

Macromolecular Features of Amaranth Starch

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ABSTRACT

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High-performance size-exclusion chromatography (HPSEC), static light scattering (SLS) and dynamic light scattering (DLS) techniques were used for the structural characterization of amaranth starch, solubilized in water by microwave heating in a high-pressure vessel. Apparent average molar mass (M_w), gyration radius (R_G), and hydrodynamic radius (R_H) values were obtained from Berry and Zimm treatment of light-scattering data. When heating time increased from 35 to 90 sec, the M_w , R_G , and R_H decreased, demonstrating a possible polymer degradation due to temperature. Apparent M_f values from HPSEC at 35 sec ($27 \pm 2 \times 10^7$ g/mol) and 50 sec ($20 \pm 2 \times 10^7$ g/mol) were lower than those determined by SLS (35 sec = 69×10^7 g/mol, 50 sec = 56×10^7 g/mol). However, at 70 and 90 sec, the inverse pattern was obtained. The fractal dimensions (d_f')

from HPSEC study for samples dissolved for 35 (3.26), 50 (3.24), and 70 sec (3.14) are characteristic of a particle that has the internal structure of hard sphere, and for samples dissolved for 90 sec (2.19), are characteristic of a fully swollen, randomly branched macromolecule. From SLS, d_f' decreased with increasing treatment time ($d_f' = 2.44, 2.18, 1.50,$ and 1.03 for 35, 50, 70, and 90 sec, respectively). The particle-scattering factors and Kratky plots, well-suited for studying the internal structure of a macromolecule, showed a sample degradation when treatment time increased. Results from DLS showed bimodal distributions with differences in the peak locations when treatment time increased. The ratio of R_G to R_H (ρ) for samples analyzed were between 0.88 and 1.3; these values are characteristic of a sphere or globular structure.

The potential of amaranth grain as food was recognized by earlier cultures in America; the Aztecs and Incas used it commonly until it was replaced after the Spanish Conquest, for reasons not well-known, by other crops such as corn and common beans. Although there are still some farmers dedicated to its seeding and commercialization, in many places amaranth remains only as a familiar ornamental in the backyard. Interestingly, the word amaranth means "everlasting" in Greek, which seems adequate for the history of this crop (Lozoya-Gloria 1994). For some years now, amaranth has been "rediscovered" as a useful and promising plant, mainly because it looks like an interesting candidate to alleviate the increasing need for food by some countries of the developing world.

Amaranth is resistant to drought, hot climate, and various pests (Paredes-López et al 1988). The grain contains protein, lysine, fat, fiber, ash, and minerals in higher amounts than in common cereal grains (Uriyapongson and Rayas-Duarte 1994). The seeds contain $\approx 67\%$ of uniform starch granules. Also, they are only between 0.8 and 2.5 μm in diameter and are spherical or polygonal in shape (López et al 1994). Therefore, they have a very large surface area per unit of weight when compared to that of most other starches; this suggests a wide variety of possible applications (Zhao and Whistler 1994). Amaranth has been classified as a "waxy type" starch; waxy starches are characterized by high amylopectin content. This composition confers very useful and unique properties, such as high viscosity and gelatinization at high temperatures (López et al 1994).

Diverse methods have been reported for amaranth starch isolation (Paredes-López et al 1989, Paredes-López and Hernández-López 1991, Pérez et al 1993, Uriyapongson and Rayas-Duarte 1994, Zhao and Whistler 1994), and some differences were found among different species in the physicochemical and functional properties of those starches.

Starch is widely used in many industries where its functional properties require dissolution or dispersion in aqueous media under nondegradative conditions (pH 4–10). Functional properties are directly influenced by the average molar mass of amylose and

amylopectin, as demonstrated in gels (Clark et al 1989), extrusion products (Della Valle et al 1996), and starch pastes (Doublier et al 1986). Recent advances in scientific instrumentation have triggered works on the structure and properties of native, nondegraded starch components (Aberle et al 1994, Fishman and Hoagland 1994, Galinsky and Burchard 1995). Most structural studies of starch components have been realized after physical (extrusion, autoclaving, pasting) or enzymatic (isoamylase, pullulanase, α -amylase and β -amylase) degradation. The different structural elements can then be studied using either high-performance size-exclusion chromatography (HPSEC) methodologies (Takeda et al 1993, Shibnuma et al 1994, Ong and Blanchard 1995, Striegel and Timpa 1995), high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) (Shi and Seib 1995, Hanashiro et al 1996), or sedimentation field flow fractionation combined with multiangle laser-light scattering (MALLS) (Hanselmann et al 1995). But a prerequisite step to the determination of the molecular weight distribution of amylose and amylopectin by any technique, is the complete dissolution of the starch sample in an appropriate solvent without degradation of the constitutive macromolecules. To fulfill this requirement, it is necessary to get information representative of the initial starch sample.

Until now, very few studies on the structural characterization of amaranth starch have been reported. Studies have been made using gel filtration after debranching amaranth starch, and a bimodal distribution of chains was reported (Sugimoto et al 1981, Konishi et al 1985, Paredes-López et al 1994). Recently, HPSEC with refractive index (RI) and HPAEC-PAD have been used to elucidate the profiles of amaranth amylopectin after debranching with isoamylase and pullulanase enzymes (Bello-Pérez et al 1996b). Additionally, HPSEC and light-scattering (LS) techniques were utilized for structural characterization of amaranth amylopectin after debranching with isoamylase and pullulanase enzymes and hydrolysis with α -amylase and β -amylase (Bello-Pérez et al 1996a). However, up to now, there were no data available on the structure of nondegraded amaranth starch.

