Structures and Physicochemical Properties of Starches from Acha (*Digitaria exilis*), Iburu (*D. iburua*), and Tamba (*Eleusine coracana*)

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ABSTRACT

Structures and physicochemical properties of starches from three African cereal grains, acha (*Digitaria exilis*), iburu (*D. iburua*), and tamba (*Eleusine coracana*), were determined. The starches were fractionated into amylose and amylopectin. The amylose contents (%), determined from iodine affinity, of acha (18.7), iburu (19.6), and tamba (19.8) starches were similar to those reported for rice (17–19), wheat (21.7), and corn (21.5) starches. The values of iodine affinity (IA) (g/100 g), blue value, and λ_{max} (nm) obtained for the three starches fall within the range reported for most cereals. Analyses of reducing and nonreducing residues showed that the amyloses of acha, iburu, and tamba were small molecules with number-average degree of polymerization (DP_n) 1,040, 1,120, and 1,420, respectively, each with an average of six chains. Acha amylose had the highest amount of branched molecule (≈79 mol%) with lower average number of chain (NC ≈7) than those of others (53–34 mol%, NC ≈10–13). The amylopectins from the three cereals had IA values of 1.3–1.5 g/100 g, average chain lengths of 20–21, and β-amylolysis limits of 60–62%, which are similar to those of the rice and corn amylopectins. Rapid viscograph analysis (RVA) at 9% (w/w) starch suspension of acha, iburu, and tamba showed a lower peak pasting viscosity and considerably lower breakdown than did the RVA of corn starch. Unlike the other starches, the viscosity of tamba starch did not change during the hold at 92.5°C. Granules from the three starches were of the A crystalline type, as in those of rice, wheat and corn starches, with diameter in the 2.0–14.3 μm range.

Starches from various plant sources, such as wheat, maize and rice, have received extensive attention in relation to structural and physicochemical properties (Hizukuri and Maehara 1990, Suzuki et al 1992, Takeda and Preiss 1993). Molecular structures of amylose and amylopectin from several sources have been analyzed in detail by newly developed methods. Similar studies are scanty on cereal starches from developing countries. Accumulating studies on the structures of amylose and amylopectin isolated from starches by established methods (Lansky et al 1949, Takeda 1993) have shown that the components from different plant origins are unique in structure (Takeda et al 1987).

Acha (*Digitaria exilis*), iburu (*D. iburua*), and tamba (*Eleusine coracana*, a Nigerian variety of finger millet) (Rachie and Peters 1977), are three underutilized African cereal grains. The properties of acha and iburu have received some attention (Carbiener et al 1960; de Lumen et al 1993; Jideani et al 1994a,b) due to some outstanding nutritional properties. Two sulphur-amino acid-rich proteins in acha have been identified and their N-terminal sequences obtained (de Lumen et al 1993). The proteins in acha have also been characterized by electrophoresis and gel-permeation chromatography (GPC) (Jideani et al 1994b). The carbohydrates of acha, iburu, and tamba have not been rigorously investigated. Preliminary investigation on some physical and chemical properties of acha and iburu flours and starches have been reported (Jideani and Akingbala 1993). Acha and iburu starches are typical of nonwaxy cereal starches with a two-stage swelling property. There is need for detailed information on the structure and physicochemical properties of starches from these cereal grains to further their use for food and other purposes. An understanding of the various physical and functional properties of amylose and amylopectin requires elucidation of their molecular structures. The present investigation was initiated to determine the structure and physicochemical properties of starch from acha, iburu, and tamba, including isolation of starch, fractionation into amylose and amylopectin, and characterization of structures and physicochemical properties of starch and its components. The structures of amyloses and amylopectins from acha, iburu, and tamba, as well as some physicochemical properties are presented.

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MATERIALS AND METHODS

Materials

The three grain samples, acha (A94), iburu (I94), and tamba, were purchased from Jos market in Nigeria. Starch was prepared from each grain by a conventional method in the laboratory (Takeda et al 1983) involving alkaline steeping to soften the protein-starch matrix and to denature enzymes. Samples of different acha (A85) and iburu (I85) starches from an initial investigation (Jideani and Akingbala 1993), prepared by steeping in acid solution, were analyzed along with fresh starch preparations. Corn starch was used as a reference in some of the analyses because it remains the dominant source of cereal starch (Seib 1994). β-Amylase was prepared (Takeda and Hizukuri, 1969) from sweet potato and recrystallized from aqueous ammonium sulfate to improve its stability on storage. Crystalline grade of isoamylase from *Pseudomonas amyloderamosa* was obtained from Hayashibara Biochemical Laboratories Ltd. (Okayama, Japan). Toyopearl HW-75F was obtained from Tosoh Co. Ltd. (Tokyo, Japan). All chemicals of the highest grade available were purchased from Wako Pure Chemicals Ind. (Osaka, Japan).

Fractionation of Amylose and Amylopectin

Starches (A94, A85, I94, I85, and tamba) were defatted by three replicates of dissolution in hot dimethyl sulfoxide solution and precipitation with ethanol to remove trace amounts of lipids, that interfere with complete dispersion. Fractionation of defatted starch (10 g) into amylose and amylopectin was performed by the method of Lansky et al (1949) with modifications (Takeda et al 1986a). Amyloses were purified by ultracentrifugation, followed by repeated recrystallization from 10% aqueous 1-butanol. Ultracentrifugation was required to remove microgel-like amylopectin (MGA) from amylose. Significant quantities of MGA were obtained from A85 and tamba starches.

The purity of amylose was confirmed by GPC on Toyopearl HW-75F. After removal of amylose precipitates by centrifugation, supernatant solutions of the amylopectin fractions (Ap fractions) were concentrated to 0.10 volume in a rotary evaporator at 40–45°C and the Ap fractions were precipitated by addition of a threefold volume of ethanol, washed with ethanol and ether, and dried over $CaCl_2$. β-Limit dextrin (β-LD) of amylose was prepared as previously described (Takeda et al 1987).

