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Characterization of starches dissolved in water by microwave heating in a high pressure vessel

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Starch granules derived from four corn varieties were dispersed in water and depending on variety about 49–71% of the granules dissolved by microwave heating in a high pressure vessel (MWHPV). The apparent ratios of amylopectin to amylose were 1:0, 3:1, 1:1 and 3:7. High performance size exclusion chromatography (HPSEC) was carried out using two μ Bondagel† and one Synchropak† HPSEC columns placed in series. These had size exclusion limits specified by their manufacturers as 400 nm, 100 nm, and 10 nm, respectively. The mobile phase was 0.05 M $NaNO_3$. For each starch composition, refractive index and viscosity chromatograms were obtained and fitted with the same six Gaussian components by nonlinear regression analysis. Calibration of the column set with pullulan and dextran standards in hydrodynamic volume and root mean square radius of gyration (R_g) enabled calculation of the intrinsic viscosity (IV), molecular weight (M), and R_g for each component in addition to global values of these quantities for the entire distribution. Analysis of the data revealed that as starches eluted from the column set, there were large changes in M and R_g and rather small changes in IV. Furthermore, MWHPV containing water as employed here produces starch of relatively large molecular weight and size but low intrinsic viscosity leading to the conclusion that dense starch granule fragments were solubilized.

INTRODUCTION

Since many of the applications of starch involve dissolving or dispersing it in aqueous media, investigation of its properties in aqueous solution would aid in elucidating structure–function relationships in such solvent systems. Developing generalizations about the behavior of starch in water is difficult in that starch, for the most part, is comprised of two chemically homogeneous polysaccharides containing D-glucopyranosyl residues which are heterogeneous with respect to linkage and molecular weight distribution. In the case of corn starch, amylopectin is a group of polymers which are highly branched structures containing (1 → 4)-α-D-glucose and (1 → 6)-α-D-glucose linkages, whereas amylose is much more linear with long stretches of (1 → 4)-α-D-glucose linked monomer units (Manners, 1989). Adding to the complexity of characterizing starch solutions are differences arising from the method of gelatinizing (or melting) and solubilizing amylose and amylopectin from their native starch granules. Also, the limited stability of starch polymers in neutral aqueous solution presents problems.

In spite of the difficulties outlined above, a rapid, relatively reproducible and comprehensive characterization of the aqueous solution properties of starches is now possible. Sample preparation has been accelerated by the development of a high pressure, microwave gelatinization procedure (Delgado *et al.*, 1991). Sample analysis has been facilitated by the development of a method for the simultaneous characterization of molecular weight (M), intrinsic viscosity (IV) and root mean square radius of gyration (R_g) by high performance size exclusion chromatography (HPSEC) with online viscosity detection (DP) and differential refractive index detection (ΔRI) (Fishman *et al.*, 1991*a*,*b*, 1992, 1993). In this paper we characterize the solution properties of corn starches with apparent amylopectin to amylose ratios of 1:0, 3:1, 1:1 and 3:7.

† Reference to a brand or firm name does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

MATERIALS AND METHODS

Solubilization of starch

Commercial corn starches, waxy, common, amylomaize V, and amylomaize VII, were obtained from American Maize Products Co., Chicago, IL. The apparent amylose content of these starches was 0%, 25%, 50% and 70%, respectively (Delgado *et al.*, 1991). The microwave solubilization procedure consisted of placing 88 mg of starch and 20 ml of HPLC grade water in the Teflon cup of a model 4782 polycarbonate microwave bomb (Parr Instr. Co. Moline, IL). After thoroughly shaking, the capped Teflon cup was fitted into the bomb which had its own pressure cap and the entire apparatus was centered inside a model R321T 700 watt microwave oven (Amana Refrigeration Inc., Amana IA). The bomb was heated for 90 s at the highest power setting and allowed to cool in a water bath for 0.5–0.75 h prior to removing the pressure cap. Then the sample was centrifuged at 43 500*g* for 10 min at ambient temperature. The supernatant was quantitatively diluted 4:1 with 0.25 M $NaNO_3$, filtered with a 0.40 μm Nucleopore membrane filter (Costar Corp., Cambridge, MA), and immediately chromatographed. The entire procedure was repeated in triplicate for each corn variety.