Thurn and Burchard (1985) postulated that the various structural elements of a macromolecule, such as polydispersity, overall molecular dimensions, hydrodynamic behavior, and internal mobility, should exert a marked influence on its properties in solution. For this reason, they became interested in the structure and properties of native, nondegraded starch, which can be determined by LS. There are two types of LS measurements, dynamic and static (DLS and SLS, respectively). The foundation of DLS is based on the scattering of light by moving particles (Dalglish and Hallet 1995). In SLS, the intensity of scattered radiation is aver-

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aged over a fairly long time (≈ 2 sec), and this is in most cases, long enough to smooth out all internal mobility (Burchard 1992). SLS gives information about the average molecular weight (M_w), the root mean square z-average radius of gyration (R_G), and the LS second osmotic virial coefficient (A'_2) of macromolecules in dilute solution (Anthonsen et al 1994). Measurements at different molar masses reveal an unexpected weak increase of the R_G , which is

due to lateral (side-by-side) aggregation of chains. The conclusion could be confirmed by DLS, according to which, an increase in segment density occurs as the molar mass increases (Burchard 1993). Recent developments of DLS techniques allow a determination of the relaxation time distribution over a large time range. If different components of the system have characteristic relaxation times that are not too close, they can be determined individually in one measurement (Ousalem et al 1993).

The present study was undertaken to characterize the dimensions and structural properties of amaranth starch using successive steps of pretreatment of the sample, followed by a solubilization in water by microwave heating, and analysis by SEC-MALLS-RI and LS techniques.

MATERIALS AND METHODS

Starch Isolation and Sample Pretreatment Preparation

Amaranth starch (waxy type) was isolated as reported previously (Paredes-López and Hernández-López 1991). It contained 2.0% amylose. The sample (1 g) was first dissolved in 95% dimethyl sulfoxide (DMSO) (20 mL) with magnetic stirring for three days at room temperature. After the sample was precipitated with ethanol (60 mL) and stored one night at 4°C, it was filtered over a glass filter and washed successively with acetone (10 mL) and diethyl ether (10 mL). The precipitate was maintained under a hood for few hours to eliminate the solvents and finally dried in a vacuum at 45°C for 18–24 hr.

Sample Solubilization

The solubilization procedure consisted of weighing 10 mg of sample inside a microwave bomb and adding 20 mL of water previously filtered through a 0.1- μm membrane (Anotop, Whatmann, Maidstone, England). The solution was heated for 35, 50, 70, and 90 sec in a microwave (900W) oven. After cooling in an ice bath (30 min), the solution was centrifuged ($31,200 \times g$ for 10 min) and filtered (5 μm). Dilution series were made at room temperature, yielding four lower concentrations. Carbohydrate concentration was measured by the sulfuric acid-ornicol colorimetric method (Planchot et al 1996).

HPSEC and Data Analysis

The HPSEC system included a programmable HPLC-pump (Waters 590, Waters, Milford, MA), an autosampler (Waters 717), and a degasser (ERC-3312, Erma Optical Works, Japan). Dual detection of solutes was realized by a MALLS detector (Dawn DSP-F, Wyatt Technology Corp., Santa Barbara, CA), and a differential RI detector (ERC-7510) in series. Photodiode coefficients were normalized with pullulan as a reference. The size-exclusion system was composed of three columns (TosoHaas, Stuttgart, Germany): TSK gel SWXL guard column, 6-mm i.d. \times 4-cm.; TSK gel G4000 SWXL 7.8-mm i.d. \times 30-cm.; and TSK gel G3000 SWXL 7.8-mm i.d. \times 30-cm. All three columns were maintained at 30°C. The eluent was water with 0.02% sodium azide carefully degassed and filtered before use through durapore GV membranes (0.2 μm , Millipore). The mobile phase had a flow rate of 0.5 mL/min. Sample (100–500 μL) was injected into the HPSEC system. Operation of the MALLS was checked by running dextran as a standard. The M_w and R_G were established using ASTRA software for Macintosh (v. 1.2) (Wyatt 1993).

The lowest error for the determination of M_w and R_G were obtained using the Berry method (ASTRA); only the lower angles were used (from 22 to 90°) with a second-order polynomial fit.

SLS

Experiments were performed at 25°C in the angular range from 30 to 150° in steps of 15° in the homo-dyne mode with full photon-

