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RESEARCH NOTE

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Molecular Structure of Sorghum and Waxy Sorghum Starches

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Abstract Amylose contents and amylopectin chain architecture of sorghum and waxy sorghum starches were determined and compared with those of other common cereal and tuber starches. Also, *in vitro* digestibility of sorghum starch was estimated using a novel methodology. The absolute amylose content of sorghum starch was similar to that of corn and wheat starches. The side chain length distribution patterns for sorghum and waxy sorghum amylopectin were very similar to those of corn and waxy corn, respectively. The k_{cat}/K_m values for sorghum and potato amylopectin did not show a significant difference. The kinetic parameters could be used as novel indicators for starch digestibility.

Keywords: sorghum starch, waxy sorghum starch, amylopectin side chain length distribution, amylose content, starch digestibility

Introduction

Starch is the dominant component of reserves in cereal and some root crops. Two types of glucan polymers, amylopectin and amylose compose starch molecules. Amylopectin is a highly branched polymer, consisting of α(1,4)-linked linear glucans branched with α(1,6)-linkages. Another component, amylose is a primarily α(1,4)-linked linear glucan with fewer branches. The ratio of amylose to amylopectin and the structure of amylopectin affect the functional properties of starches and their products. It is widely known that starches contain 20-30% amylose and 70-80% amylopectin, but the exact ratio varies with their botanical sources. The amylose-to-amylopectin ratio of starch greatly affects the starch functional properties. In most starches, amylopectin is the major component and its fine structure is reported to be related to important characteristics of starch such as crystalline structure, gelatinization, retrogradation, and pasting properties (1-6).

Sorghum (*Sorghum bicolor* L. Moench) is an important food cereal especially in Africa, Asia, and the semi-arid tropics worldwide. Due to its drought-resistant characteristic, sorghum is cultivated as a staple food crop in these regions, providing a principal source of energy, protein, vitamins, and minerals for millions of people living in these regions (7). Sorghum is the 5th important cereal crop throughout the world, and its utilization become increasing not only for food but also for animal feed and other purposes such as wax production (8,9).

Sorghum is known to have a characteristic of poor digestibility, because of its grain organization and interactions between protein and other components (7). There has been a significant amount of work conducted on the protein digestibility of sorghum in comparison with other cereals (7). Also, its positive effect on cholesterol metabolism has been studied (10). However, very little work has been carried out on the characteristics and properties of sorghum starch. Therefore, generating information on sorghum starch will help better understanding of the physicochemical properties of sorghum products in relation to its nutrient availability.

In this study, amylose contents, amylopectin chain length distributions, and average amylopectin chain length of sorghum and waxy sorghum starches were determined and compared with those of other common cereal and tuber starches. Also, a novel methodology to evaluate *in vitro* starch digestibility was proposed and the digestibility of sorghum starch was estimated according to the method.

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Materials and Methods

Starches Sorghum and waxy sorghum starches were provided by Dr. Bruce Hamaker's laboratory in the Department of Food Science, Purdue University, IN, USA. Potato, sweet potato, wheat, corn, and waxy corn starches were provided by Samyang Genex Co. (Seoul, Korea) and rice starch was provided by Bangkok Interfood Co. (Bangkok, Thailand).

Enzymes and reagents Debranching enzyme (isoamylase) was purchased from Hayashibara Biochemical Laboratories Inc. (Okayama, Japan). Saccharification enzyme (amyloglucosidase) was purchased from Novozyme Co. (Copenhagen, Denmark). Porcine pancreatic α-amylase (A-4268) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All the reagents used in this study were analytical grade, which were purchased from Sigma-Aldrich Co.

Measurement of amylose contents Amylose content of various starches was determined colorimetrically according to the method of Juliano (11). Five mL of starch solution was pipetted into a 100 mL volumetric flask, and 1 mL of 1 N acetic acid and 2 mL of iodine solution (0.2 g iodine and 2.0 g potassium iodine in 100 mL of aqueous solution) were added. The volume of the solution was brought to 100 mL with distilled water. The solution was well-shaken and left to stand for 20 min. Absorbance of the solution at 620 nm was measured using a spectrophotometer (Ultrospec 4000; Amersham Bioscience, Buckinghamshire, UK). Amylose content was determined by referring to a standard curve previously obtained with potato amylose of varied contents.

