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Amylopectin—properties and fine structure

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Five amylopectins from different sources were studied to compare their physicochemical properties and structure. Physicochemical properties such as iodine binding capacity (IBC), maximum absorption (λ_{max}) and intrinsic viscosity ([η]) were assayed. Normal corn amylopectin and potato amylopectin gave high values of IBC whereas waxy corn, corn commercial and amaranth exhibited low values of this parameter; all values for λ_{max} were in the range 525–595 nm and those for [η] varied from 23 to 104 ml/g. Results suggested structural differences in the amylopectins. These macromolecules were eluted by high-performance size exclusion chromatography (HPSEC) with refractive index (RI) showing different elution times, which indicate structural differences among such components. The five amylopectins were debranched with isoamylase and pullulanase, and the fractions, studied both by HPSEC with RI and by high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD), showed structural differences among amylopectins and differences in debranched patterns of isoamylase and pullulanase. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Starch is composed of two polymers of different structure; amylose is essentially linear, whereas amylopectin is a branched component, and starch owes much of its functionality to these macromolecules (French, 1984). Amylopectin is one of the biggest molecules in nature being the principal component in the majority of starches and perhaps the most important in terms of their functional properties (Manners, 1989).

The estimation of the chain length distribution is of primary importance for characterizing the molecular structure of amylopectin (Koizumi *et al.*, 1991). Substantial progress in investigating its fine structure has become possible by the use of highly purified amylolytic enzymes (e.g. pullulanase, isoamylase, β-amylase). The debranching enzymes isoamylase and pullulanase have been very widely used for such structural analyses and specifically hydrolyse the α-(16)-D-glycosidic inter-chain linkages, but have no action on the major α-(14)-D-glycosidic bonds (Ong *et al.*, 1994). Guilbot & Mercier (1985) reported that the rate of hydrolysis, liberating linear chains, is dependent on the length of the branching and also on the specificity of the enzyme. Some authors have found differences in debranching profiles of isoamylase and pullulanase (MacGregor & Morgan, 1984; Thurn & Burchard, 1985).

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Research on the fine structure of amylopectin has been achieved using different methodologies such as gel filtration chromatography (Biliaderis *et al.*, 1981; MacGregor & Morgan, 1984; Bertoft & Avall, 1992; Jane & Chen, 1992; Reddy *et al.*, 1993; Paredes-López *et al.*, 1994), high-performance size-exclusion chromatography (HPSEC) using a differential refractive index detector (RI) (Hizukuri, 1985, 1986; Kobayashi *et al.*, 1986; Taki *et al.*, 1988; Bradbury & Bello, 1993; Yuan *et al.*, 1993; Fishman & Hoagland, 1994; Ong *et al.*, 1994; Wang & White, 1994), high-performance liquid chromatography (HPLC) with a multi-angle laser-light-scattering detector (Hizukuri, 1985; Suzuki *et al.*, 1992; Ong *et al.*, 1994) and high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) (Koizumi *et al.*, 1991; Suzuki *et al.*, 1992). Three distribution modes have been published up to now from those studies, bimodal, trimodal and polymodal distributions, although HPAEC–PAD determines the individual components. It is also known that all of these different distributions are consistent with the cluster model, which best explains the amylopectin structure (Robin *et al.*, 1974); this model applies to all amylopectins, irrespective of their botanical source (Manners, 1989). Some investigators (Kobayashi *et al.*, 1986; Enevoldsen & Juliano, 1988) have reported that there are no differences in the structure of amylopectin of the same source independently of the amylose and amylopectin levels. However, other authors have reported

variations in the amylopectin structure even within the same source (Taki *et al.*, 1988; Hizukuri *et al.*, 1989; Sanders *et al.*, 1990; Reddy *et al.*, 1993); this could partially explain the diverse functional properties of these starches. These investigations have attempted to relate the structure of amylopectins with functional properties (Suzuki *et al.*, 1992; Reddy *et al.*, 1993; Yuan *et al.*, 1993; Bello-Pérez & Paredes-López, 1994, 1995; Paredes-López *et al.*, 1994; Wang & White, 1994). The present study was undertaken to assess some of the basic physicochemical properties of different amylopectins, and to characterize their fine structure using two chromatographic techniques combined with the debranching action of isoamylase and pullulanase.