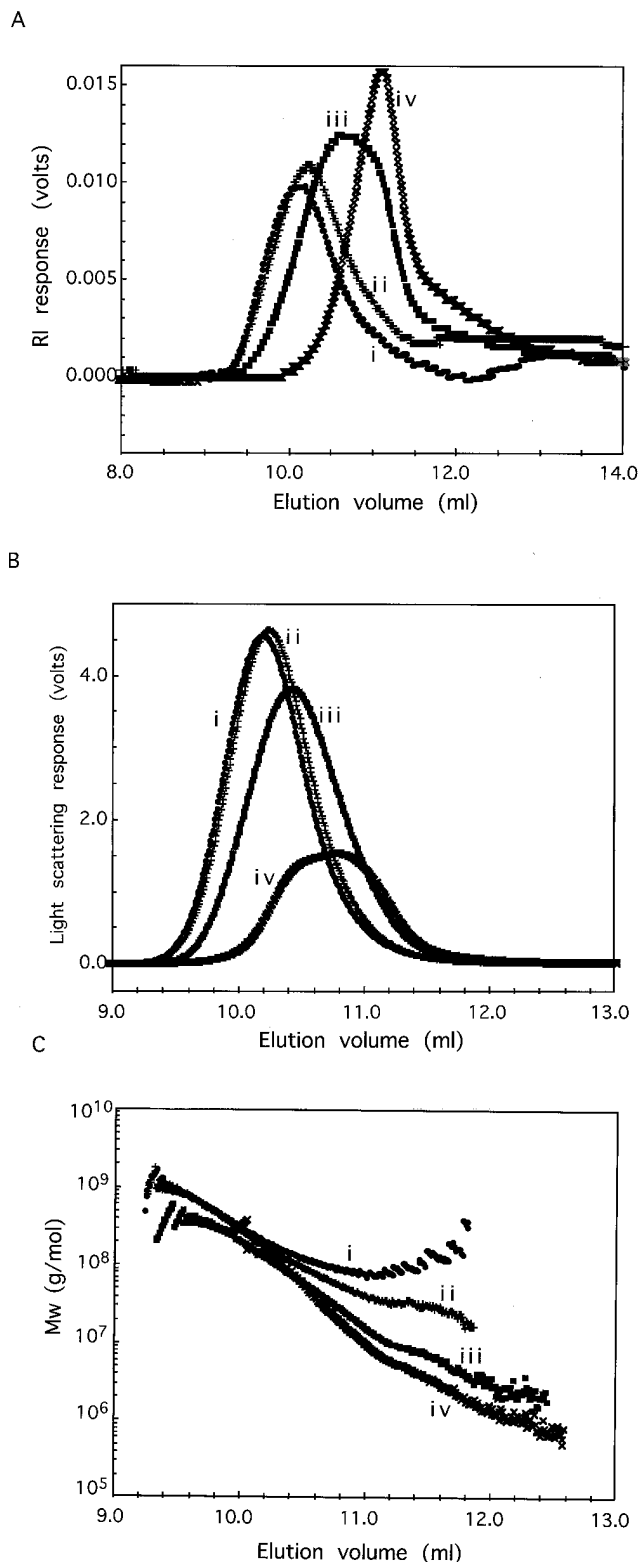


Fig. 1. High-performance size-exclusion chromatography profiles of amaranth starch dissolved for different time periods (i–iv = 35, 50, 70, and 90 sec, respectively). **A**, Refractive index (RI) response; **B**, light scattering at 90°; **C**, molecular mass (M_w) vs. elution volume.

counting detection and a 128-channel K7025 Malvern correlator. Incident radiation at 514.5 nm was obtained from a 3W Ar Ion Spectra-Physics laser vertically polarized. The RI increment (dn/dc) was 0.146 mL/g (Roger and Colonna 1993, Fishman et al 1996). Optical alignment was checked over the angular range described using filtered (0.1 μ m) benzene. Monitoring programs were written in Basic and run on an HP9300 microcomputer. All fittings were performed using a Berry plot with a homemade program.

DLS

Measurements were made with an ALV 5000 correlator (ALV-Laser, Vertriebsgesellschaft mbH Lagen, Germany) from 30 to 150° in steps of 30° using the laser equipment listed above. For the data treatment, a cumulative analysis was used to get R_H (ALV-5000 software, v. 5.0), and the REPES method (Stepánek 1993) was used to determine relaxation time distributions.

RESULTS AND DISCUSSION

Solubilization Extent

Solubilization extent corresponds to the percentage of initial material recovered in the supernatant after centrifugation and filtration. Solubilization extent for amaranth starch was: 83.7, 86.0, 97.5, and 100% for 35, 50, 70, and 90 sec of microwave heating, respectively. Lower values were reported by Fishman and Hoagland (1994) using microwave heating for 90 sec: 48.9% for waxy corn, 67.3% for common corn, 61.1% for amylo maize V, and 71.2% for amylo maize VII. Recently, for starches solubilized using a microwave oven for 80 sec, Fishman et al (1996) found recovery percentages of 63.0% for waxy corn, 65.0% for common corn, 72.0% for amylo maize V, and 81.0% for amylo maize VII.

As indicated above, a prerequisite step to the determination of the molecular weight distribution of amylose and amylopectin by any technique is the complete dissolution of the starch sample in an appropriate solvent without any degradation of the constitutive macromolecules. Absence of sample degradation with microwave heating for 35 sec was obtained (data not shown).

Macromolecular Features Determined by HPSEC-MALLS

Aqueous starch analysis by HPSEC using polyhydroxy-methyl-methacrylate-based SEC columns gave a low percentage of mass recovery (Roger and Colonna, unpublished results). Problems arose mainly with amylopectin, which is not eluted quantitatively from the columns, in contrast to amylose (Roger and Colonna 1993). Amylopectin is the principal component of amaranth starch (98.6%), and there are problems with complete starch solubilization and determination with confidence of macromolecular features (features important in determining its functional properties) (Doublier et al 1986, Della Valle et al 1996).

The RI chromatograms of amaranth starch (Fig. 1A) showed that at longer heating times, chromatograms are shifted to a higher elution volume (V_e). The peak maxima were 10.1, 10.2, 10.7, and 11.1 mL, respectively, for heating times of 35, 50, 70, and 90 sec. Furthermore, the plots of M_w against V_e (Fig. 1B) showed differences. For the same V_e (10.8 mL), the sample at 35 sec had a higher M_w value than samples at 50, 70, and 90 sec. For LS traces at

90° (Fig. 1C), at approximately the same total concentration, signal intensity generally decreased when treatment time increased.

The determination of M_w and R_G are based on SEC-MALLS results. The M_w and R_G values are shown in Table I. The general tendency was that M_w and R_G values decreased as a function of heating time, demonstrating that molecular degradation occurred. This conclusion can be drawn because the complete solubilization at 70 and 90 sec of heating in our procedure rules out changes in the molecular weight of the fractions solubilized. The M_w value at 35 sec was higher than those found for corn amylopectin ($2.2 \pm 0.2 \times 10^8$ g/mol) and waxy corn starch ($2.0 \pm 0.2 \times 10^8$ g/mol) using the same method (Bello-Pérez et al, unpublished results). However, the R_G value for amaranth starch (176 nm) was lower than those for corn amylopectin (229 nm) and waxy corn starch (234 nm). These results point out that amaranth starch has a more compact structure that could be related to the branching differences between waxy corn starches and amaranth starch. The M_w value for amaranth starch dissolved for 50 sec ($2.0 \pm 0.1 \times 10^8$ g/mol) is the same as that for corn amylopectin heated at the same treatment time. Again, the R_G value for amaranth (166 nm) was lower than that for corn amylopectin (208 nm). At longer treatment times, M_w and R_G decreased. These values were lower than those found for corn amylopectin at the same heating times. These results show that amaranth starch is more easily degraded by heating than is corn amylopectin.

