

Molecular Weight and Chain Conformation of Amylopectin from Rice Starch

Zhaocheng Ma,¹ Siming Zhao,² Ke Cheng,² Xufeng Zhang,¹ Xiaojuan Xu,¹ Lina Zhang¹

¹Department of Chemistry, Wuhan University, Wuhan 430072, China

²Food Science and Technology Department, Hua Zhong Agricultural University, Wuhan 430072, China

Received 3 March 2006; accepted 13 November 2006

DOI 10.1002/app.26141

Published online 5 March 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: By using laser light scattering (LS) and size exclusion chromatography combined LS, we have investigated the molecular weight and chain conformation of amylopectin from rice of India (II-b), japonica (IJ-b), and glutinous (IG-b) in dimethyl sulfoxide (DMSO) solution. The weight-average molecular weight (M_w) and radius of gyration ($\langle S^2 \rangle^{1/2}$) of amylopectin were determined to be 4.06×10^7 and 128.5 nm for India rice, 7.41×10^7 and 169.6 nm for japonica rice, 2.72×10^8 and 252.3 nm for glutinous rice, respectively. The $\langle S^2 \rangle^{1/2}$ values were much lower than that of normal polymers, indicating a small molecular volume of amylopectin, as a result of highly branched structure. Ignoring the difference of degree of branching, approximated

dependences of $\langle S^2 \rangle^{1/2}$ and intrinsic viscosity ($[\eta]$) on M_w for amylopectin in DMSO at 25°C were estimated to be $\langle S^2 \rangle^{1/2} = 0.30M_w^{0.35}$ (nm) and $[\eta] = 0.331M_w^{0.41}$ (mL g⁻¹) in the M_w range studied. Moreover, from the $\langle S^2 \rangle^{1/2}$ values of numberless fractions obtained from many experimental points in the SEC chromatogram detected with LS, the dependence of $\langle S^2 \rangle^{1/2}$ on M_w for the II-b sample was estimated also to be $\langle S^2 \rangle^{1/2} = 0.34 M_w^{0.347}$, coinciding with the above results. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 104: 3124–3128, 2007

Key words: amylopectin conformation; light scattering; molecular weight; SEC

INTRODUCTION

Starches from various cereal sources, such as wheat, corn, rice and their different varieties, have received extensive attention in relation to their structural and physicochemical properties.^{1–3} Amylose and amylopectin are the main components of starch, and the front is mainly a linear macromolecule with a few branches, whereas the latter is a highly branched macromolecule. The differences in structure of amylopectin, such as molecular weight and its distribution, as well as the chain conformation, can influence the physicochemical behavior of the onset swelling and gelatinization of starches and the texture of food products.^{4,5} Amylopectin is one of the largest molecules in nature, and weight-average molecular weight (M_w) for waxy maize amylopectin^{6,7} lies usually in the range from 5.30×10^7 to 7.69×10^7 . The sedimentation-coefficient data⁸ have confirmed the M_w of amylopectin in dimethyl sulfoxide (DMSO) to be $\sim 10^7$, irrespective of the source of the starch. Stacy and Foster⁹ have summarized some of the M_w values of amylopectin determined by using light scattering (LS) to show in the range from 1.0×10^7 to 4.2×10^8 , depending on their source. Banks et al.¹⁰ have reported the M_w val-

ues ranging from 6.5×10^7 to 5.0×10^8 . It is noted that the pulsed field-gradient nuclear magnetic resonance (PFG-NMR) technique shows that, in aqueous systems, amylopectin has a volume having 400 times larger than the single molecule.¹¹ This implies that the amylopectin may form aggregates in aqueous solution, resulting in an unreliable result.

Another matter of debate has concerned the conformation of amylopectin in solution. Early¹² have believed that amylopectin is not spherical, but 'more extended' from LS measurements. However, the solvent diffusion experiments¹¹ have showed that amylopectin could be described as an oblate ellipsoid in water, with an axial ratio of semimajor to semiminor axes of eight. Additional evidence for the planarity of amylopectin has been provided by analytical ultracentrifugation⁸ and from theoretical modeling of the relationship between the concentration dependence of a polymer's self-diffusion coefficient and its macromolecular shape.¹³

In view of the results reported in the literatures, there are some disputes in chain conformation and unreliable values in M_w . In this present work, we focused on the study of molecular weight and the conformation of amylopectin isolated from three rice types. We measured molecular weight of amylopectin using the relatively new technique of multiple angle laser light scattering (MALLS) and size elution chromatogram (SEC) combined with LLS (SEC-MALLS).