HPSEC

Details of chromatography, curve fitting, data reduction, and calculations are given elsewhere (Fishman *et al.*, 1992). Three HPSEC columns which had size exclusion limits specified by the manufacturer as 400 nm, 100 nm and 10 nm were placed in series. These were a μ Bondagel E High Å; (300 × 3.9 mm), μ Bondagel E 1000 (300 × 3.9 mm), Waters Association, Milford, MA; and a Synchropak GPC 100 (250 × 4.6 mm), Synchrom, Inc., Linden, IN. The mobile phase was 0.05 M $NaNO_3$, the injected sample volume was 100 μl, and nominal flow rate was 0.5 ml/min. Detection of solutes as they emerged from the column set was by a model 100 differential viscometer (Viskotek Corp., Houston, TX) and a model 7510 differential refractometer (ERMA Optical Co., Tokyo) in series. Operation of the viscometer was checked by running dextran standards. Intrinsic viscosities agreed within three digits in the second decimal place. The cells of the detectors were thermostatted at 45°C. Columns were immersed in a water bath thermostatted at 45 ± 0.01°C. Since dextrans were more stable in neutral salt solutions than starch and both are polyglucoses, the percentage of initial starch recovered was obtained by chromatographing a series of standard T-70 dextran concentrations and plotting the area of refractive index response under the chromatogram against the weight of dextran injected. The linear response curve generated enabled the starch recovered to be calculated from the area under its refractive index chromatogram. Calibration of the column set was established with a combination of pullulan and dextran standards ranging in MW from 853 000 to 9300 and in R_g from 38.9 to 2.76 nm (Fishman *et al.*, 1987). Log R_g against partition coefficient (K_{av}) (Fishman *et al.*, 1991*a*) and log $IV(M)$ against K_{av} (Fishman *et al.*, 1991*b*) were convergent when plotted for these standards and column set. Fits for component analysis were obtained by assuming the chromatograms to be comprised of a linear combination of partially resolved components. The envelope of the experimental chromatogram is fitted with a preset number of Gaussian shaped components found by minimizing the deviation between the experimental and calculated values of the chromatogram on a point by point basis. Best fits for chromatograms from all the starch varieties were obtained with six components. One iterated value of the quarter width at half height (σ) for the components sufficed for all six components, 0.292 ml. This value was reasonably close to the average experimental value of 0.254 ± 0.007 ml obtained by eluting a series of narrow fractions of pullulans on the column set and measuring the (σ) of the eluted peaks. A nonlinear least squares computer program was used to iteratively minimize the sum of the square of the residuals between the area generated by the components and the experimental chromatogram (Fishman *et al.*, 1992). For the starches under study, we found that once having determined the component peak positions, number of components, and (σ) all from the refractive index chromatogram, the viscosity chromatograms could be fitted with rapid convergence by fixing these values and iterating peak heights with the aid of the computer software.

RESULTS

The percentage of initial starch recovered as supernatant was 48.9 ± 9.9, waxy, 67.3 ± 6.1, common, 61.1 ± 5.9, amylomaize V, and 71.2 ± 0.9, amylomaize VII.

Figures 1–4 contain superimposed traces from the differential refractive index (ΔRI) detector, differential viscosity (DP) detector and IV as a function of K_{av} for each of the starches studied. The relative positions of the two traces have been corrected for offset due to the dead volume between detectors by measuring the volume of eluant between the leading edges of narrow fractions of pullulans measured by the two detectors. The IV was calculated from the areas of the DP and ΔRI response (Fishman *et al.*, 1992). The amylopectin:amylose ratio decreases among starch varieties in the order waxy (Fig. 1) < common (Fig. 2) < amylomaize V (Fig. 3) < amylomaize VII (Fig. 4). The waxy variety which is almost pure amylopectin is dominated

by one large peak and shows a very small change in *IV* with change in size (K_{av}). As the proportion of amylose increases (Figs 2–4), a second peak appears and increases in relative area. Furthermore, the change in *IV* with size also increases with decrease in the amylopectin:amylose ratio. Thus the two detectors give almost superimposable traces in the case of waxy (Fig. 1) and the relative shapes of the two traces diverge with an increasing proportion of amylose (Figs 2–4).

From a visual examination of the chromatograms in Figs 1–4, it appeared that the size distributions are bimodal rather than monomodal in nature with the exception of waxy maize (Fig. 1) . Accordingly, we have analyzed the chromatograms by component analysis, an approach first applied to pectin also a polysaccharide whose size distribution appears to be multimodal in nature (Fishman *et al.*, 1991*a*,*b*, 1992). For the starches under study, we found that once having determined the component peak positions, number of components, and standard deviation (σ) all from the refractive index chromatogram, the viscosity chromatograms could be fitted with rapid convergence by fixing these values and iterating peak heights with the aid of the computer software.

Figures 5–8 contain chromatograms for the four starch varieties which have been analyzed by component analysis. The solid line is the experimental or expected chromatogram whereas the light dotted lines represent the fitted components. The calculated envelope obtained from the point by point sum of these components is represented by the heavy dotted line. As shown by the data in the figures, the calculated envelope

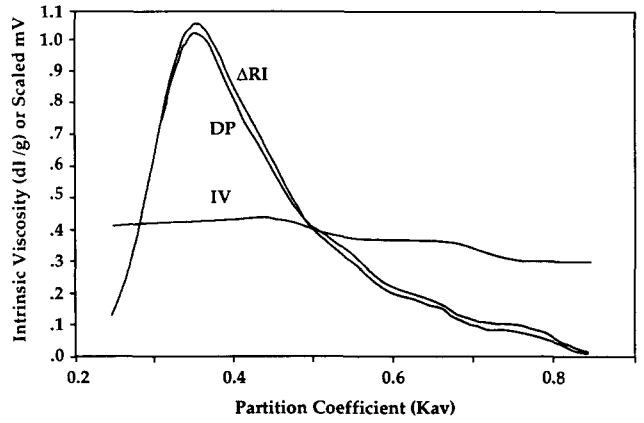

Fig. 1. Waxy maize intrinsic viscosities superimposed on chromatograms. Apparent ratio of amylopectin to amylose (1:0); ΔRI, response from viscosity detector; DP, response from viscosity detector; *IV*, intrinsic viscosity (dl/g); mobile phase, 0.05 M $NaNO_3$; nominal flow rate, 0.5 ml/min; Injection volume 100 μl.