General Analytical Methods

The iodine affinity (IA, g/100 g) was determined by the amperometric titration method of Larson et al (1953) with modifications (Takeda et al 1987). The blue value (absorbance at 680 nm of iodine-glucan complexes) and λ_{max} (the maximum wavelength of iodine-glucan complexes) were determined as previously described (Takeda et al 1983). Carbohydrate and reducing terminii of debranched amylopectin were determined by the phenol-sulphuric acid method (Dubois et al 1956) and the Somogyi method (Somogyi 1952), respectively. The latter used the colorimetric reagent of the method described by Nelson (1944), but the heating time with the Somogyi reagent was extended to 30 min (Hizukuri et al 1970). The reducing and nonreducing terminal glucosyl residues were determined by the modified Park-Johnson method and the rapid Smith-degradation method (Hizukuri et al 1981), with minor modifications (Takeda et al 1984), respectively. The number-average degree of polymerization (DP_n) and average chain length (CL) values were (carbohydrate equivalent to glucose)/(reducing terminus) and (carbohydrate equivalent to glu-

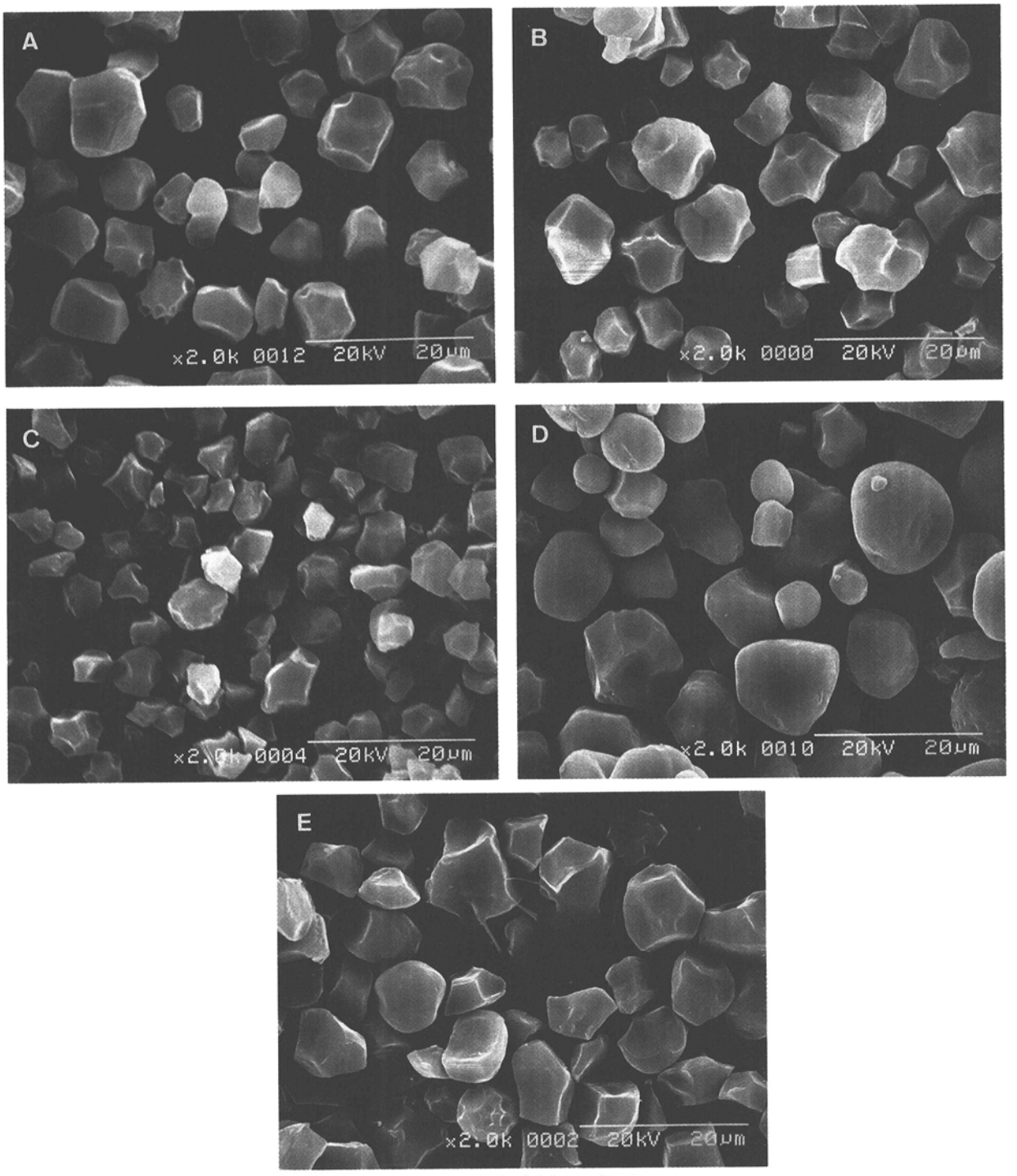

Fig. 1. Scanning electron micrographs of starches: **A,** acha A94; **B,** acha A85; **C,** iburu I94; **D,** iburu I85; and **E,** tamba.

cose)/(nonreducing terminii), respectively. The average number of chains per molecule (NC) was calculated as DP_n/CL.

Gel-Exclusion HPLC

The weight-average degree of polymerization (DP_w) of amylose and the DP distribution were determined by size-exclusion high-performance liquid chromatography (SE-HPLC) monitored with a low-angle laser-light-scattering (LALLS) photometer (Tosoh LS-8) and a differential refractometer (Ri, Tosoh RI-8000). A series of columns of TSK-gel G6000PW, G4000PW, and G3000PW (each 7.5 × 600 mm) was used as described previously (Hizukuri and Takagi 1984). The weight-average chain length (CL_w) and CL distribution of the amylopectin were determined by SE-HPLC using connected columns of Asahipak GS-520 (7.6 × 500 mm), GS-320 (7.6 × 500 mm) (Asahi Chemical Industry Co., Ltd, Tokyo) and TSK-gel G2000PW (7.5 × 600 mm) (Tosoh) with Ri and LALLS (Tosoh LS-8000) detectors (Hizukuri and Maehara 1990). The columns were eluted with 0.1*M* phosphate buffer (pH 6.0) containing 0.02% sodium azide at 0.5 ml/min. The column temperature was maintained at 35 ± 0.1°C in a water bath with a thermostat. Sample solutions (3.0 mg, 0.2 ml) for SE-HPLC were prepared as described by Hizukuri and Maehara (1990).