Correspondence to: L. Zhang (lnzhang@public.wh.hb.cn).

Moreover, DMSO as solvent, a more-powerful solvent, in which polysaccharide aggregation can be avoided.

EXPERIMENTAL SECTION

Materials

The three kinds of rice including indica rice, japonica rice, and glutinous rice, were purchased from Wuhan market in China. Starch sample was prepared from each rice by with 0.3% NaOH first to remove protein, and then defatted with ether in a Soxhlet apparatus for 8 h to obtain pure starch powder. All chemicals were of analysis grade.

Preparation of amylopectin samples

Each purified starch powder (10 g) was soaked in 300 mL of an alcohol/1-butanol mixture of 2 : 1 (v/v) in boiling water bath for 1 h, then cooled to room temperature. Amylose-alcohol complex was removed by centrifuging at 6000 rpm for 20 min, and the amylopectin supernatant was carefully decanted. The resulting amylopectin supernatants were stored in 4°C for about 24 h. Amylopectin was purified by repeatedly centrifuging, and extracting the supernatant in a boiling water bath 8 times. The final supernatant was concentrated to 50% volume in a vacuum rotary evaporator at 40°C. Three amylopectin samples were prepared by precipitating from their aqueous solution to ethanol, then vacuum-dried at room temperature, coded as II-b, IJ-b, and IG-b for India rice, Japonica rice, and Glutinous rice, respectively.

Characterization

Intrinsic viscosity measurement

Intrinsic viscosities ($[\eta]$) of the samples in DMSO were measured at $(25 \pm 0.1)^\circ\text{C}$ by using a capillary viscometer. The kinetic energy correction was always negligible. Huggins and Kraemer plots were used to get intrinsic viscosity by

$$\eta_{sp}/c = [\eta] + k'[\eta]^2c \quad (1)$$

$$(\ln \eta_r)/c = [\eta] - k''[\eta]^2c \quad (2)$$

where k' and k'' are constant for a given polymer at a given temperature in a given solution, η_{sp}/c the reduced specific viscosity, $(\ln \eta_r)/c$ the inherent viscosity.

Laser light scattering

In static LLS, the scattering light intensity known as Rayleigh ratio (R_θ) of a polymer solution at angle (θ) and concentration (c) is related to the weight-average molecular weight (M_w) by

$$\frac{Kc}{R_\theta} = \frac{1}{M_w P(\theta)} + 2A_2c \quad (3)$$

where $K = 4\pi^2 n_0^2 (dn/dc)^2 / (N_A \lambda_0^4)$, with N_A , n_0 , and λ_0 being Avogadro's number, the refractive index of the solvent, and the wavelength of light in vacuum, respectively. $P(\theta)$ is a function of the particle scattering, radius of gyration $\langle S^2 \rangle^{1/2}$, angle (θ), shape and structure. The appropriate representation is the following

$$P(\theta)^{-1} = 1 + (1/3)(4\pi\eta/\lambda)^2 \langle S^2 \rangle_z \sin^2(\theta/2) \quad (4)$$

The scattering light intensities were measured with multi-angle laser light scattering instrument (MALLS, DAWN[®] DSP, Wyatt Technology, St. Brabara, USA) equipped with a He-Ne laser ($\lambda = 632.8\text{nm}$) at 25°C. The refractive indexes increments (dn/dc) for the amylopectin solution in DMSO were measured with a double beam differential refractometer (DRM-1020, Otsuka Electronics, Japan) to be $0.060 \text{ cm}^3 \text{ g}^{-1}$ at 632.8 nm and 25°C. All the solutions used were filtered with a $0.45 \mu\text{m}$ filter (PTFE, Puradisc[™] 13 mm Syringe Filters, Whatman, England) two times into scintillation vial for the LS measurement. Astra software was utilized for data acquisition and analysis.