Fractionation of amylopectin Amylopectin was separated from amylose according to Jane and Chen (3). A starch suspension (1.33%, w/v, in water) was heated and stirred in a water bath at 96°C until starch was fully gelatinized. The starch solution was then filtered through 5.0 μm disposable syringe filter to remove insoluble residues. The pH of the solution was adjusted to 5.9-6.3 with a phosphate buffer and the solution was autoclaved at 121°C for 3 hr. The flask containing the starch solution was stirred in a water bath at 96°C for 2 hr to disperse starch molecules. *n*-Butyl alcohol (20% by volume) was added, and the solution was stirred at 96°C for 1 hr. The mixture was transferred to a pre-warmed flask, sealed, and allowed to cool down to room temperature over 24-36 hr. An amylose-butylalcohol complex was formed during cooling. The crude amylose-butylalcohol complex was separated by centrifuging (5°C, 8,700×g, 30 min). The amylopectin remaining in the supernatant was concentrated with a rotary evaporator and then treated twice with *n*-butyl alcohol to remove amylose residues. The solution was further concentrated and precipitated with methyl alcohol.

Analysis of chain length distribution of amylopectin For the analysis of amylopectin chain length distribution, amylopectin solutions were incubated with a debranching enzyme, isoamylase (Hayashibara Biochemical Laboratories Inc.) 0.36 U/mg, in 25 mM NaOAC buffer (pH 4.3) at 60°C for 63 hr. The reaction mixture was boiled for 5 min to stop the debranching reaction. The amylopectin chain length distribution was analyzed by using high-performance anion-exchange chromatography (HPAEC) system (Dionex-300; Dionex, Sunnyvale, CA, USA) with an electrochemical detector (ED40; Dionex). A CarboPac^{TM} PA-1 anion-exchange column (250×4 mm, Dionex) and a guard column were used for the separation of debranched samples. After column was equilibrated with 150 mM NaOH, the sample was eluted with varied gradients of 600 mM sodium acetate in 150 mM NaOH at a flow rate of 1 mL/min. The applied gradients of sodium acetate was as follows: a linear gradient 10-30% for 0-10 min, 30-40% for 10-16 min, 40-50% for 16-30 min, 50-60% for 30-52 min, 60-65% for 52-82 min.

Measurement of average amylopectin chain length The average side chain length of amylopectin was calculated by dividing the total glucose content by the number of reducing end. The number of reducing end of debranched amylopectin was measured using copper-bicinchoninate method (12). The reaction with the addition of boiled enzyme was used as a blank. Two-hundred μL of reaction mixture was added to an equal volume of copperbicinchoninate working agent (solution A: 97.1 g of disodium 2,2'-bicinchoninate, 3.2 g of sodium carboninate monohydrate and 1.2 g of sodium bicarboninate in 50 mL water, solution B: 62 mg of copper sulfate pentahydrate and 63 mg of L-serine dissolved in 50 mL of water) and incubated in a water bath at 80°C for 30 min. The sample was then cooled for 5 min and the absorbance was measured at 570 nm using an EL340 automated microplate reader. The total glucose content was measured after isoamylase and amyloglucosidase treatment using glucose oxidase/peroxidase method with slight modification (12). The reaction mixture contained 100 μL reaction mixture, 500 μL of 50 mM acetate buffer (pH 4.5), amyloglucosidase (AMG, Novozyme, Denmark) 50 μL, and double distilled water 350 μL. The mixture was incubated at 55°C for 4 hr. The reaction was stopped by boiling for 5 min. The 40 μL reaction mixture was then quenched with 360 μL of glucose oxidase reagent from the glucose-kit (Asan Pharmaceutical Co., Seoul, Korea). This was then incubated at 37°C for 30 min and absorbance was measured at 505 nm. The amount of glucose was calculated from a glucose calibration curve. The equivalent of hydrolyzed glycosidic bonds was quoted as the glucose equivalents using a maltose equilibration curve.