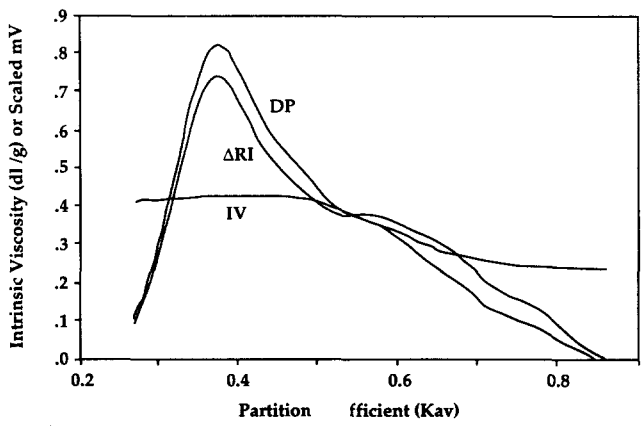

Fig. 2. Common maize intrinsic viscosities superimposed on chromatograms. Apparent ratio of amylopectin to amylose (3:1); ΔRI, response from viscosity detector; DP, response from viscosity detector; *IV*, intrinsic viscosity (dl/g); mobile phase, 0.05 M $NaNO_3$; nominal flow rate, 0.5 ml/min; injection volume 100 μl.

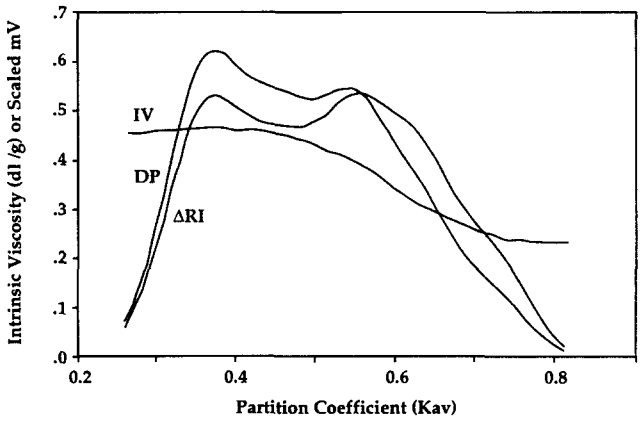

Fig. 3. Amylomaize V intrinsic viscosities superimposed on chromatograms. Apparent ratio of amylopectin to amylose (1:1); ΔRI, response from refractive index detector; DP, response from viscosity detector; *IV*, intrinsic viscosity (dl/g); mobile phase, 0.05 M $NaNO_3$; nominal flow rate, 0.5 ml/min; injection volume 100 μl.

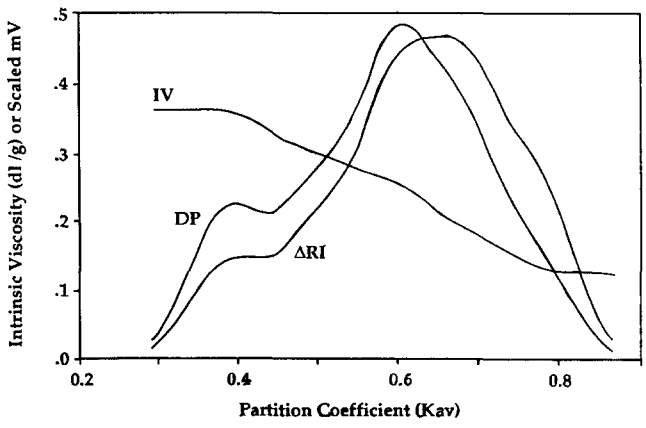

Fig. 4. Amylomaize VII intrinsic viscosities superimposed on chromatograms. Apparent ratio of amylopectin to amylose (3:7); ΔRI, response from refractive index detector; DP, response from viscosity detector; *IV*, intrinsic viscosity (dl/g); mobile phase, 0.05 M $NaNO_3$; nominal flow rate, 0.5 ml/min; injection volume 100 μl.

coincided fairly well with the experimental envelope. The average percentage error between experimental and calculated results for the ΔRI chromatograms of waxy maize, common maize, amylomaize V and amylomaize VII were 6.3, 3.4, 2.3 and 1.1, respectively. Whereas the average percentage error for the experimental and calculated results for the DP chromatograms of waxy maize, common maize, amylomaize V and amylomaize VII were 1.3, 1.4, 1.6 and 0.8, respectively.

Table 1 contains the weight percentages of the components for the four starch varieties. The components are numbered in order of elution with component 1 eluting first and 6 last. As the percentage of amylose increases, the weight fraction of components 1 and 2 decrease whereas the weight fraction of components 3–6 increase.

Table 2 gives the R_g of the components. All components decrease in size with increase in elution volume as would be expected in separation by HPSEC. Components 1 and 2 and possibly 3 decrease in size with increasing percentage of amylose whereas components 4–6 show little if any trend with percentage of amylose. Furthermore, the size differences are rather small or non-existent with percentage of amylose for the last three components. Nevertheless, because the weight fraction of smaller sized starch components increased with increasing percentage of amylose, global averages of size decrease with increasing amylose content (see Table 3).