Physical Analysis

X-ray diffraction was performed on wet specimens (Rotaflex RV-20013; Rigaku Denki Co., Tokyo, Japan) under the conditions described by Hizukuri et al (1988). Scanning electron micrographs were taken with a JEOL JSM-25/LaB6 III scanning electron microscope (Tokyo, Japan). Starch samples were successively treated with glutaraldehyde and osmium tetraoxide and dehydrated in ethanol. Samples were applied to a micro-coverglass placed over adhesive tape attached to specimen stubs and coated in vacuo with gold-palladium. Mounted specimens were examined at an accelerating voltage of 5 kV. Representative micrographs were taken of each starch granule type at a magnification of 2,000×. Images were photographed on Neopan-SS (Fuji) film. Starch granule diameter was estimated by averaging the dimension of 100 random starch granules from five micrographs for each starch type. Scanning electron micrographs were also taken with a Hitachi scanning electron microscope PE S-2150 (Tokyo, Japan). Pasting characteristics of 8, 9, and 10% (w/w, dwb) starch suspensions were determined using the Rapid Visco-Analyzer (RVA) (model RVA-3D; Newport Scientific Pty, Ltd., Australia) with RVA data analysis software. Heating and cooling was at 3°C/min.

RESULTS AND DISCUSSION

Size Distribution

Electron photomicrographs of acha, iburu, and tamba starch granules (Fig. 1) revealed polygonal granules similar to those of rice (Juliano 1972), oats (Wang and White 1994), and amaranth (Zhao and Whistler 1994). The granules, in general, have shapes that are similar and range in size from 2.0 to 14.7 μm (Table I). On the basis of starch granule size, acha, iburu, and tamba have very large surface area per unit of weight compared to that of most other starches, which suggests a wide variety of possible applications. The average granule sizes of acha, iburu, and tamba were significantly smaller than the 15 μm reported value for normal maize starch (Lineback 1984). The polyhedral form may be due to compression of the starch granules during grain development. Some granules also have some hemispherical grooves as observed for rye starch granules (Lii and Lineback 1977).

X-ray Diffraction

Granules of acha, iburu, tamba and maize starches were of the A crystalline type, in keeping with the A-type diffraction pattern of cereal starches, although amylomaize starch is of B-type (Hizukuri et al 1983). No difference in crystallinity was observed for starches prepared from kernels steeped in acid solution (A85 and I85) or in alkaline solution (A94, I94, and tamba). It has been observed that alkaline-steeping tends to slightly reduce the crystallinity of starch granules (Takeda et al 1988). Generally, it is accepted that crystallinity is a property of the amylopectin fraction (Hizukuri et al 1983, Morrison 1995). Amylopectin with long average chain length has been suggested to crystallize into the B-type and that with short chain length into the A-type during starch biosynthesis (Hizukuri 1983).

Amylose Content

The amylose contents of acha, iburu and tamba starches were calculated from the iodine affinity (IA) values of starch, amylose, and amylopectin (Table II). The values were between 19.3% (tamba) and 26.9% (A85). The IA value and amylose content of A85 were higher than those of A94. Major differences between A85 and A94 were observed in most of the properties analyzed in this investigation. The amylose content (19.3) of tamba was slightly lower than the 20–22% reported for pearl millets (Beleia et al 1980), indicating differences in the structural characteristics within the millet family. Values for actual amylose content, calculated on the basis of IA of amylose and amylopectin, were lower than the apparent contents conventionally calculated without consideration of amylopectin IA values. The values for apparent amylose content (20–29%) were similar to that of normal maize (23%) but lower than that of sugary-2 maize (44%) starch (Takeda and Preiss 1993). The apparent amylose contents are higher than the actual amylose contents due to the amylopectin binding with iodine. The apparent content values for A85 (29%) and I85 (26.5%) were similar to that (28%) previously reported (Jideani and Akingbala 1993) for both starches. The reason for the slight difference in value may be due to the published values being determined by a colorimetric method using different standards.

Structures of Acha, Iburu, and Tamba Amyloses

The purity of each amylose preparation was confirmed to be free from amylopectin on HW-75F gel-chromatography (Takeda et

TABLE I
Size Distribution of Acha, Iburu, and Tamba Starch Granules

Sample	Range (μm)	Average ± SD[a] (μm)
Acha		
A94	11.0–2.0	5.5 ± 1.9
A85	13.1–3.3	6.6 ± 1.8
Iburu		
I94	9.8–2.0	5.0 ± 1.4
I85	13.5–2.9	8.0 ± 2.4
Tamba	14.3–3.3	7.3 ± 2.1

[a] Values are the average ± standard deviation of 100 starch granules, 20 each from five scanning electron micrographs.

TABLE II
Iodine Affinities (IA) and Amylose Content for Acha, Iburu, and Tamba Starches[a]

		Amylose Content (%)	
Sample	IA (g/100 g)	Actual[b]	Apparent[c]
Acha			
A94	5.3	22.1	26.5
A85	5.8	26.9	29.0
Iburu			
I94	5.0	19.3	25.0
I85	5.1	21.2	25.5
Tamba	5.0	19.3	20.0

[a] Defatted by repeated dissolution in dimethyl sulphoxide by heating and precipitation with ethanol.
[b] Calculated as: $[(IA_{starch} - IA_{amylopectin})/(IA_{amylose} - IA_{amylopectin})] \times 100$.
[c] Calculated as: $(IA_{starch} / IA_{amylose}) \times 100$, where $IA_{amylose}$ was assumed to be 20.

al 1984) (elution profiles not shown). The yields of amylose from starches were 20.8, 21.3, 20.3, 19.0, and 19.2% for A94, A85, I94, I85, and tamba, respectively.