Kinetic studies on amylopectin digestion The kinetic parameters of separated amylopectin were measured as follows. Porcine pancreatic α-amylase (0.07 mL) in an appropriate concentration was mixed with substrates (0.7 mL) at various concentrations in 50 mM sodium phosphate buffer (pH 7.0) with 0.9% sodium chloride. The reaction was carried out at 37°C. To analyze the kinetic properties of the enzymes, aliquots (0.07 mL) of α-amylase reaction mixture were taken at every 30 sec for 4 min. After adding the same volume of 0.1 M NaOH solution to stop the reaction, reducing sugar formed during the reaction was measured using the copper-bicinchoninate method (12). One unit for hydrolyzing the substrate was defined as the amount of enzyme producing 1 mM of maltose per min. Kinetic parameters were determined using Lineweaver-Burk plot.

Results and Discussion

Amylose contents of various starches Amylose contents of sorghum and other common starches measured using iodine affinity method are presented in Table 1. Generally, the measured values appeared to be overestimated compared to those from literature (3,4). Jane *et al.* (13) have reported the similar values for the overestimated 'apparent' amylose content measured using iodine affinity method. They attributed the discrepancy to the iodine complex formation with long chain amylopectin as well as amylose. The discrepancy was roughly proportional to the amount of long chain amylopectin (DP >36). According to their suggestion, it was concluded that absolute amylose content of sorghum starch was similar as that of corn and wheat starches, because they showed similar values of measured

Table 1. Analysis of molecular structure of various starches

	Amylose content (%)	% Distribution				Average chain length
		A	B1		B2	
		DP 6-12	DP 13-24	DP 25-36	DP >36	
Potato	33.69±1.86	18.82	51.03	14.86	14.48	28.68±1.45
Corn	30.91±1.84	24.48	54.92	11.93	7.37	24.93±0.91
Wheat	30.57±2.82	29.80	50.72	10.72	7.29	27.39±1.26
Sorghum	30.43±1.59	23.88	56.01	11.60	7.61	24.34±1.93
Sweet potato	28.86±1.35	22.82	53.82	13.83	8.96	26.43±0.54
Rice	26.15±1.18	26.39	56.55	9.66	6.46	22.30±0.40
Waxy sorghum	-	22.58	56.16	14.66	5.82	22.11±0.82
Waxy corn	-	21.65	53.59	17.48	6.59	23.22±1.67

amylose contents and the amount of amylo-pectin long chains (DP >36). The reported absolute amylose contents of corn and wheat are around 22 and 25%, respectively (3,4).

Molecular structure of amylopectin In the traditional classification, A-chains are defined as unsubstituted, whereas B-chains are substituted by other chains. The macromolecule contains one C-chain possessing single reducing end. The B-chains are further subdivided into short B1-chains, existing within cluster units, whereas B2-chains are long chains that span over two or more clusters, thereby interconnecting them (14). The amylopectin side chain length distribution of various starches was analyzed