Table 4 contains the *IV* of the components. For all starch varieties, the decrease in *IV* with increasing order of component elution is extremely small in view of the

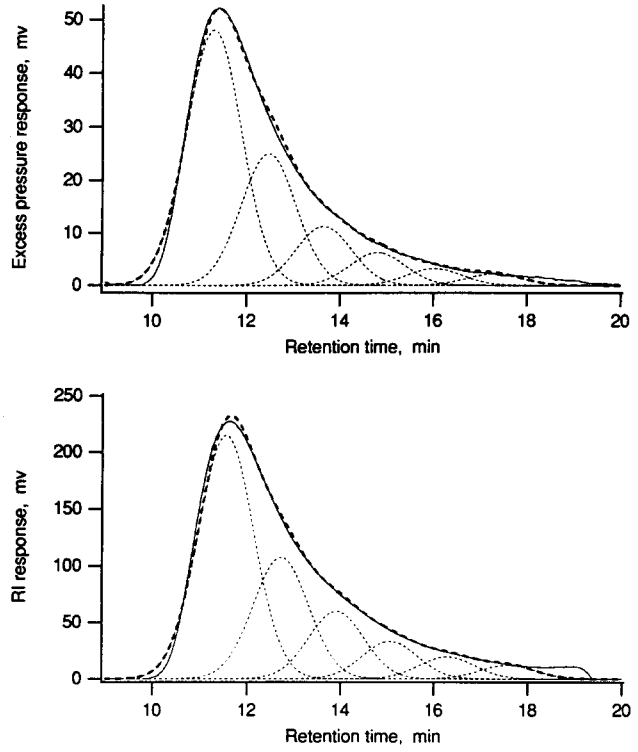

Fig. 5. Waxy maize chromatograms analyzed for components. Solid line, experimental chromatogram; heavy dotted line, chromatogram calculated from sum of components; light dotted line, fitted components. Upper chromatogram, response from viscosity detector. Lower chromatogram, response from refractive index detector.

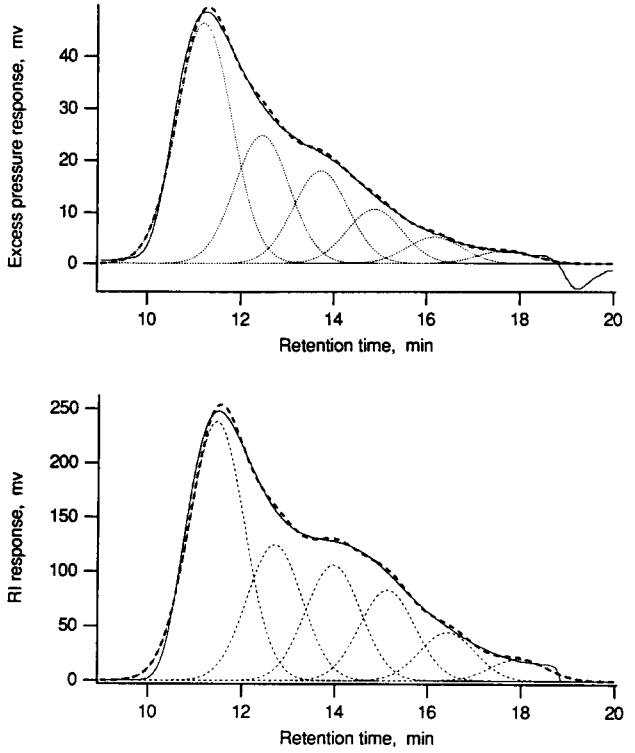

Fig. 6. Common maize chromatograms analyzed for components. Solid line, experimental chromatogram; heavy dotted line, chromatogram calculated from sum of components; light dotted line, fitted components. Upper chromatogram, response from viscosity detector. Lower chromatogram, response from refractive index detector.

Table 1. Calculated percentage by weight of starch components

Sample	Components					
	1	2	3	4	5	6
Waxy	46 ± 1[a]	24 ± 1	14 ± 1	8 ± 1	5 ± 1	3 ± 1
Common	38 ± 2	21 ± 1	17 ± 1	14 ± 1	8 ± 1	3 ± 1
AMY V	24 ± 2	19 ± 1	22 ± 1	19 ± 1	11 ± 1	5 ± 1
AMY VII	11 ± 1	13 ± 1	27 ± 1	25 ± 2	16 ± 1	7 ± 1

[a] ± Standard deviation.

rather large decreases in R_g for the same components. As noted when comparing the continuous change in *IV* with partition coefficient (Figs 1–4), the magnitude of the reduction in *IV* increases with increasing amylose content in the starch variety. The small change in *IV* with size is reflected in the small differences between global moments within varieties (Table 5). The global values of *IV* clearly reveal that *IV* decreases with increasing amylose content of the starch.

Table 6 contains the component molecular weights. Trends in molecular weight are comparable with those found for R_g. Namely, molecular weight decreases with increasing order of component elution and decreases with increasing amylose content for components 1–3. Differences among varieties for components 4–6 are not significant with the exception of waxy maize components whose molecular weights are lower than for the other three varieties. Global molecular weight averages (Table 7) decrease with increasing amylose content.