The properties of amyloses are summarized in Table III. All the amyloses showed iodine affinities (IA), blue values, and λ_{max} of iodine coloration within the range of other amyloses (Takeda 1993), but slightly lower than those of corn (Takeda and Preiss 1993). Iburu and tamba amyloses have IA values similar to that of amylomaize amylose (19.4) (Takeda et al 1989a). The DP_n values of acha and iburu amyloses were similar to those of rice and corn amyloses (DP_n 920–1,110) (Takeda et al 1986a, 1987). The exceptionally low values of A85 and I85 may be due to long and improper storage of the starches, thereby resulting in degradation. However, the DP_n values for acha and iburu were lower than the amyloses of wheat (DP_n 1,290), kuzu (1,540), chestnut (1,690), nagaimo (2,000), lily (2,310), tapioca (2,660) (Takeda et al 1987), sweet potato (3,280–4,400) (Takeda et al 1986b), and potato (4,920–6,340) (Hizukuri et al 1981, Takeda et al 1984). The DP_n value for tamba amylose was slightly higher than that reported for wheat (830–1,180) (Shibanuma et al 1994).

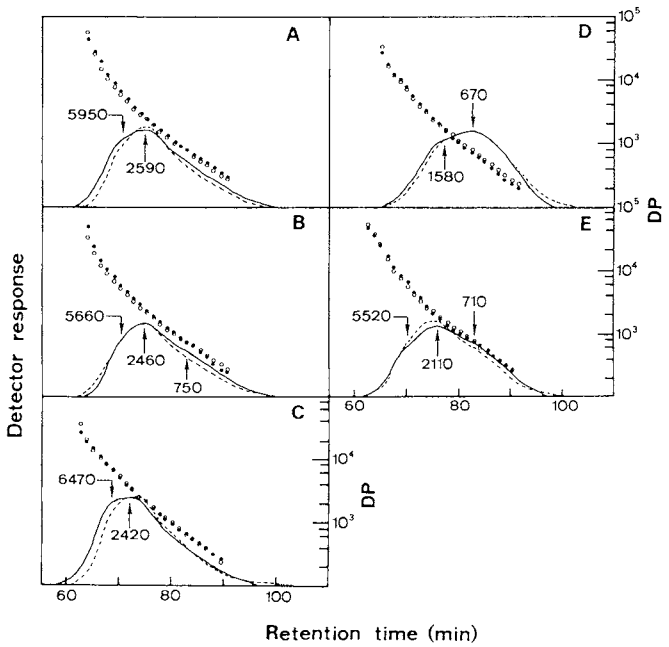

Fig. 2. Size-exclusion high-performance liquid chromatography profiles of **A,** acha A94; **B,** iburu I94; **C,** tamba; **D,** acha A85; and **E,** iburu I85 amyloses, and their β-limit dextrins (β-LD) on the connected columns of TSKgel G6000PW, G4000PW, and G3000PW. Conditions were as previously described (Hizukuri and Takagi, 1984). Response of differential refractometer for the amyloses and β-LD are shown as — and --- , respectively. Degrees of polymerization (DP) values of the amyloses and β-LD, respectively, are shown as ○ and ●, respectively. Numbers with arrows are DP values of the peaks or shoulders.

Cereal amyloses are generally smaller molecules than those of other origins. The CL of acha, iburu, and tamba amyloses were similar to those of the wheat (150–255) amyloses (Shibanuma et al 1994), but lower than those of rice, kuzu, sweet potato, tapioca (310–340), chestnut (375), lily (475), nagaimo (525) (Takeda et al 1987), and potato (670) (Takeda et al 1984) amyloses. The number of chains per molecule (NC) of acha, iburu, and tamba amyloses were similar to those of wheat amyloses, lower than those of potato, tapioca, and sweet potato (7–13) (Takeda et al 1987), but higher than those of corn, rice (3.5), lily, chestnut, kuzu and nagaimo (4–5) amyloses. The β-amylolysis limits of acha, iburu, and tamba amyloses (71–81%) were higher than those of the sweet potato, tapioca, and kuzu amyloses (72–76%), but lower than those of corn, wheat, rice (79–85%), chestnut (86%), and lily (89%) amyloses (Takeda et al 1983, 1987). The values obtained for acha and tamba amyloses suggest that they contain branched molecules. To understand the functional behavior of macromolecules, it is essential to know the molecular weight and molecular weight distribution. These parameters, detected by means of SE-HPLC, are shown in Table III for amyloses. The amyloses showed higher DP_w/DP_n values (2.86–4.28) than corn (2.44–2.66) (Takeda et al 1988), potato (1.29), and sweet potato (1.31) amyloses (Hizukuri and Takagi 1984), suggesting wide DP distributions (DP_w ≈300–17,400).

The elution profiles of the amyloses and β-LD in Figure 2 indicated that the branched amyloses have one or two peaks. This finding suggested that acha, iburu, and tamba branched amyloses had two components differing in molecular size. The multicomponent nature has been observed for sago (Takeda et al 1989b) and water chestnut (Hizukuri et al 1988) amyloses. Slightly concave curves of log DP versus retention time plots showed steeper slopes for the shorter retention times, suggesting that these amyloses may be comprised of two components, a highly branched, high molecular weight fraction, and a less highly branched, low molecular weight fraction. The curvature of the DP plot also suggested that the larger components were more spherical molecules due to higher degree of branching as reported by Hizukuri et al (1988). The lower amylose contents and a higher amount of the branched amylose with a lower NC, particularly in A94 (Table IV), may render these grains useful in the manufacture of food products such as noodles. These structural properties, in