Information about the structure of polymers can be obtained from the particle-scattering factor $P(q)$: $P(q) = R(\theta)/R(\theta = 0)$, which describes the angular distribution of the scattered light. In Fig. 2, the particle-scattering factors from six different fractions of the amaranth starch sample, heated for 35 sec, are plotted on a double logarithmic scale against the dimensionless parameter u ($\equiv qR_G$), where q is the scattering vector: $q = (4\pi n/\lambda) \sin(\theta/2)$. The

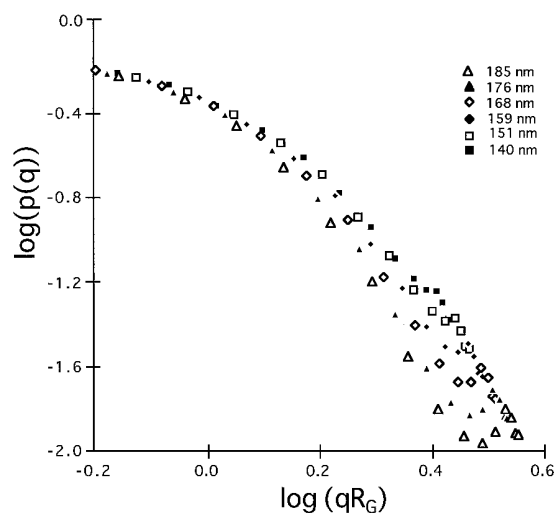


Fig. 2. Double logarithmic plot of six particle-scattering factors vs. the normalized scattering vector. Scattering factors are for six different slices of the molecular weight distribution of amaranth starch dissolved for 35 sec during microwave heating.

TABLE I
Macromolecular Features^a of Amaranth Starch Dissolved at Different Treatment Times and Determined by Laser-Light Scattering (LS) and High-Performance Size-Exclusion Chromatography (HPSEC)

Time (sec)	LS			ρ	d_f'	HPSEC		
	M_w ($\times 10^{-7}$) (g/mol)	R_G (nm)	R_H (nm)			M_w ($\times 10^{-7}$) (g/mol)	R_G (nm)	d_f'
35	69	334	273	1.2	2.44	27 ± 2	176 ± 12	3.26
50	56	314	236	1.3	2.18	20 ± 1	166 ± 12	3.24
70	4.5	147	152	0.97	1.50	6.1 ± 0.2	120 ± 5	3.14
90	0.76	114	130	0.88	1.03	1.4 ± 0.3	60 ± 3	2.19

^a d_f' = Fractal dimension (e.g., slopes of Figs. 2 and 6); R_G = gyration radius; R_H = hydrodynamic radius; $\rho = R_G/R_H$.

scattering factors correspond to starch molecules from six different slices of the size distribution of molecules which, therefore, have different R_G values. One common curve can be drawn, and the theory of fractals relates the asymptotic slope of this curve as a fractal dimension (d_f'). Very similar behavior was found for amaranth samples heated for 50 and 70 sec, but the derived exponents (Table I) differed slightly. The sample heated for 90 sec presented the lowest d_f' value. In general, d_f' values decreased with increased treatment time. The values of d_f' for samples dissolved at 35, 50, and 70 sec (3.26, 3.24, and 3.14, respectively) are characteristic of a particle that has the internal structure of a hard sphere ($d_f' = 3.0$). However, the d_f' value for the sample dissolved for 90 sec (2.19) is characteristic of a particle that has an internal structure between that of a hard sphere and a fully swollen randomly branched macromolecule in a thermodynamic good solvent ($d_f' = 2.0$).

Additional information was obtained when $P(q)$ was plotted in a Kratky plot: $u^2 P(q)$ vs. u . The scattering curves from six slices of the distributions are compared in Fig. 3. The different fractions from the sample heated for 35 sec gave the same response at low u values (0–1.0), but the function increased at higher u values as R_G decreased. This increase could be interpreted as an effect of the presence of linear chains. However, the sample heated for 90 sec (Fig. 3B) showed a different behavior than that of Fig. 3A. All slices of the distribution gave the same response at low u values (0–1.5) and were slightly different at higher u values. When the

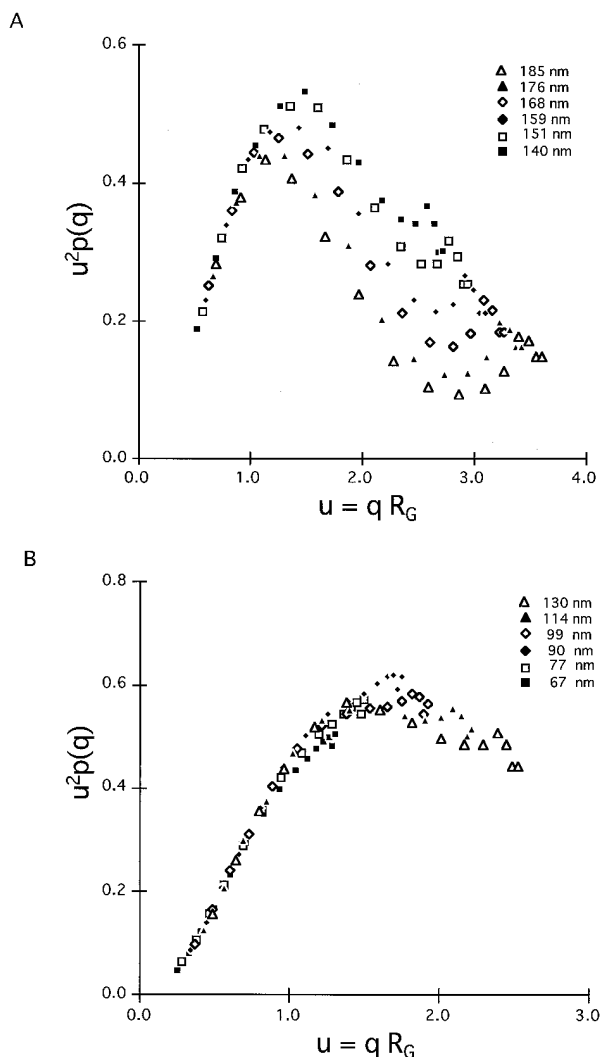


Fig. 3. Kratky plot of six particle-scattering functions, each obtained from a different slice of the molecular weight distribution of amaranth starch dissolved for 35 sec (A) and 90 sec (B) during microwave heating.

results from the four treatment times are compared at $u = 2.0$, the sample dissolved for 90 sec showed a higher value for $u^2 P(q)$ than the samples dissolved at lower treatment times; this effect results from sample degradation.