DISCUSSION

Starch polymers have often been characterized by one of two general approaches. They have been isolated and characterized separately or investigated as mixtures as a function of amylopectin:amylose ratio. When investigating mixtures, the physical properties of the individual polymers are inferred by relating changes in global physical properties to changes in composition. By analyzing whole starch samples with component analysis in conjunction with HPSEC and online viscometric

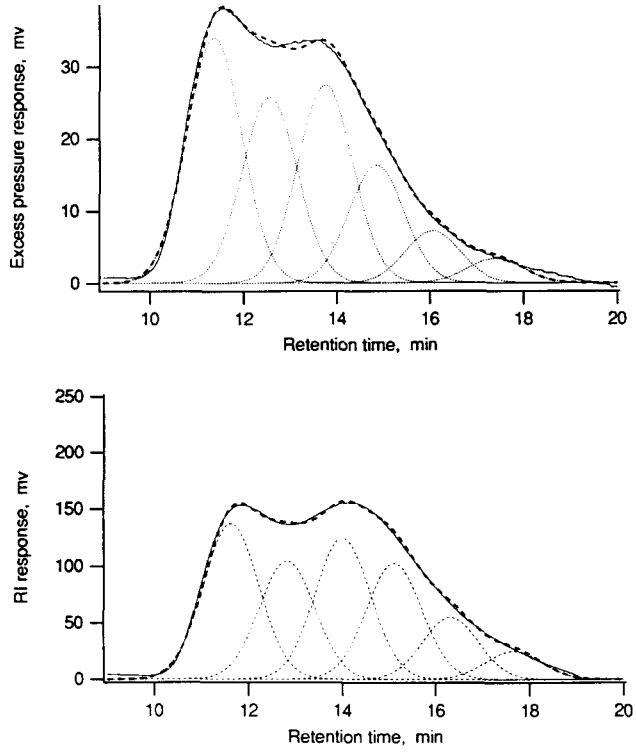

Fig. 7. Amylomaize V chromatograms analyzed for components. Solid line, experimental chromatogram; heavy dotted line, chromatogram calculated from sum of components; light dotted line, fitted components. Upper chromatogram, response from viscosity detector. Lower chromatogram, response from refractive index detector

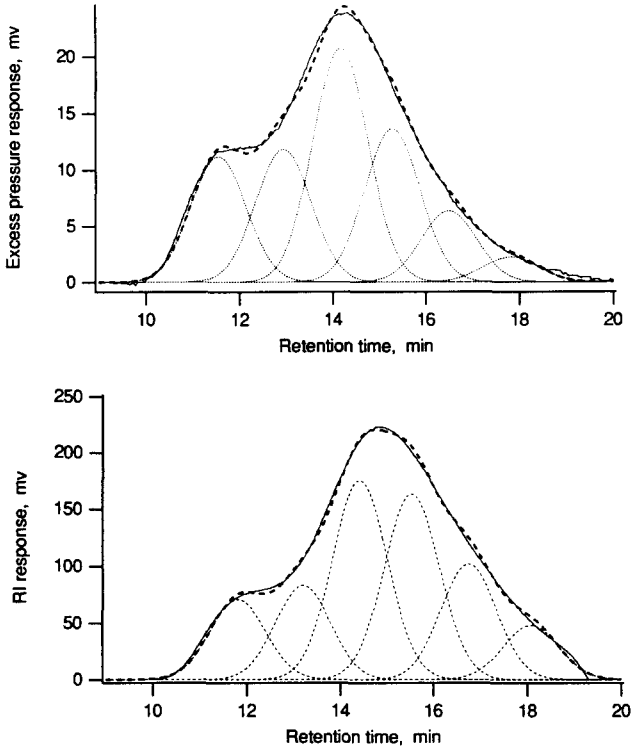

Fig. 8. Amylomaize VII chromatograms analyzed for components. Solid line, experimental chromatogram; heavy dotted line, chromatogram calculated from sum of components; light dotted line, fitted components. Upper chromatogram, response from viscosity detector. Lower chromatogram, response from refractive index detector.

Table 2. Radius of gyration of starch components (nanometers)

Sample	Components					
	1	2	3	4	5	6
Waxy	95 ± 2[a]	44 ± 2	19 ± 1	9·5 ± 0·5	5·2 ± 0·3	2·5 ± 0·4
Common	89 ± 6	41 ± 5	19 ± 2	10 ± 1	6·1 ± 0·6	3·6 ± 0·5
AMY V	84 ± 5	38 ± 3	17 ± 2	9·4 ± 1	5·6 ± 0·6	3·3 ± 0·4
AMY VII	73 ± 5	29 ± 2	14 ± 1	8·0 ± 0·4	4·9 ± 0·2	3·0 ± 0·1

[a] ± Standard deviation.

Table 3. Global average radius of gyration of starches (nanometers)

Sample	R_{gn}[a]	R_{gw}[b]	R_{gz}[c]
Waxy	28 ± 1[d]	60 ± 2	81 ± 2
Common	22 ± 2	48 ± 3	72 ± 4
AMY V	16 ± 2	35 ± 3	61 ± 3
AMY VII	10 ± 1	20 ± 2	41 ± 4

[a]Number average.
[b]Weight average.
[c]Z-average.
[d]± Standard deviation.

detection (Haney, 1985), we can simplify data interpretation somewhat and possibly eliminate the need for performing time consuming isolation of components. At the same time we can retain the speed, convenience, and the ability to measure structures with properties intermediate between 'linear' amylose and highly branched amylopectin as may be found when measuring mixtures. Recently, a similar conclusion appears to have been reached by another group of researchers (Klingler & Zimbalski, 1992).

The percentage of starch recovered was higher for starches containing amylose than for waxy maize. The time of microwaving was selected to maximize both molecular weight and recovery for the waxy maize variety. One possible interpretation of these results is that amylopectin is more difficult to solubilize when pure than when in the presence of amylose and that once amylopectin is solubilized, it is degraded by further heating. The difficulty of completely solubilizing starches without reducing the molecular weight has been reported before (Jackson *et al.*, 1988; Delgado *et al.*, 1991).

