circ$  and the other from  $\approx 22^\circ$  to  $26^\circ$ . Also, the enthalpy of Residue 2 was lower than that of Residue 1 and stored starch (Table III). These results indicated that enzyme hydrolysis occurred not only in an amorphous region but also in a crystalline region. Therefore, SDS appeared to contain amorphous components and double helical components with partially ordered structure.

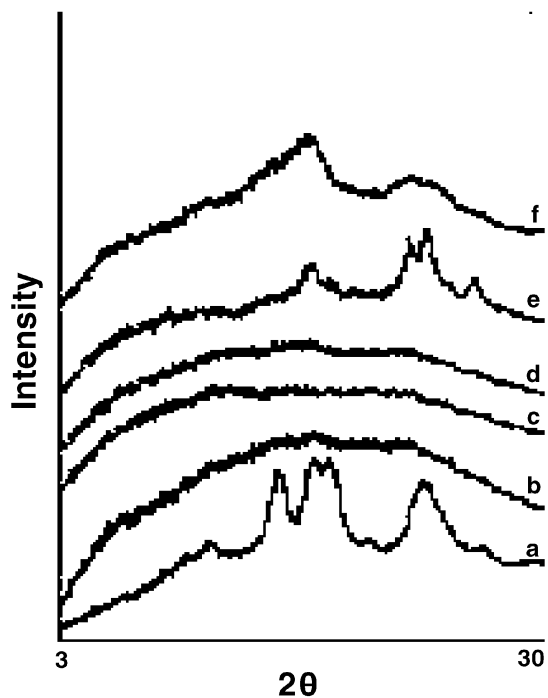


Fig. 2. X-ray diffraction patterns of raw waxy sorghum starch (a), cooked waxy sorghum starch (b), cooked-debranched waxy sorghum starch (c), stored starch (d), Residue 1 (e), and Residue 2 (f).

### CONCLUSIONS

The optimal conditions for SDS production from waxy sorghum starch were debranching with isoamylase for 8 hr followed by storage at  $1^\circ\text{C}$  for three days. DSC analysis showed that removal of RDS from stored starch to give Residue 1 did not cause significant decrements of  $T_p$  and  $\Delta H$ , whereas removal of RDS and SDS to give Residue 2 resulted in dramatic decrements. The X-ray diffraction pattern of stored starch was mostly amorphous, while the crystallinity of Residues 1 and 2 increased. However, the peaks in Residue 2 were much broader than those in Residue 1. These results indicate that RDS and SDS mainly consist of amorphous regions, but SDS also has a small portion of imperfect crystallites.

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