When a Kratky plot was made for the particles with radii between 130 and 140 nm (Fig. 4), the four functions agreed well at $u < 1.2$, but differed in the asymptotic region. For the samples heated for 35, 50, and 70 sec, the function value decreased as treatment time increased. Similar behavior was found by Hanselmann et al (1996) for waxy corn samples treated at different heating periods. The cited effect was produced by sample degradation. However, the function values for the sample heated for 90 sec at $u > 1.2$ were higher than those found for samples dissolved at lower treatment times; this effect may be due to a higher sample degradation.

SLS

The M_w and R_G values were measured by SLS using a second-order Berry plot because the Zimm plot does not allow a sufficiently accurate extrapolation at zero scattering angle when molecules of high M_w are studied (Aberle et al 1994). In general, M_w and R_G values decreased (Table I) when heating time increased during the sample solubilization step. This tendency demonstrates a degradation of starch components for long treatment periods. The M_w and R_G of amaranth samples dissolved for 35 sec (69×10^7 g/mol and 334 nm) and 50 sec (56×10^7 g/mol and 314 nm) are higher than those for corn amylopectin at 35 sec (27×10^7 g/mol and 259 nm) and 50 sec (24×10^7 g/mol and 260 nm). However, M_w and R_G of amaranth samples dissolved for 70 sec (4.5×10^7 g/mol and 147 nm) and 90 sec (7.6×10^6 g/mol and 114 nm) are lower than for corn amylopectin at 70 sec (11×10^7 g/mol and 190 nm) and 90 sec (7.8×10^7 g/mol and 170 nm). These results suggest that amaranth starch dissolved for longer treatment times is more susceptible to degradation, and that this degradation is likely because amaranth starch has a more compact structure with a higher level of branching than does corn amylopectin, which demonstrates structural differences in waxy type samples from diverse sources. The values of M_w and R_G obtained with SLS were slightly higher than those determined with HPSEC-MALLS; the differences in the experimental procedure and data treatment could be responsible for this behavior. However, both techniques may be complementary in polymer analysis.

Figure 5 shows the M_w dependence of the R_G for amaranth starch treated at the four different time periods. Within the limits

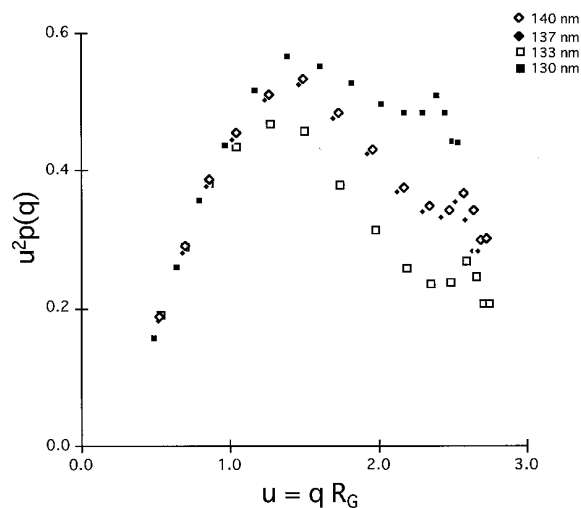


Fig. 4. Kratky plot of particle-scattering functions, each obtained from other slices of the molecular weight distribution of amaranth starch samples dissolved for different time periods ($\diamond = 35$ sec, $\blacklozenge = 50$ sec, $\square = 70$ sec, $\blacksquare = 90$ sec).

of experimental error, R_G to M_w relationship follows a common straight line in the double logarithmic plot ($r = 0.94$) (Kaledigraph v. 2.1.3 Abelbeck Software) describing a power law behavior $R_G = K M_w$ as already reported by Hanselmann et al (1996), who stated the molar mass dependence of the R_G according to the power law:

$$M = K' R_G^{1/\nu} R_G = K' R_G^{df} \quad (1)$$

The slope value (νR_G) was 0.39 and $d_f = 2.56$. For instance $d_f = 3.0$ would define a globular homogenous structure, and $d_f = 2.0$ would define a planar structure. Galinsky and Burchard (1995), using SLS reported $d_f = 2.54$ for partially degraded potato starches. In another study (Bello-Pérez et al, unpublished results) we found $d_f = 2.77$ and 2.70 for corn amylopectin and normal corn, respectively. These values demonstrate structural differences in starches from diverse botanical origins.

In Fig. 6, the particle scattering factor $P(q)$, which describes the angular distribution of the scattered light from the sample heated for 35 sec, is plotted against the dimensionless parameter u ($\equiv qR_G$), where q is a scattering vector (Brown and Nicolai 1993): $q = (4\pi n/\lambda) \sin(\theta/2)$. Information about the structure of polymers can be obtained from $P(q)$, which describes the angular distribution of the scattered light. The theory of fractals (Stauffer 1979) relates this asymptotic slope to a fractal dimension. In this experiment, d_f' is focusing rather the internal structure in contrast to d_f , which is related to the global structure (Hanselmann et al 1996). These slopes showed dependence on treatment time. In general, d_f' values decreased with increasing treatment time. Values of d_f' for samples heated for 35 sec ($d_f' = 2.44$) and 50 sec ($d_f' = 2.18$) are characteristic of a particle that has an internal structure between that of a hard sphere ($d_f' = 3.0$) and a fully swollen randomly branched macromolecule in a thermodynamically good solvent ($d_f' = 2.0$) (Hanselmann et al 1996). We conclude that the sample dissolved for 50 sec can swell up to a larger extent than the sample heated at 35 sec.