Comparison of the *IV*s in Table 4 with the R_gs in Table 2 and molecular weights in Table 6 reveals that components 1 and 2 of all varieties except amylomaize VII exhibit large changes in R_g and molecular weight at practically constant *IV*. Constant *IV* values and higher *IV*, R_g, and *M* values for components 1 and 2 as compared to the remaining components in all varieties but amylomaize VII tend to suggest that these two components are amylopectin in these varieties. Banks *et al.* (1972) found that starch isolated from waxy maize by boiling in water and stirring at high speeds gave values of 10×10^6, 0.35 dl/g, and 105 nm for M_w (weight average molecular weight), *IV*, and R_{gz} (*z*-average radius of gyration), respectively, whereas they found values of 400×10^6 and 1.0 dl/g for M_w and *IV*, respectively when the sample was solubilized with dimethylsulfoxide. They attributed the lower values to shear degradation as a result of high speed stirring. We found values of 19.5×10^6, 0.40 dl/g and 81 nm for M_w, *IV*, and R_{gz}, respectively for waxy maize. Component 1 of waxy maize had values of 38.1×10^6, 0.42 dl/g and 95 nm for M_w, *IV*, and R_g, respectively. Our samples were heated for 90 s under conditions which were comparable to heating under rapid pressurization followed by cooling for 30–45 min. Samples were not stirred or sonicated. Even though our values for M_w, *IV*, and R_{gz} compare well with the values obtained by Banks *et al.* (1972) when they heated starch in water, shear degradation during the microwave procedure appears unlikely. Nevertheless, molecular weight reduction during microwaving through breaking of covalent bonds by heating is possible. Nevertheless, as indicated by what follows, gelatinization by the microwave method may have resulted in the solubilization of fragments of intact dense amylopectin such as found within the thick lamella or growth rings of common starch (French, 1984).

In another study (Young, 1984), the M_w and *IV* were reported for starch from the same four varieties of corn as were under investigation in this study. Starch granules were gelatinized with dimethyl sulfoxide (DMSO) for 1 h at 150°C. The M_w was determined by size exclusion chromatography on low performance porous glass bead columns. The M_w values were 21.8, 14.5, 5.75, and 3.96×10^6 for waxy, common, amylomaize V and amylomaize VII, whereas the *IV* values in 1 M KOH at 35°C were 1.30, 1.53, 1.01 and 0.92 dl/g for starch from the same four varieties. Comparison of these values with the M_ws in Table 7 and the global weight average *IV* values in Table 5, reveals that the molecular weights for various starches from the two studies were comparable, whereas the *IV*s were considerably larger in KOH. The larger *IV* found for starch in a strong base as compared to a neutral salt may reflect

Table 4. Intrinsic viscosity of starch components (dl/g)

Sample	Components					
	1	2	3	4	5	6
Waxy	0·42 ± ·01[a]	0·43 ± ·01	0·36 ± ·01	0·36 ± ·01	0·33 ± ·03	0·21 ± ·04
Common	0·42 ± ·01	0·43 ± ·01	0·37 ± ·01	0·29 ± ·02	0·25 ± ·02	0·21 ± ·03
AMY V	0·43 ± ·04	0·43 ± ·04	0·37 ± ·06	0·27 ± ·05	0·22 ± ·04	0·17 ± ·06
AMY VII	0·35 ± ·01	0·32 ± ·03	0·26 ± ·01	0·18 ± ·01	0·14 ± ·01	0·10 ± ·01

[a]± Standard deviation.

Table 5. Global average intrinsic viscosity of starches (dl/g)

Sample	$[\eta]_n$[a]	$[\eta]_w$[b]	$[\eta]_z$[c]
Waxy	0·40 ± 0·01	0·40 ± 0·01	0·40 ± 0·01[d]
Common	0·37 ± 0·01	0·38 ± 0·01	0·38 ± 0·01
AMY V	0·31 ± 0·05	0·33 ± 0·05	0·35 ± 0·05
AMY VII	0·22 ± 0·01	0·24 ± 0·01	0·26 ± 0·02

[a] Number average.
[b] Weight average.
[c] Z-average.
[d] ± Standard deviation.

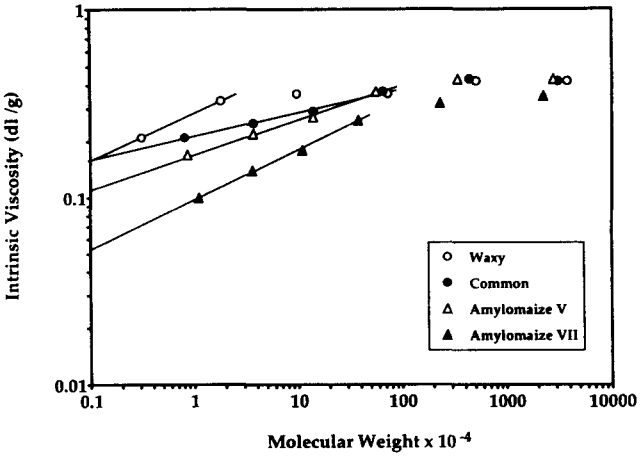

Fig. 9. Mark–Houwink plot of starch components from four varieties of corn.

the greater solvating power of base over neutral salt. Amylose alone appears to behave in a similar fashion when dissolved in base or neutral salt (Roger & Colonna, 1992).