TABLE III
Properties of Acha, Iburu, and Tamba Amyloses

	Acha		Iburu		
Property	**A94**	**A85**	**I94**	**I85**	**Tamba**
Iodine affinity (g/100 g)	18.7	18.0	19.6	19.2	19.8
Blue value	1.52	1.37	1.46	1.45	1.61
λ_{max} (nm)[a]	656	636	656	655	659
DP_n[b]	1,040	520	1,120	890	1,420
DP_w[c]	4,450	1,480	3,190	3,500	4,640
DP_w/DP_n	4.28	2.86	2.84	3.95	3.27
Apparent DP_w distribution[d]	320–20,400	260–6,300	290–12,400	360–16,800	380–17,400
β-Amylolysis limit (%)	80	81	71	77	80
CL[e]	175	100	200	160	240
NC[f]	6.0	5.2	5.6	5.6	5.9

[a] Iodine reaction.
[b] Number-average degrees of polymerization.
[c] Weight-average degrees of polymerization.
[d] DP_w values of the subfractions (10% by weight) with the lowest and highest molecular weights.
[e] Average chain length.
[f] Average number of chains (DP_n/CL).

some wheat amyloses, have been associated with superior processing performance and eating quality of noodles.

The branched molecule in amylose was analyzed with the β-LD. The β-LD was isolated from the β-amylolyzate by chromatography on a Bio-Gel P-4 column. The properties of the β-LD from acha, iburu, and tamba amyloses, summarized in Table IV, show iodine-binding and staining properties similar to those of the respective parent amyloses as reported for other β-LD (Takeda et al 1987). The IA values that ranged from 14.6 (A85) to 20.1 (tamba) were significantly higher than those of corresponding amylopectin (1.3–1.5), implying that the branched molecule was clearly distinguishable from amylopectins and proved that the branching was not due to contamination by amylopectin. The DP_n values were in the 460–1,190 range and differed with the origin of the amylose. Judging from these values and those obtained for DP_w and CL, the amyloses have small branched molecules as in corn and chestnut amyloses (Takeda et al 1987). The DP_n and DP_w of the β-LD of acha, iburu, and tamba were lower than the parent amyloses, except the DP_w of I94 β-LD, which is higher (3,340) than the parent amylose (3,190). The molecular size of the β-LD are often similar or even slightly greater than the parent amylose because the size of linear molecule is smaller than that of the branched molecule. Similarity in β-LD molecular sizes (DP_n and DP_w) and the parent amyloses suggests that the amyloses comprised large branched and small unbranched molecules, as observed for the maize (Takeda et al 1988), rice, and wheat amyloses (Takeda et al 1987).

The CL of acha, iburu, and tamba β-LD were similar to those of some wheat cultivars (50–94) (Takeda et al 1987, Shibanuma et al 1994), and lower than that of maize (160) (Takeda et al 1988), indicating small amylose molecules with a short inner chain length, because the β-LD originated from the branched molecules in the amyloses. These CL are closely related to the iodine affinities of the β-LD, indicating similar modes of branching. The NC value of A94 β-LD (the branched molecule) was lowest (≈7), while those of tamba and I85 β-LD were highest (≈13). A85 and I95 β-LD had medium NC (≈11). These NC values compare with those reported for corn (≈5), kuzu (8), rice, nagaimo, lily and chestnut (9–11), sweet potato, wheat, and tapioca (14–17) (Takeda et al 1987). The higher numbers of chains of the β-LD than those of the parent amyloses confirmed that the amyloses are mixtures of branched and unbranched molecules.

The β-LD is very useful for the analysis of branched natures of amylose and amylopectin because it preserves intact all the branch linkages of these polysaccharides. The molar fractions of the branched and unbranched molecules in amylose were calculated from the numbers of chains of amylose and the β-LD (Takeda et al 1987). Table V shows that the A85, I85, and tamba amyloses contain low molar fractions (39–41%) of branched molecules, whereas the A94 amylose has a high molar fraction (81%) as did sweet potato (70%) (Takeda 1993). I94 amylose is composed of nearly equal numbers of branched and unbranched molecules. The ratio of DP_n values for the β-LD and the parent amylose (0.57–0.89) (Table IV), suggest that the branched molecules in most amyloses are larger than the unbranched molecules. The A94 amylose appears to have fewer larger unbranched molecules than the others.

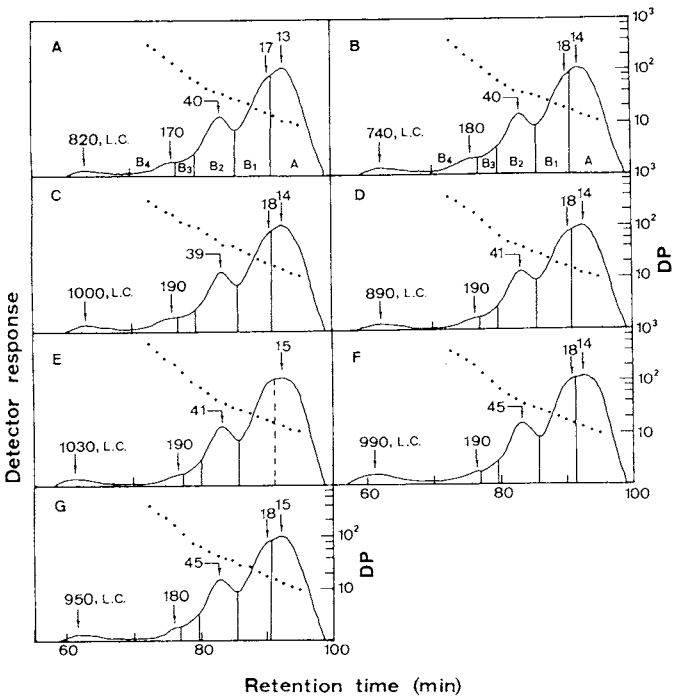

Fig. 3. Size-exclusion high-performance liquid chromatography profiles of isoamylase-debranched amylopectins of **A,** acha A94; **B,** acha A85; **C,** iburu I94; **D,** iburu I85; **E,** tamba; **F,** tamba-G; and **G,** corn. Conditions were as described previously (Hizukuri and Maehara 1990). DP = degree of polymerization; numbers with arrows are DP values of the peaks. LC = long chain; A and B_1 to B_4 are fraction designations.