MATERIALS AND METHODS

Amylopectin isolation

Amylopectins were isolated from amaranth starch, and from waxy and normal corn starches using the methodology reported by Banks & Greenwood (1967). Corn commercial amylopectin and potato amylopectin were purchased from Sigma Chemical Co. (St Louis, MO, USA); no information was provided on the type of corn and potato used to isolate these amylopectins.

Physicochemical properties

The iodine binding capacity (IBC) was determined by standard potentiometric titration (Schoch, 1964) at 2°C using a titrator TTT80 Radiometer (Copenhagen, Denmark). The IBC was expressed as milligrammes of iodine bound to 100 mg of amylopectin.

The maximum absorbance wavelength (λ_{max}) between 750 and 400 nm of amylopectin–iodine complex was determined using a spectrophotometer DU-40 (Beckman Instruments, Inc., Fullerton, CA, USA).

The intrinsic viscosities ([η], ml/g) were obtained at 25°C in 0.1 M KOH, pH 12.5–13.0, using an automatic Ubbelholde viscosimeter (Amtec, France) and were calculated using a double extrapolation to zero concentration based on the Huggins and Kraemer equations (Van Krevelen, 1990).

Glucose concentration was assayed with the glucose oxidase–peroxidase method using a SLT Spectra reader (Tecan, France) (Banks & Greenwood, 1971); in all cases carbohydrate concentration was checked by the sulphuric acid–orcinol colorimetric method (Tollier & Robin, 1979).

Debranching of amylopectin

Amylopectins were debranched with isoamylase and pullulanase enzymes (Hayashibara Shoji, Inc., Okayama, Japan). Samples (8 mg) of amylopectins were dissolved in 1 ml of 0.1 M acetate buffer and heated for 20 min at 100°C. After cooling, 15 μl of isoamylase or 16 μl of pullulanase were added and the solutions were incubated for 24 h at 37°C. The digested samples were heated for 20 min at 100°C to inactive the enzyme. Insoluble material was removed by centrifugation (10 000*g* for 20 min) and the supernatant was filtered through a 0.45-μm filter. The blanks without enzyme samples were filtered through a 5-μm filter.

High-performance size-exclusion chromatography

Total amylopectins and the chains distribution of debranched amylopectins were estimated by an HPSEC system that includes a programmable HPLC-pump (Waters 590, Waters, Milford, MA, USA), an autosampler (Waters 717), a degasser (Erma ERC-3312, Erma Optical Works Ltd, Japan) and a differential refractive index detector (Erma ERC-7510); the following were used: a guard column (TSK gel SWXL guard column, 6 mm ID×4 cm, TosoHaas, Stuttgart, Germany), a column (TSK gel G3000 SWXL 7.8 mm ID×30 cm, TosoHaas) and two columns (TSK gel G2000 SWXL 7.8 mm ID×30 cm). The columns were maintained at 37°C and the detector at 40°C. The water used was taken from a Milli-RO-6-plus and Milli-Q-plus water purification system (Millipore, Bedford, MA, USA) and the eluent was 0.1 M acetate buffer with 0.02% sodium azide, carefully degassed and filtered before use through durapore GV (0.45 mm) membranes (Millipore). The mobile phase had a flow rate of 0.5 ml/min. A 100-μl sample was injected into the HPSEC system. The software used for the acquisition, storage and processing of data was the Apex Chromatography workstation (Autochrom Inc., France).

High-performance anion-exchange chromatography with pulsed amperometric detection

The chain length distribution of debranched amylopectins was analyzed also by a HPAEC–PAD system (Dionex BioLC model 4000i, Sunnyvale, CA, USA). A Dionex CarboPac PAI column (250×4 mm) was used.