To further investigate the relative amounts of amylose and amylopectin in the components, a Mark–Houwink plot was constructed for the components of each variety (Fig. 9). In the case of common, amylomaize V, and amylomaize VII, components 3–6 appear to give a linear plot at molecular weight values less than 1×10^6. As suggested previously, the *IV* of components 1 and 2 of waxy, common and amylomaize V were uncorrelated with molecular weight which may indicate a behavior in solution which is similar to the behavior expected for dense spherically shaped structures. Components 1 and 2 of amylomaize VII had significantly lower values of *IV* and somewhat lower molecular weights than comparable components from the other varieties indicating that these components may have contained a significant amount of amylose in addition to amylopectin. A linear Mark–Houwink plot was not found for components 3–6 of waxy maize. Mark–Houwink plots were constructed for components 3–6 for all varieties but waxy (Fig. 9). The values of K derived from the intercept, the slope, α, and the correlation coefficient are included in Table 8. The relatively low values of the Mark–Houwink exponents, α, for components 3–6 in common, amylomaize V and VII as compared with the α values found for isolated amylose in neutral salt solution (i.e. 0.12–0.27 against 0.50–0.530) may indicate that a portion of the amylose is significantly branched or that it is associated with amylopectin in these components (Young, 1984). A third possibility is that several linear amylose chains are aggregated with considerable side by side overlap. Pectin, a linear polysaccharide, exhibits a lower than expected Mark–Houwink exponent because of side by side aggregation (Fishman *et al.*, 1993). A plot of log *IV* against log R_g (not shown) was found to be similar to Fig. 9 in its functionality.

Another method of measuring shape is to plot log R_g against log M (Yau *et al.*, 1979). In such a plot, a value of 0.5 for the exponent of M is indicative of a non-draining coil, whereas an exponent of 1 for M is indicative of a rod. In Fig. 10, log R_g is plotted against log M for the components of all the starch varieties. Surprisingly, the points from all varieties showed a high degree of linear correlation. The exponential value of M was 0.397, the pre-exponential term was 0.0910 and the correlation coefficient was 0.998. Thus the scaling of R_g on M for all components is less than expected for a non-draining coil, a result generally consistent with the scaling of *IV* on M.

Roger and Colonna (1992) determined that 0.1 M KCl was a psuedo ideal (Θ) solvent for a series of monodispersed synthetic, linear amyloses by plotting R_g^2/M against $M^{0.5}$ and observing that the slope of this linear plot was zero. A 'good' solvent for amylose,

Table 6. Molecular weight of starch components $\times 10^{-5}$

Sample	Components					
	1	2	3	4	5	6
Waxy	381 ± 24[a]	51 ± 4	7·3 ± 0·7	0·97 ± 0·01	0·18 ± 0·04	0·031 ± 0·009
Common	317 ± 48	44 ± 12	6·5 ± 2·1	1·4 ± 0·4	0·37 ± 0·12	0·081 ± 0·036
AMY V	282 ± 14	34 ± 12	5·6 ± 0·6	1·4 ± 0·2	0·37 ± 0·05	0·086 ± 0·007
AMY VII	224 ± 38	23 ± 2	3·8 ± 0·7	1·1 ± 0·2	0·36 ± 0·02	0·11 ± 0·01

[a] ± Standard deviation.

Table 7. Global average molecular weight of starches $\times 10^{-5}$

Sample	M_n[a]	M_w[b]	M_z[c]
Waxy	2·6 ± 0·5[d]	195 ± 13	358 ± 22
Common	2·8 ± 0·6	133 ± 18	294 ± 41
AMY V	2·0 ± 0·3	80 ± 4	251 ± 11
AMY VII	1·3 ± 0·3	32 ± 8	192 ± 35

[a] Number average.
[b] Weight average.
[c] Z-average.
[d] ± Standard deviation.

Table 8. Mark–Houwink constants for components 3–6 of the starches

Sample	$K \times 10^4$ (dl/g)	α	Corr. coeff
Waxy	—	—	—
Common	656	0·13	0·996
AMY V	320	0·18	0·997
AMY VII	84·6	0·27	0·999

0.1 M NaOH, gave a linear plot with a positive slope. The function plotted was derived on the assumption that the expansion of amylose with increasing molecular weight was due strictly to the excluded volume effect. We constructed a similar plot for the components of our four varieties (Fig. 11). Physically, the term R_g^2/M is the cross sectional area through the center of a sphere generated by the Brownian motion of a molecule with a radius of gyration R_g and normalized by its molecular weight. In the case of our samples, analyzing changes in this area is somewhat more complicated than analyzing the area expansion of pure linear amylose. Changes in cross sectional area in our samples may arise from changes in the amylopectin:amylose ratio and in chain branching of amylose molecules in addition to changes in the excluded volumes. Nevertheless, analysis of the data in Fig. 11 proved informative. Component 1 exhibited virtually no change in cross sectional area with starch variety. The same could be said of components 2 and 3. Yet considered as a group component 1 has a slightly smaller area than 2 which is slightly smaller than component 3. The probability is strong that component 3 contains some amylose or some amylose/amylopectin intermediate(s). Mark–Houwink plots (Fig. 9) indicate that components 1 and 2 of amylomaize VII are different from the same components of the other three varieties, also possibly indicating the presence of amylose or an amylose/amylopectin intermediate. Therefore we must conclude that $NaNO_3$ is a Θ solvent for all species in addition to amylopectin in components 1 and 2 of amylomaize VII and component 3 of all varieties. Furthermore, the increase in cross sectional areas with decreasing molecular weight probably arises from a decrease in chain branching particularly in the case of components 3–6 since the percentage of amylose is also increasing. In the case of waxy maize, components 4–6 represent about 16% of its weight, which is far in excess of any possible contamination by amylose. Interestingly, the chains of waxy maize components 4–6 have larger cross sectional areas (i.e less branched) than the chains of comparable components from the other starch varieties. Therefore, based on standard amylose/amylopectin classifications, waxy maize components 4–6 might be considered linear fragments of amylopectin. Whereas components 4–6 of common maize have smaller cross sectional areas than comparable components from waxy maize, they have larger areas than comparable components from amylomaize V or VII. Components 4–6 comprise about 25% of common maize by weight which is the weight percent attributed to the amylose portion of that starch. Components 4–6 comprise about 35% of amylomaize V whereas they comprise about 48% of amylomaize VII which is well below the 50% and 70% of amylose reported for these two starches, respectively. Therefore these components should contain primarily amylose