TABLE IV
Properties of β-Limit Dextrins from Acha, Iburu, and Tamba Amyloses

	Acha		Iburu		
Property	**A94**	**A85**	**I94**	**I85**	**Tamba**
Iodine affinity (g/100 g)	17.6	14.6	18.1	17.6	20.1
Blue value	1.39	1.15	1.44	1.35	1.51
λ_{max} (nm)[a]	651	627	650	645	653
DP_n[b]	590	460	910	820	1,190
DP_w[c]	3,350	1,360	3,340	3,090	3,470
DP_w/DP_n	5.66	2.94	3.66	3.77	2.92
Apparent DP_w distribution[d]	430–14,000	160–5,600	300–12,500	340–12,900	300–11,200
CL[e]	82	41	87	62	89
NC[f]	7.2	11.2	10.5	13.2	13.4
CL/CL(amylose)	1.2	2.3	1.8	2.2	2.2
DP_n/DP_n(amylose)	0.57	0.89	0.81	0.92	0.84

[a] Iodine reaction.
[b] Number-average degrees of polymerization.
[c] Weight-average degrees of polymerization.
[d] DP_w values of the subfractions (10% by weight) with the lowest and highest molecular weights.
[e] Average chain length.
[f] Average number of chains (DP_n/CL).

Structures of Acha, Iburu, and Tamba Amylopectins

The properties of acha, iburu, and tamba amylopectins are summarized in Table VI. The yields (%) of amylopectin from A94, A85, I94, I85, and tamba starches were 70.3, 59.3, 70.1, 65.2, and 76.7%, respectively. The IA of the amylopectins (1.3–1.5 g/100 g) were slightly higher than the IA of corn amylopectin (1.14 g/100 g) (Takeda et al 1988, Takeda and Preiss 1993) and fall within intermediate range when compared with those of japonica (0.37–0.89 g/100g) and indica (1.62–2.62 g/100 g) amylopectins (Hizukuri et al 1989). These relatively high IA were due to their own structures and not to contamination by amylose. The CL of A94 (21), I94 (20), and tamba (20) amylopectins were similar to those reported for wheat (18.5), rice (17–19), corn (19.5), and sweet potato (20–21). The CL were shorter than that for potato amylopectin (22.5), and longer than that of waxy rice (17.1). DP_n values of 3,000 (A85), 34,000 (A94), 8,600 (I85), 20,000 (I94), and 16,000 (tamba) were observed. The amylopectin of A94 is a bigger molecule than the others (Table VI). A85 and I85 had an especially low DP_n, possibly due to small amylopectin molecules resulting from degradation of the starches during long storage of the starches at ambient temperature and not by acid steeping when the starches were prepared (Takeda et al 1986b). A85 amylose also had a low DP_n value (Table III). The methods of rapid Smith-degradation and debranching by isoamylase gave the same CL in the range 18-21, indicating complete debranching by isoamylase. The CL values of acha, iburu, and tamba amylopectins were similar to those (18.6) of waxy corn (Hizukuri et al 1983) and (19–20) of japonica rice (Takeda et al 1987) amylopectins, and lower than those of indica rice (21–22) (Takeda et al 1987), kuzu (21.1) (Suzuki et al 1981), tapioca (21.1) (Suzuki et al 1985), sweet potato (21–22) (Takeda et al 1986b), corn (20–22) (Takeda and Preiss 1993), amylomaize (29–32) (Takeda et al 1993b), potato (23.7) (Suzuki et al 1981), lily (23.6) (Takeda et al 1983), and nagaimo (24.0) (Suzuki et al 1986) amylopectins. Both the external and internal CL values (13–15 and 4–6, respectively) for acha, iburu, and tamba (Table VI) were within the same range as those (14–15 and 5–6, respectively) of rice, corn and wheat amylopectins (Hizukuri 1993). The β-amylolysis limits of the amylopectins were comparable with those of other cereal amylopectins.

The CL distributions of acha, iburu, tamba and corn amylopectins, debranched with isoamylase and analyzed by SE-HPLC-LALLS, are shown in Figure 3 and Table VII. The chromatograms show tetramodal distributions. The chains were fractionated into long chain (LC), B_4–B_1 and A, in order of elution (Hizukuri 1986). A-chains are those chains in amylopectin that are unbranched, and B-chains are those chains to which another chain is attached by an α-D-(1→6) linkage (Peat et al 1952). Some slight differences in carbohydrate amounts (expressed as wt% and mol%) and CL_w of the fractions were observed (Table VII). The LC fraction of each sample is a real component of the amylopectin. This has been confirmed for rice, wheat, and lotus amylopectins (Hizukuri 1986, Shibanuma et al 1994). The amounts of the LC fraction were in the 1.7–4.0% range (Table VII), and compare with that in corn (2.4%), and wheat (4–6%) amylopectins (Hizukuri 1993). The MGA in tamba (tamba-G) contained ≈60% higher amount of the LC fraction than did the others. This higher amount of the LC fraction, 4.0% by weight, in tamba amylopectin compared with the others (Table VII), may have significantly contributed to the functional properties, as was observed in the

TABLE V
Molar Fractions of Branched and Unbranched Molecules in Acha, Iburu, and Tamba Amyloses

	Acha		Iburu		
Molecule	A94	A85	I94	I85	Tamba
Branched[a]	0.81	0.39	0.53	0.41	0.40
Unbranched	0.19	0.61	0.47	0.59	0.60

[a] $(NC_{amylose} - 1)/(NC_{\beta\text{-limit dextrin}} - 1)$, where NC = average number of chains (DP_n/CL).