SEC-MALLS measurements

SEC-LLS measurements were carried out on size exclusion chromatography combined with multiangle laser photometer aforementioned combined with a P100 pump (Thermo Separation Products, San Jose, CA) equipped with columns of G6000H8 (MicroPak, TSK) and a differential refractive index detector (RI-150, Japan) at 25°C. The carrier solution was redistilled DMSO. The samples were dissolved in DMSO overnight with stirring. The DMSO and polymer solution were purified by a $0.45 \mu\text{m}$ filter (PTFE, Puradisc 13-mm Syringe Filters, Whatman, England) and degassed before use. The injection volumes were 200 μL with a concentration of 0.2% for each sample, and the flow rate was 1.0 mL/min. Astra software was utilized for data acquisition and analysis.

RESULTS AND DISCUSSION

Molecular weight and parameters from MALLS in the batch mode

From LLS data, we were able to obtain the M_w , $\langle S^2 \rangle^{1/2}$ of the sample in dilute solution from Zimm plot by eqs. (3) and (4). It is worth nothing that when the molecular mass of sample is greater than 1×10^6 the plot of (Kc/R_θ) versus $\sin^2(\theta/2)$ for experimental data becomes practically nonlinear in a broad angular range yielding $\langle S^2 \rangle^{1/2}$ unreliable. In LS measurements dust particles can contribute as scatters and the pres-

ence of small amounts of microgels may also complicate the interpretation of LS measurement. Therefore, difficulties in interpreting LS results for large macromolecules of amylopectin have been reported in the investigations using LS to measure the molecular size and shape of amylopectin from various sources.⁷ As the Zimm plot procedure did not allow sufficiently accurate extrapolation to the zero scattering angle for molecules of high molecular weight (even negative molecular weight can be obtained⁷). Berry¹⁴ has proposed the root plot with $[(Kc/R_\theta)^{1/2}]$ versus $\sin^2(\theta/2)$ plot in the presence of nonlinear scattering angular variation. However, the Berry method has been demonstrated to determine M_w of large molecules with greater accuracy.¹⁵ We also encountered this difficulty, thus second order Berry plots were used to interpret LS data. The Berry plots for amylopectin from glutinous rice (a), India rice (b), and japonica rice (c) at 25°C are shown in Figure 1. Clearly, the second order Berry method exhibits a good curve fitting with laser signals obtained at different angles. However it has noticeable downward curvatures. The phenomenon is normal and a second-order polynomial fitting in the angular extrapolation to zero angle furnishes reliable M_w and results.

The measured values of M_w and radius of gyration $\langle S^2 \rangle^{1/2}$ are summarized in Table I. The M_w values fall in the molecular weight range reported in the literature. The $\langle S^2 \rangle^{1/2}$ values reflect the molecular volume. From Table I, the values are much lower than that of normal polymers, this suggests amylopectin possesses a small volume as a result of highly branched structure. We assumed that the degree of hyperbranched starch has weakly effect on the values of M_w and $\langle S^2 \rangle^{1/2}$, so we can estimate the approximated relationship of them from the data of the three samples. The double logarithmic plot of $\langle S^2 \rangle^{1/2}$ against M_w for amylopectin in DMSO at 25°C are shown in Figure 2 and the plot can be represented by following equation

$$\langle S^2 \rangle^{1/2} = 0.30M_w^{0.35}(\text{nm}) \quad (5)$$

The exponent α of the equation is 0.35 in the M_w region studied. Yoo and Jane¹⁶ have reported also the exponent α value (0.334) for A-type starches in 90 wt % DMSO. This is in good agreement with our result (0.35), it is just a little bigger, indicating that the DMSO is better solvent than 90 wt % DMSO for amylopectin. Galinsky and Burchard¹⁷ have obtained slightly larger value (0.394) for amylopectin in 0.5N NaOH, suggesting that amylopectin is slightly extended in 0.5N NaOH than in DMSO. Usually, the exponent α reflects the chain conformation of polymer in solution. The exponent α for flexible polymer in good solvent is in the range from 0.5 to 0.6, whereas from 0.2 to 0.4 for polymer with high degree of branching, and 0.3 for highly compact spherical

shape.¹⁸ Therefore, the α value of 0.35 indicates that the amylopectins exist as compact spherical conformation in DMSO.