The eluents A and B were 100 mM sodium hydroxide solution and 100 mM sodium hydroxide solution containing 600 mM sodium acetate, respectively, and the flow rate was 0.9 ml/min. The eluents were degassed by a Dionex degas module with helium gas. The composition of the eluent B was changed as follows: 20% at 0 min, 35% at 10 min, 59% at 25 min, 77% at 40 min, 90% at 50 min and 20% at 70 min.

α-Amylase activity

The α-amylase activity was assayed using the methodology reported by MacCleary & Sheehan (1987).

RESULTS AND DISCUSSION

Physicochemical properties

The physicochemical properties of amylopectins under study are listed in Table 1. Iodine binding capacity

Table 1. Physicochemical properties of amylopectins

Sample	IBC[a] (mg/100 mg of amylopectin)	λ_{max}[b] (nm)	$[\eta]$[c] (ml/g)
Waxy corn	1.22	530	99
Corn commercial	0.39	538	104
Normal corn	5.41	595	70
Amaranth	0.55	525	73
Potato	3.23	540	23

[a]Iodine binding capacity.
[b]Maximum absorption wavelength.
[c]Intrinsic viscosity.

(mg/100 mg amylopectin) for corn commercial (0.39), amaranth (0.55) and waxy corn (1.22) were much lower than those from potato (3.23) and normal corn (5.41). The low values of IBC found for the former three samples suggest the presence of short branch chains and freedom from amylose, whereas the high values of the latter two amylopectins may imply longer branch chains, a degraded structure and/or amylose contamination (Wang *et al.*, 1993; Wang & White, 1994). Values of IA between 0.39 and 2.57 g/100 g of starch were found by Hizukuri *et al.* (1989) for different amylopectins isolated from rice and they proposed that IA is a direct function of long branch chains.

Amylopectins presented λ_{max} values ranging from 525 to 595 nm (Table 1). These values are related to the IBC; in general when the IBC increased λ_{max} tended to increase as well, except for corn commercial as in this case it is likely that the amylopectin is still inside the starch granular structure as reported previously (Paredes-López *et al.*, 1994). Wang & White (1994) reported λ_{max} values between 557 and 560 nm, which varied within a closer range than that found in our study. The $[\eta]$ parameter is not correlated with the molecular size but reflects the profile of the molecular architecture (Hizukuri *et al.*, 1989; Wang *et al.*, 1993; Wang & White, 1994). Waxy corn and corn commercial samples presented the highest $[\eta]$ values (Table 1), suggesting a more branched structure of those amylopectins; normal corn and amaranth showed intermediate values (70 and 73, respectively), implying that these amylopectins have shorter chains than the previous ones, whereas the potato amylopectin gave the lowest value probably because of its more degraded structure. In earlier works, Hizukuri *et al.* (1989) found $[\eta]$ values ranging from 134 to 170 ml/g for rice amylopectins, also Wang & White (1994) found $[\eta]$ values in the range from 124 to 146 ml/g. These values are higher than those found in our study, but they are difficult to compare since different methods of determination were used.

Molecular structure

Amylopectins were eluted in the HPSEC system and the profiles are shown in Fig. 1. Waxy (Fig. 1,A) and

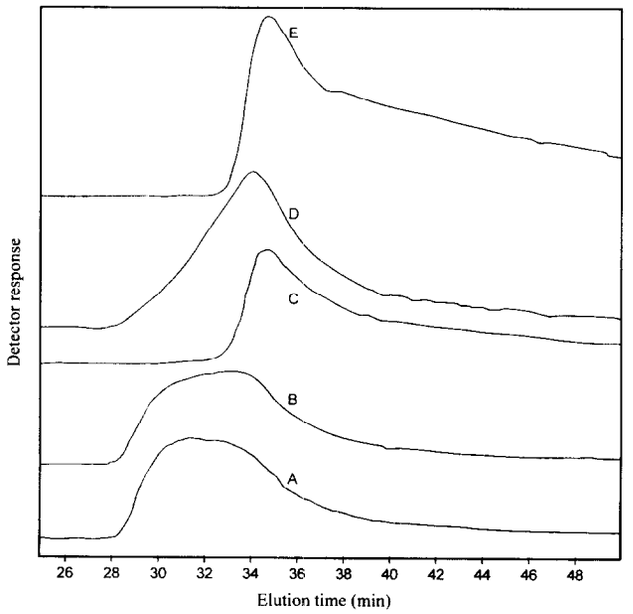

Fig. 1. High-performance size-exclusion chromatograms of amylopectins. (A) Waxy corn; (B) corn commercial; (C) normal corn; (D) amaranth; and (E) potato.