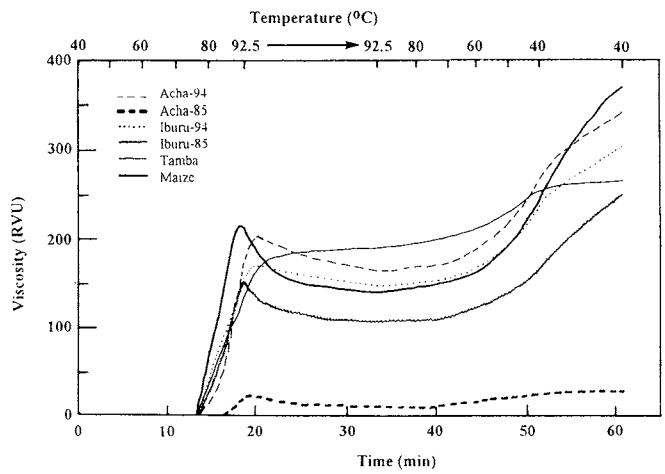

Fig. 4. Rapid viscograph pasting curves for 9% (w/w) slurries of acha, iburu, tamba, and corn starches.

TABLE VI
Properties of the Amylopectin (Ap) Fraction from Acha, Iburu, and Tamba Starches

	Ap Fraction[a]						
Property	A94	A85	A85G	I94	I85	T	TG
Iodine affinity (g/100 g)	1.5	1.3	···	1.5	1.3	1.4	···
Blue value	0.18	0.19	0.19	0.19	0.16	0.18	0.19
λ_{max} (nm)[b]	581	566	569	575	565	574	579
CL[c]							
Smith degradation	21	19	18	20	21	20	20
Isoamylolysis	20	19	18	20	21	20	19
DP_n[d]	34,000	3,000	···	20,000	8,600	16,000	16,000
β-Amylolysis limit (%)	60	61	62	60	57	61	60
NC[e]	1,700	160	···	1,000	410	780	780
External CL[f]	15	14	13	14	14	14	14
Internal CL[g]	5	4	4	5	6	5	5

[a] A, I, and T represent acha, iburu and tamba respectively; A85G and TG are microgel-like amylopectins.
[b] Iodine reaction.
[c] Average chain length.
[d] Number-average degrees of polymerization.
[e] Average number of chains (DP_n/CL).
[f] Calculated as: (CL × β-AL/100) + 2, where β-AL is β-amylolysis limit (Takeda et al 1993b).
[g] Calculated as: CL – (external CL) – 1 (Takeda et al 1993b).

cooking behavior. The LC component of amylopectin has considerable effect on some functional properties, such as viscosity, retrogradation tendency, and, possibly, inclusion capacity of hydrophobic materials (Hizukuri et al 1989). These properties are important for use in food products and industrial applications of starch. The amylopectins of acha, iburu, and tamba contain proportions of B_3-A fractions similar to that of corn amylopectin. The wt% of B_4 fraction in corn was lower (2.5%) than the similar fraction in acha, iburu, and tamba (3.0–4.7%). It is possible that the B chains of A94 amylopectin linked increased number of A chains (68 mol%). However there was no increase in chain length as reported for the B chains in wheat and rice amylopectins (Hizukuri 1993). The CL between two neighboring clusters, from the difference in average CL between fractions B_3 and B_2 (Hizukuri 1986), would be 49, 42, 55, 55, 57, 48, and 43 for A94, A85, I94, I85, tamba, tamba-G, and corn, respectively.

The CL distribution of amylopectin is known to be an important factor in the properties of food. Structural differences in amylopectin, such as molecular weight and its distribution, as well as shape, are known to influence the physicochemical behavior of starches. For instance, the onset of swelling and gelatinization, has been related to the molecular weight and shape of the whole amylopectin molecule (Juliano et al 1987, Takeda et al 1989, Tester and Morrison 1990). It has been suggested that high amylose and longer amylopectin chains may give firmer texture and resistance to disintegration of food products (Salomonsson and Sundberg 1994). It is very likely that grain products will continue to serve as both a staple food and an effective means to improve the nutritional health of the world for some time to come. Therefore, research is needed on the utilization of acha, iburu, and tamba starches and flours in more modern cereal products.

Pasting Behavior

The pasting behavior in water during heating and cooling of acha, iburu, and tamba starches compared with that of maize starch at 9% (w/w) concentration using the Rapid Visco-Analyzer is shown in Figure 4. Corn starch had the highest pasting viscosity (213 RVU) followed by A94 (200), tamba (181) and A94 (168). I85 and A85 had the lowest pasting viscosities (150 and 23 RVU, respectively). All starches showed similar pasting temperature (76.2 to 77.3°C), except a higher temperature of 85°C for A85 starch. Tamba starch showed considerable stability in viscosity

TABLE VII
Carbohydrate Amounts and Chain-Length Distribution of the Fractions of Isoamylase-Debranched Amylopectins

	Fraction[a]					
	LC	B_4	B_3	B_2	B_1	A
CL_w[b]						
A94	1,000	270	89	41	21	12
A85	800	240	83	41	21	12
I94	1,100	270	97	42	21	13
I85	1,000	290	98	43	22	13
Tamba	1,300	300	98	41	16[c]	
Tamba-G[d]	1,020	300	94	45	22	13
Corn	1,200	290	91	48	23	13
Weight (%)[e]						
A94	2.0	3.5	3.9	20.3	29.0	41.3
A85	1.7	4.7	5.1	20.1	28.8	39.7
I94	2.5	4.1	4.1	20.3	28.4	40.2
I85	2.6	3.0	3.8	19.8	30.5	40.3
Tamba	2.7	3.0	3.6	20.2	70.5[c]	
Tamba-G[d]	4.0	3.2	3.5	19.1	31.8	38.4
Corn	2.4	2.5	4.6	20.5	29.4	40.7
Mole (%)[f]						
A94	0	0.2	0.8	9.4	25.5	68.0
A85	0	0.4	1.2	9.4	26.5	62.5
I94	0	0.3	0.8	9.7	27.0	62.2
I85	0	0.2	0.8	9.0	27.8	62.0
Tamba	0	0.2	0.8	10.1	89.0[c]	
Tamba-G[d]	0.1	0.2	0.7	8.5	28.9	61.6
Corn	0	0.2	1.0	8.8	26.0	64.0

[a] Chains were fractionated into long chain (LC), B_4–B_1 and A, in order of elution (Hizukuri 1986).
[b] Weight-average chain length.
[c] B_1 and A fractions gave a single peak.
[d] Microgel-like amylopectin.
[e] Percentages of each fraction within each sample type as measured by peak area and as calculated from weight %.
[f] Percentages of each fraction within each sample type as measured by peak area and as calculated from CL_w.