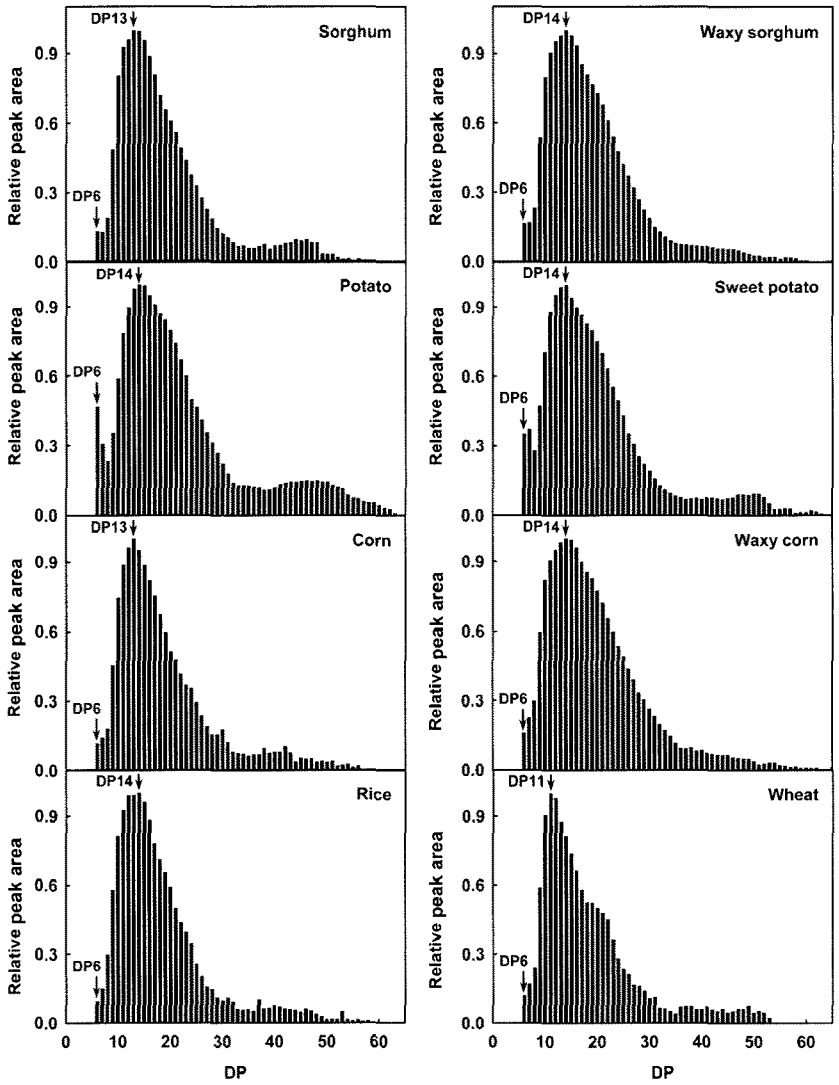

Fig. 1. HPAEC analysis of amylopectin side chain length distribution of various starches.

Table 2. Kinetic parameters of porcine pancreatic α-amylase for potato amylopectin and sorghum amylopectin

	K_m (mg/mL)	k_{cat} (1/sec)	k_{cat}/K_m (mL/sec · mg)
Potato	0.32	1,625.18	5,036.48
Sorghum	0.24	1,187.99	4,863.48

using HPAEC and the relative proportion of each chain was estimated as shown in Fig. 1. Each starch showed its characteristic distribution pattern. Potato and sweet potato had more long chains (DP >36) and DP 6 chain compared to other cereal starches, which is consistent with the result of Jane *et al.* (13). Interestingly, the side chain length distribution pattern for sorghum and waxy sorghum amylopectin appeared to be very similar to that of corn and waxy corn, respectively.

The structure of amylopectin was analyzed by classifying chains into 4 groups by chain length such as DP 6-12 (A or B1 chains), DP 13-24 (B1 chain), DP 25-36 (B1 chain), and beyond DP 36 (B2 chain), according to Jane *et al.* (13). Rough assignment of A, B1, or B2 chains were followed by Hizukuri (15). Even though the percentage values in Table 1 were simply obtained from peak areas, thus did not reflect absolute contents, it provided useful information in investigating variations of chain components among different starches. Potato starch showed the highest B2 chain content and the lowest A chain content among starches. Waxy sorghum showed the lowest B2 chain content. Again, the relative content of each group for sorghum and corn amylopectin, and waxy sorghum and waxy corn amylopectin were similar.

Average chain length The average side chain length of amylopectin was calculated by dividing the total glucose content by the number of reducing end. The average chain length of various amylopectin data are shown in Table 1. Waxy sorghum has the shortest average chain length with DP 22 among the tested starches and the average chain length of sorghum, waxy sorghum, corn, waxy corn, and rice was in the relatively short range of DP 22.1-24.0. On the other hand, potato has the longest average chain length with DP 28.7. The average chain length measured generally correlated with the relative proportion of amylopectin long chains.

Based on the relative content of side chains in each group and the average chain length estimated in Table 1, it could be proposed that potato starch has a coarse structure with relatively long chains while sorghum and waxy sorghum have a more packed structure with shorter chains. Physicochemical properties of starches might be influenced by the proposed structure of the amylopectins.

Digestibility To identify a relationship between starch digestibility and side chain distribution, potato and sorghum amylopectin, because of their contrastive side chain distribution, were selected for experiment. To measure the rate of amylopectin digestion α-amylase from porcine pancreas was incubated with potato and sorghum amylopectin at 37°C. The values of K_m and k_{cat} values are listed in Table 2. The K_m, k_{cat}, and k_{cat}/K_m values for potato amylopectin were 0.32 mg/mL, 1,625.18/sec and 5,036.48 mL/sec · mg, respectively, while those for sorghum amylo-pectin were 0.24 mg/mL, 1,187.99/sec and 4,863.48 mL/sec · mg. The k_{cat}/K_m values for sorghum and potato did not show a significant difference.

Acknowledgments

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