Additional information was obtained when $P(q)$ was plotted in a Kratky plot (Fig. 7). The samples heated for different heating periods gave the same responses at low $u = qR_G$ (0–1.5), but differed at higher u values. In this type of diagram, the values in the asymptotic region (at u values >2) increased. Therefore, even small changes, in the particle-scattering factor in this u domain, should be related to the internal structure (Burchard 1993, Hanselmann et al 1996). In general, the longer the treatment time of these samples, the higher the function increased as u becomes larger. This increase could be interpreted as an effect of the presence of linear chains coming from the sample degradation occurring preferentially in the weakly branched do-

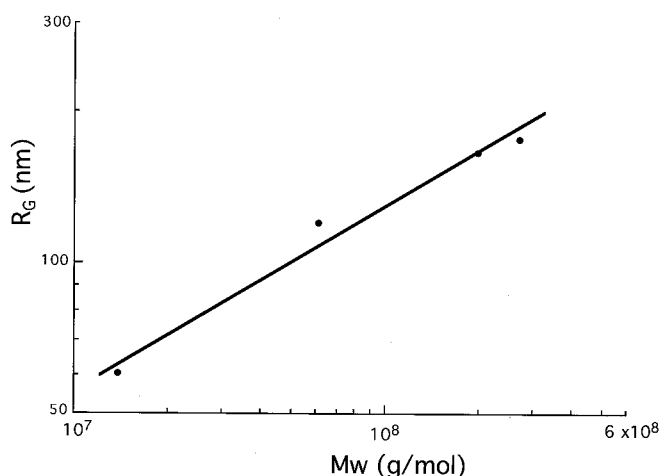


Fig. 5. Molecular weight dependence of the gyration radius (R_G) of amaranth starch dissolved for different time periods.

mains (Hanselmann et al 1996). The curve obtained for the sample heated for 90 sec demonstrates again the high degradation level of the internal structure because this behavior is found for linear chains.

DLS

Hydrodynamic radii values (R_H) of the samples studied (Table I) were obtained using extrapolation of Zimm plot. With cumulative analysis, it is possible to determine for each angle and each concentration, one average translational diffusion coefficient (D_t). Then, using the Stokes-Einstein relationship, R_H may be evaluated:

$$R_H = K_B T / (6\pi\eta_0 D_t) \quad (2)$$

where K_B is the Boltzmann constant, T is the temperature, and η_0 is the solvent viscosity.

The dynamic Zimm diagram plots apparent D_t as a function of concentration and scattering vector (q). Then, D_t of the solution can be evaluated at 0 angle and 0 concentration. With this D_t value and the Stokes-Einstein relationship, R_H is obtained (Stepánek 1993).

In general, R_H values decreased when treatment time increased. The R_H value decreasing with higher treatment times reflects a macromolecular degradation. The R_H values for the samples dissolved for 35 sec (273 nm) and 50 sec (236 nm) were higher than those found for corn amylopectin. However, R_H values determined for samples heated for 70 sec (152 nm) and 90 sec (130 nm) were

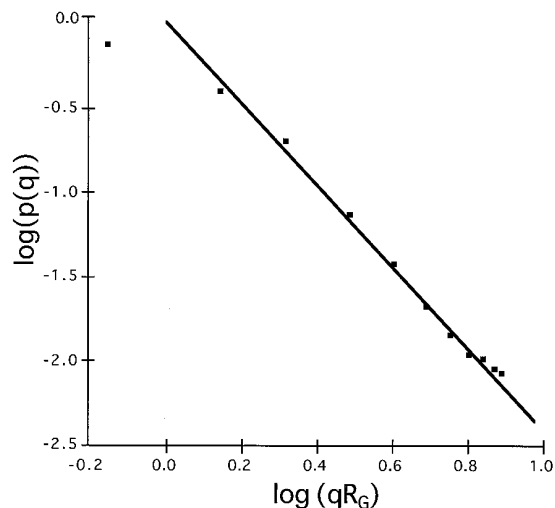


Fig. 6. Double logarithmic plot of particle-scattering factor vs. the normalized scattering vector of amaranth starch dissolved for 35 sec.

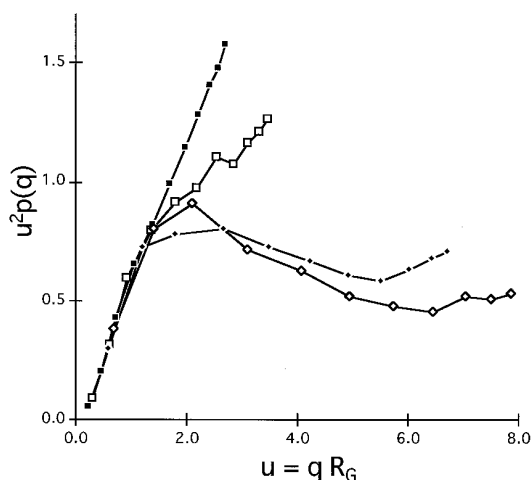


Fig. 7. Kratky plot of amaranth starch samples dissolved for different time periods ($\diamond = 35$ sec, $\blacklozenge = 50$ sec, $\square = 70$ sec, $\blacksquare = 90$ sec).

lower than those for corn amylopectin dissolved at the same treatment times. These results showed higher molecular degradation of amaranth starch samples when they are heated at longer treatment times.

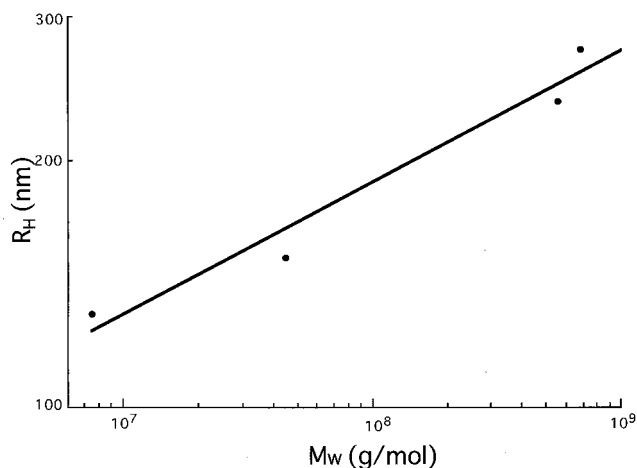


Fig. 8. Molecular weight dependence of the hydrodynamic radius (R_H) of amaranth starch dissolved for different time periods.