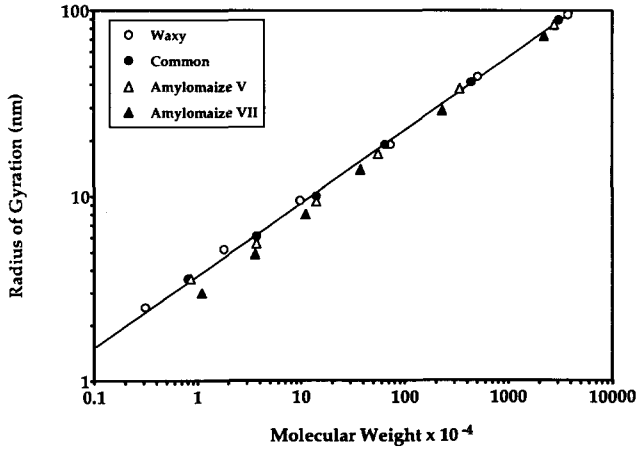

Fig. 10. Radius of gyration plotted against molecular weight of starch components from four varieties of corn.

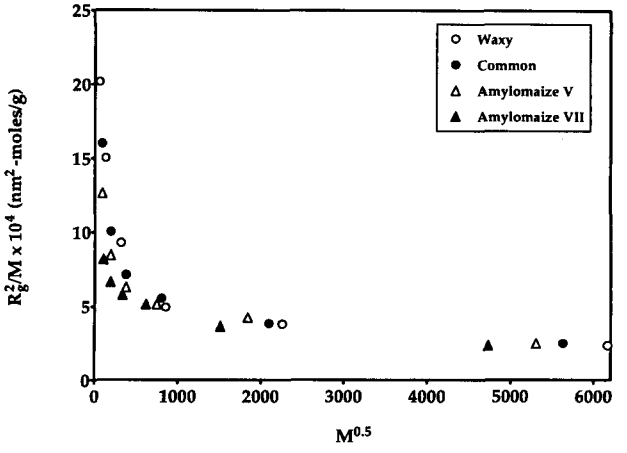

Fig. 11. Cross-sectional area plotted against square root of molecular weight of starch components from four varieties of corn.

whereas component 3 for amylomaize V and VII, as suggested above, should be a mixture of amylose and amylopectin since components 3–6 are about 57% and 75% of the total starches, respectively.

Because the method employed to fit curves does not provide a unique solution to the problem, the 'good' fits obtained in Figs 5–8 do not in themselves constitute evidence that starch distributions are multimodal in nature. The main justification for employing the method is the enhanced insight gained over merely calculating the conventional global averages such as number, weight and z-average. Nevertheless, there is circumstantial evidence indicating the possibility of multimodal starch distributions. Recently, the advantages of materials with structural hierarchy have been discussed and it was noted that many biological materials possess structural hierarchy (Lakes, 1993). Hierarchical structures are often accompanied by structures which are self similar over several orders of size magnitude. For example bone is a composite with a hierarchical structure over multiple size levels. Furthermore it possesses a concentric fibrous laminar structure with cement lines. These and other features of its hierarchical structure, it has been suggested, contribute to bone stiffness, toughness, and its ability to withstand mechanical stress. Electron micrographs of starch granules indicate that they too possess a concentric fibrous laminar structure termed growth rings (French, 1984). At a lower size level, Hizukuri (1986) showed that debranched amylopectins possessed a polymodal distribution of chain lengths when subjected to HPSEC. In other research, Hizukuri's group fractionated amylose into three subfractions by preparative size exclusion chromatography (Takeda *et al.*, 1992). The parent and the chromatogram reconstructed from the subfractions appeared to be trimodal in nature.

CONCLUSIONS

In this study we have found that the IV of starch solubilized by microwave radiation with rapid heating and pressurization in water was considerably less than by conventional dissolution in solvents such as dimethyl sulfoxide or alkali, whereas M and R_g have been reduced to a much lesser extent. On the basis of these results we have concluded that this method as employed here solubilizes dense starch granule fragments of relatively large size and molecular weight. Furthermore, we have demonstrated the potential of component analysis in conjunction with HPSEC and online viscometric detection to rapidly characterize amylopectin and amylose when each is present in gelatinized starch.

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