TABLE VIII
Viscosity Properties for 9% (w/w, dwb) Slurries of Acha, Iburu, and Tamba Starches[a,b]

Sample[c]	T_p	V_m	T_m	T_m (min)	V_r	V_e	$V_m - V_r$	$V_e - V_r$	% Breakdown
A94	77.3	200	92.45	19.8	163	258	37	95	18.5
A85	85.0	23	92.60	18.9	11	24	12	13	52.2
I94	77.4	168	92.65	19.8	145	229	23	84	13.7
I85	77.8	150	92.15	18.5	105	160	45	55	30.0
Tamba	76.7	181	92.60	22.7	191	246	−10	55	0
Corn	76.2	213	90.83	18.1	141	235	72	94	33.8

[a] Data obtained from Rapid Visco Analyzer (RVA); viscosity is expressed in RVA units.
[b] T_p = pasting temperature (°C); V_m = peak pasting viscosity; T_m = temperature at peak pasting viscosity (°C); T_m (min) = time at peak pasting viscosity; V_r = minimum viscosity; V_e = cooling viscosity (viscosity on cooling to 40°C); $V_m - V_r$ = breakdown (stability of the sample); $V_e - V_r$ = consistency; % breakdown = $(V_m - V_r) \times 100/V_m$.
[c] Each specimen was analyzed in triplicate.

TABLE IX
Viscosity Properties for 9% (w/w, dwb) Slurries of Acha, Iburu, and Tamba Starches in 5% 3-methyl-1-butanol[a,b]

Sample[c]	T_p	V_m	T_m	T_m (min)	V_r	V_e	$V_m - V_r$	% Breakdown
A94	65.0	258	84.7	16.1	140	261	118	45.7
A85	68.8	17	71.5	11.6	2	5	15	88.2
I94	67.7	178	86.4	16.6	115	255	63	35.4
I85	65.1	178	82.5	15.3	75	208	103	57.9
Tamba	66.9	261	84.1	15.8	145	339	116	44.4
Corn	65.3	246	82.4	15.3	110	215	136	55.3

[a] Data obtained from Rapid Visco Analyzer (RVA); viscosity is expressed in RVA units.
[b] T_p = pasting temperature (°C); V_m = peak pasting viscosity; T_m = temperature at peak pasting viscosity (°C); T_m (min) = time at peak pasting viscosity; V_r = minimum viscosity; V_e = cooling viscosity (viscosity on cooling to 75°C); $V_m - V_r$ = breakdown (stability of the sample); % breakdown = $(V_m - V_r) \times 100/V_m$.
[c] Each specimen was analyzed in triplicate.

throughout the heating-cooling cycle, being similar in behavior to that of a chemically cross-linked starch. This is evidence of restricted swelling and solubilization and of resistance to mechanical disintegration, as revealed by the result obtained from cooking in 3-methyl-1-butanol. Tamba also showed little or no viscosity breakdown (Table VIII) during prolonged heating and stirring at the three starch concentrations (8 and 10% not shown).

Breakdown indexes the susceptibility of cooked starch to disintegration, whereas consistency measures the degree of hardening or retrogradation of cooked starch during cooling. Although pasting viscosity was highest in all samples at 10% starch concentration, there was greater tendency to retrograde than at lower concentrations. At 9% (w/w) starch concentration, tamba showed the least retrogradation tendency. Pasting characteristics of various starches are affected by amylose and amylopectin contents as well as by their arrangement in the granule (Beleia et al 1980). In general, the viscograms of acha, iburu, and tamba starches in water were similar to those of nonwaxy cereal starches. In 3-methyl-1-butanol (3MB), the pasting temperature and temperature at maximum viscosity of all the starches were lowered, with an increase in the pasting viscosity, particularly for tamba (Table IX). The behavior of acha, iburu, and tamba in 5% aqueous 3MB is typical of most cereal starches, unlike sago (Takeda et al 1989b), sweet potato (Takeda et al 1986b), kuzu (Suzuki et al 1981), lily, and potato (Takeda et al 1983) starches. Tamba showed higher pasting viscosity (261 RVU) than the other starches, implying that restricted swelling, which was observed under a microscope, was the reason for the stability in viscosity on cooking in water (Fig. 4).

The physicochemical differences, particularly among starches from *Digitaria* species (acha and iburu) and tamba, were the result of inherent molecular dissimilarities. Genetic or environmental factors may be responsible for the differences among species. Values of properties, such as blue value, IA, and molecular weight are specific to plant species and cultivars, and are variable within certain ranges due to growth conditions and postharvest treatment. These variations mostly arise from the different methods of analysis and the historical background of specimens.

CONCLUSION

Similar structural characteristics to starches from other cereal grains were observed for acha, iburu, and tamba starches. Granules from the latter starches were relatively small (2.0–14.3 μm in diameter) and had polygonal and irregular shapes. All three starches exhibited an A-type X-ray diffraction pattern. Pasting behavior of the starches, measured by Rapid Visco Analyzer, resemble those of nonwaxy cereal starches. However, the viscosity of tamba starch was relatively stable on heating and cooling in water. The amylose molecules showed a fairly wide DP distribution ($DP_w \approx 300$–17,400). Some molecules were very small, with DP less than 300, and the average CL ranged between 180 and 240. All the amyloses may be composed of two components, except iburu which contained up to three components. The external and internal CL values for acha, iburu, and tamba amylopectins were within the 13–15 and 4–6 ranges, respectively.

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