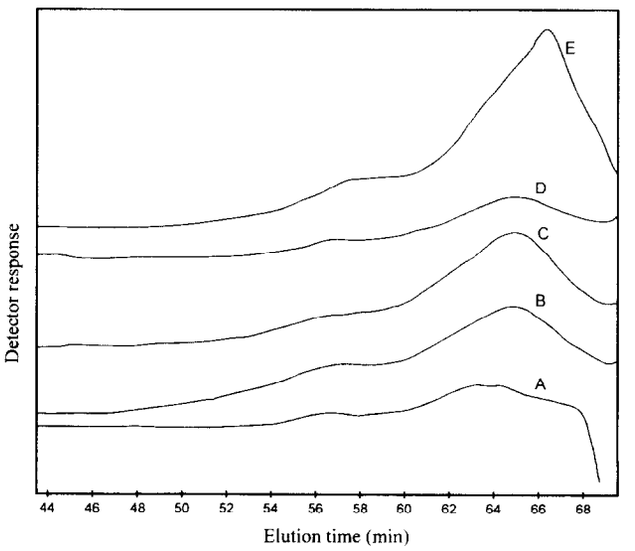

Fig. 2. High-performance size-exclusion chromatograms of isoamylase-debranched amylopectins. (A) Waxy corn; (B) corn commercial; (C) normal corn; (D) amaranth; and (E) potato.

commercial (Fig. 1,B) amylopectins, both from corn, presented somewhat similar patterns suggesting structural similarities. Normal corn (Fig. 1,C) and potato (Fig. 1,E) showed somewhat analogous profiles with higher elution times than the other samples from corn; these differences between normal and waxy corns have not previously been reported. Amaranth (Fig. 1,D) presented a pattern different from the other amylopectins studied. The different profiles reported here suggest different structures in most amylopectins with different chain distributions even in materials isolated from the same source but with different amylopectin levels. Substantial differences exist in amylopectin structures as reported by Reddy *et al.* (1993), even in starches from the same source but with different amylose and amylopectin levels.

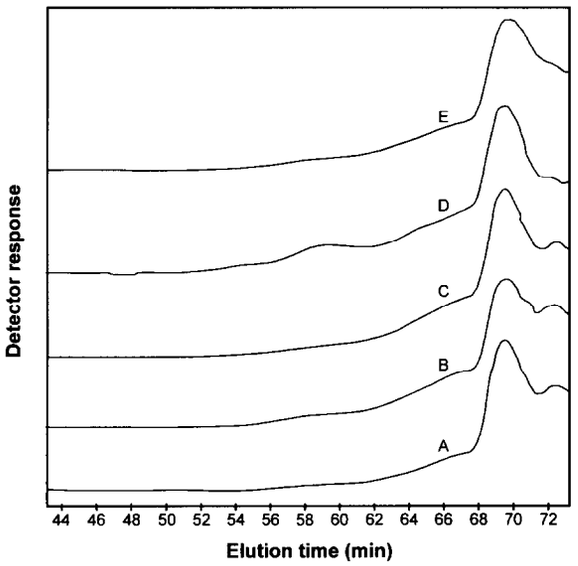

Fig. 3. High-performance size-exclusion chromatograms of pullulanase-debranched amylopectins. (A) Waxy corn; (B) corn commercial; (C) normal corn; (D) amaranth; and (E) potato.