Figure 8 shows the M_w dependencies of the R_H for amaranth starch treated for four different time periods; the slope value (νR_H) was 0.49. Galinsky and Burchard (1995) using SLS reported νR_H value of 0.476 for the partially degraded potato starch. Our νR_H value showed structural degradation of amaranth starch, because for linear chains, lines with slope of zero are generally found

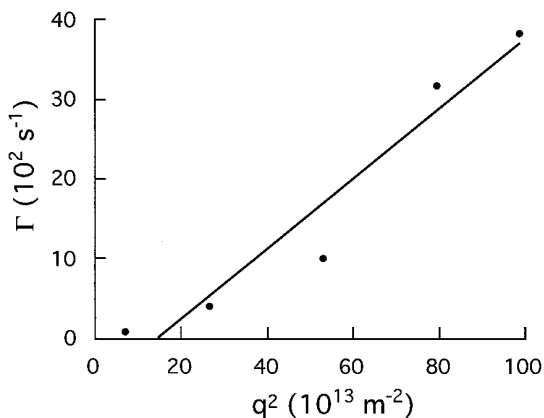


Fig. 10. Linear dependence of the relaxation rate of the main component (Γ_{slow}) on the scattering vector of amaranth starch dissolved for 35 sec.

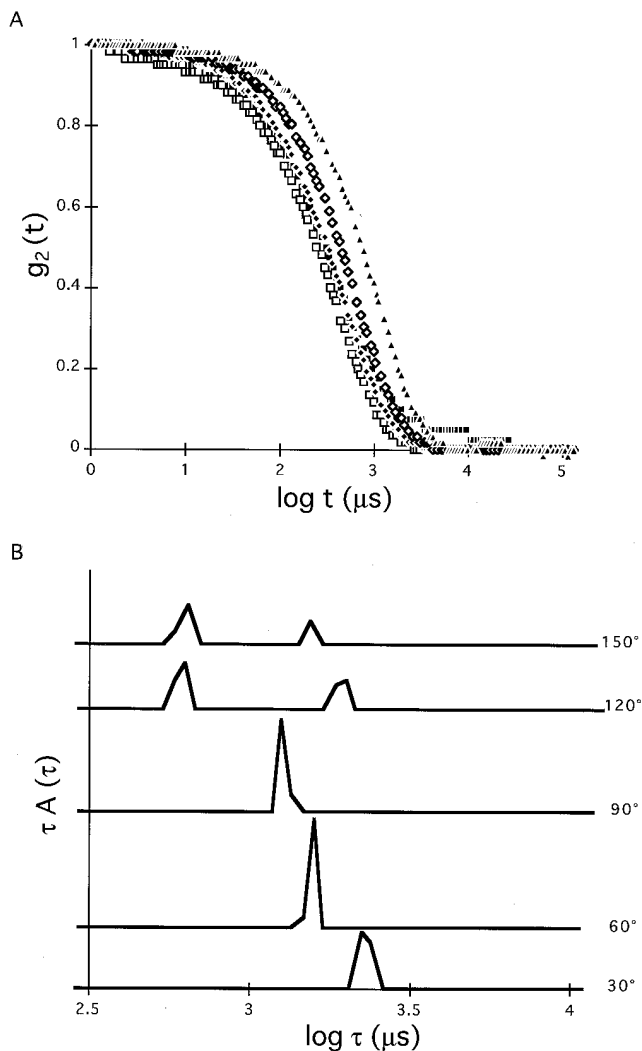


Fig. 9. Behavior of amaranth starch at the highest concentration studied (0.37 mg/mL) and dissolved for 35 sec. **A**, Angular dependence of normalized time correlation function $g_2(t)$. **B**, Relaxation time distributions ($\Delta = 30^\circ$, $\diamond = 60^\circ$, $\blacklozenge = 90^\circ$, $\square = 120^\circ$, $\blacksquare = 150^\circ$).