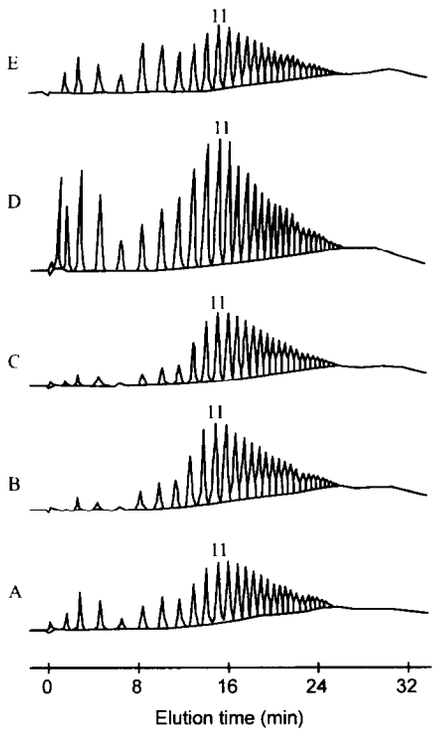

Fig. 4. High-performance anion-exclusion chromatograms of isoamylase-debranched amylopectins. (A) Waxy corn; (B) corn commercial; (C) normal corn; (D) amaranth; and (E) potato.

Amylopectins were debranched with two different enzymes to compare debranching patterns (Figs 2 and 3). Products generated after debranching are different. However, isoamylase (Fig. 2) gave a principal fraction similar to pullulanase (Fig. 3) but with a smaller elution time suggesting that isoamylase generated products with longer chains than pullulanase. In most profiles (with both enzymes) two fractions were obtained, similar to those reported by other authors using isoamylase (Hizukuri, 1985; Bradbury & Bello, 1993; Yuan *et al.*, 1993; Fishman & Hoagland, 1994), indicating bimodal

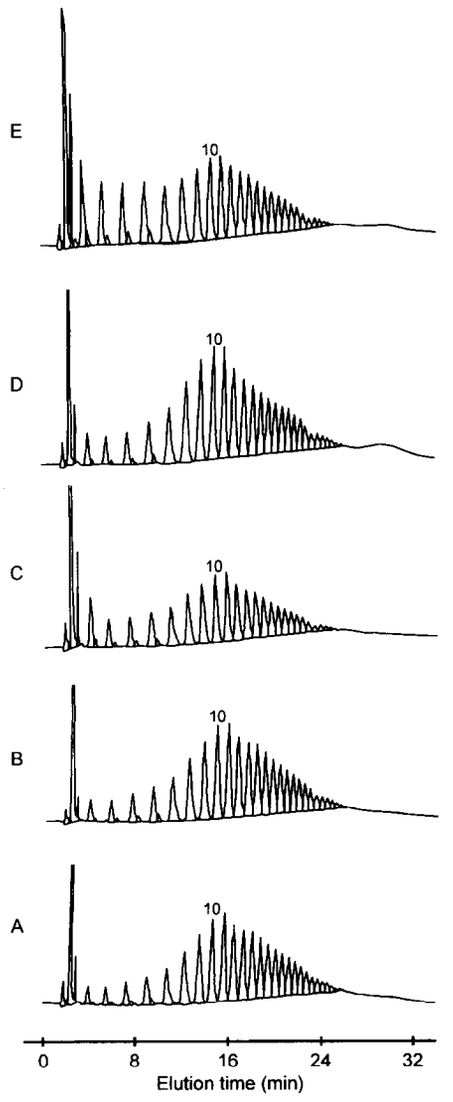

Fig. 5. High-performance anion-exclusion chromatograms of pullulanase-debranched amylopectins. (A) Waxy corn; (B) corn commercial; (C) normal corn; (D) amaranth; and (E) potato.

Table 2. Glucose concentration (mg/ml) of isoamylase-debranched and pullulanase-debranched amylopectins

Sample	Enzyme	
	Isoamylase	Pullulanase
Waxy corn	0.7	43.0
Corn commercial	0.6	45.3
Normal corn	1.0	45.9
Amaranth	1.0	44.0
Potato	0.6	43.8

distributions of chains. All patterns showed a high proportion of short chains and a lower level of long inter-cluster chains.

After the tests with HPSEC, HPAEC–PAD was used to determine the individual components in debranched amylopectins. The profiles of those components are shown in Figs 4 and 5 for isoamylase and pullulanase, respectively. Amylopectins debranched with isoamylase showed, in general, the highest levels of components between degrees of polymerization (DP) 10 and 13. The chain distributions obtained by HPSEC (Fig. 2) with isoamylase accords with that generated with the same enzyme by HPAEC–PAD (Fig. 4). The profiles of amylopectins debranched with isoamylase were similar to those found by Koizumi *et al.* (1991) for amylopectins from different sources depolymerized with the same enzyme (the highest proportion of components with 10 and 13 DP). Suzuki *et al.* (1992) also found a similar pattern for lotus amylopectin debranched with such an enzyme. The samples debranched with pullulanase (Fig. 5) showed the highest amount of glucose, and components with chains between 8 and 12 DP. These patterns, compared with those obtained with isoamylase, showed more DP components. The patterns obtained with HPAEC–PAD suggest structural differences among amylopectins exposed by the enzymes used. Also the patterns generated by HPSEC (Fig. 3) are in agreement with the profiles obtained with HPAEC–PAD (Fig. 5). The high amount of glucose found in this study could be an artifact of the system. For this reason the glucose oxidase–peroxidase method was used and a high concentration of glucose in the pullulanase samples was detected (Table 2). These results suggested two possibilities: (1) that pullulanase was contaminated with α-amylase; or (2) that isoamylase and pullulanase hydrolyse in different manners; α-amylase activity was not found in the pullulanase preparation, however, thus eliminating the first possibility.

Only the distribution of individual components generated from Figs 4 and 5 of waxy corn amylopectin for both enzymes are given in Fig. 6, because these distributions are similar to the other amylopectins analysed. In this figure differences in distribution generated by the two enzymes are illustrated.

In conclusion, corn commercial, amaranth and waxy corn amylopectins presented low IBC values whereas potato and normal corn samples showed higher iodine

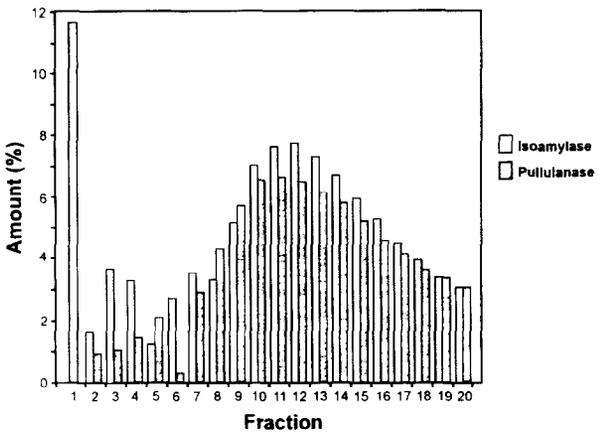

Fig. 6. The chain length distribution of isoamylase-debranched and pullulanase-debranched waxy corn amylopectin, based on HPAEC.

binding capacity which suggests the presence of longer branch chains, a degraded structure and amylose contamination. The binding capacity tended to increase for increases of λ_{max} values, except for corn commercial perhaps because of the granular structure of this material. The lowest $[\eta]$ values for potato may correspond to a more degradated structure, and to a more compact or more spherical molecule. All amylopectins gave different elution times when eluted in HPSEC. After debranching with isoamylase and pullulanase most amylopectins presented two principal fractions with evident differences among their patterns. Differences in distribution profiles of individual components of all amylopectins debranched with isoamylase or pullulanase were assessed using HPAEC–PAD. These results may well be ascribed to substantial differences in the structure among the amylopectins under study. Differences in amylopectin structure may play an important role in the functional properties of starches, supporting the importance of regulating the biosynthetic mechanisms toward specific structures (Visser & Jacobsen, 1993). However, a better knowledge of the structure–function relationship is essential for the design of novel starches.

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