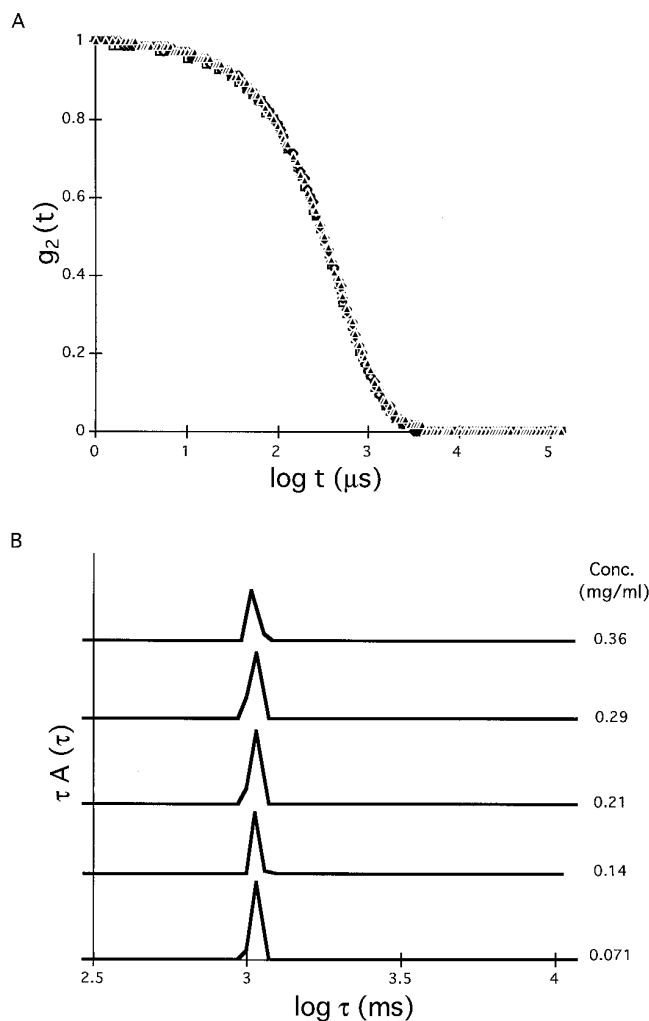


Fig. 11. Behavior of amaranth starch dissolved for 35 sec and measured at 90° . **A**, Concentration dependence of normalized time correlation function $g_2(t)$. **B**, Relaxation time distributions ($\Delta = 0.36$ mg/mL, $\diamond = 0.29$ mg/mL, $\blacklozenge = 0.21$ mg/mL, $\square = 0.14$ mg/mL, $\blacksquare = 0.071$ mg/mL).

when M_w versus R_G and M_w versus R_H are plotted (Galinsky and Burchard 1995).

The intensity time correlation function $g_2(t)$ of a dilute solution shows only one motion that is related to the translational diffusion of the branched particles. Figure 9A shows the dependence angular $g_2(t)$ for amaranth starch (0.37 mg/mL) heated for 35 sec. The relaxation times decreased with increasing scattering angle. Figure 9B depicts relaxation rate distributions at a series of angles (30, 60, 90, 120, and 150°). Amaranth starch at low angles (30, 60, and 90°) presented one population with the peak shifting when the angle increased. However, at higher angles (120 and 150°) two populations were found.

The linear dependence ($r = 0.97$) of the relaxation rate of the main component (Γ_{slow}) (Fig. 10) on the scattering vector, suggests a diffusive character for this mode.

The effect of the concentration was analyzed with samples solubilized for 35 sec and measured at a scattering angle of 90°. The intensity of time correlation function $g_2(t)$ did not show changes at the concentrations studied (Fig. 11A). One population at the same value of $\log \tau$ was found for these samples (Fig. 11B).

Then the sample was analyzed for different heating times (Fig. 12). The intensity of time correlation function $g_2(t)$ for samples dissolved at 35 and 50 sec did not generally present differences (Fig. 12A). However, when treatment time increased (70 and 90 sec),

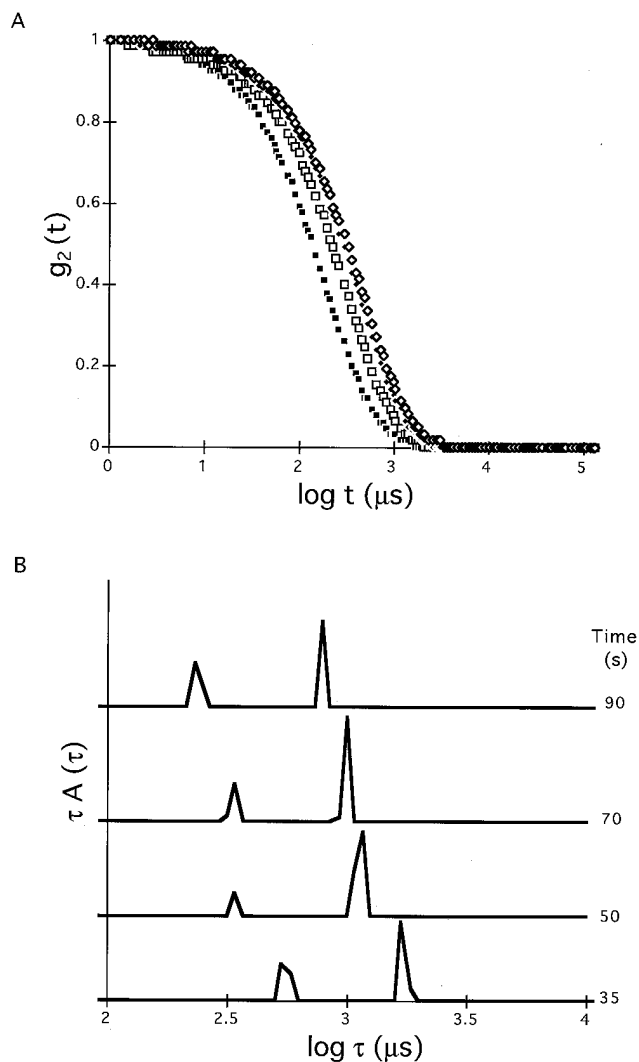


Fig. 12. Behavior of amaranth starch at the highest concentrations studied, dissolved for different time periods and measured at 90°. **A**, Sample dependence of normalized time correlation function $g_2(t)$: $\diamond = 0.36$ mg/mL and 35 sec, $\blacklozenge = 0.38$ mg/mL and 50 sec, $\square = 0.39$ mg/mL and 70 sec, $\blacksquare = 0.42$ mg/mL and 90 sec; **B**, relaxation time distributions.

the time correlation function decreased. These differences between the samples dissolved at different times are emphasized in Fig. 12B. When treatment time increased, bimodal distributions were observed with differences in the peak locations. These results show changes in the structure due to treatment time.

The dimensionless ratio $\rho = (R_G/R_H)$ (Table I) is a sensitive index of chain conformation. The ρ values for the samples dissolved at 35 sec (1.2) and 50 sec (1.3) are in the range of values reported for spheres or other globular structures (Burchard 1993). On the other hand, ρ values for samples dissolved at 70 sec (0.97) and 90 sec (0.88) are also in the range of values for spheres or other globular structures, but with a higher level of branching or linear chains than former samples.

CONCLUSIONS

HPSEC-MALLS, SLS, and DLS techniques allowed assessment of the structure and behavior of a solution of amaranth starch prepared at different heating time periods. The M_w , R_G , R_H and d_f' values obtained by HPSEC-MALLS and SLS were different as reported previously. Both techniques can be complementary for studying macromolecular features of starch samples.

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