Similarly, The $[\eta]$ values of three amylose samples in DMSO at 25°C are given in Table I. M_w dependence

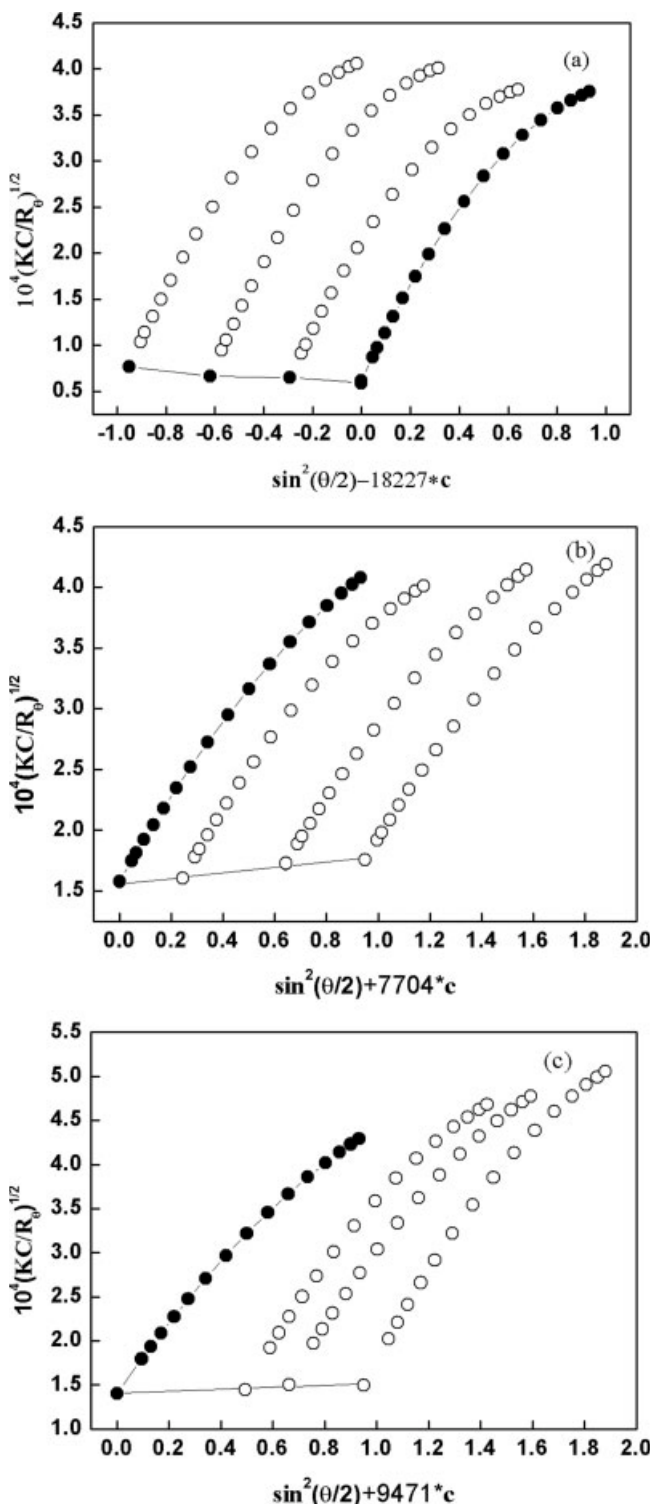


Figure 1 Berry plots of the samples IG-b(a), II-b(b) and IJ(c) in DMSO at 25°C.

TABLE I
Experimental results of M_w , branch factor, $\langle S^2 \rangle^{1/2}$ and $[\eta]$ of Amylopectin at 25°C

Sample	Source of starch	LLS		SEC-LLS	$[\eta]$ (mL/g)
		$M_w \times 10^{-7}$	$\langle S^2 \rangle^{1/2}$ (nm)	$M_w \times 10^{-7}$	
II-b	India Rice	4.06	128.5	3.31	426.7
IJ-b	Japonica Rice	7.41	169.6	6.36	539.9
IG-b	Glutinous Rice	27.2	252.3	16.1	926.1

of $[\eta]$ for the samples in DMSO at 25°C is illustrated in Figure 3. The Mark-Houwink equation for the amylopectin in DMSO in the M_w range from 4.06×10^7 to 2.72×10^8 can be represented approximately by

$$[\eta] = 0.331M^{0.41}(\text{mLg}^{-1}) \quad (6)$$

The exponent α value (0.41) is comparable to the value (0.39) for amylopectin in 0.5N NaOH reported by Galinsky and Burchard.¹⁹ Usually, the exponent α of flexible polymer in good solvent is in the range of form 0.5 to 0.8, and that of highly branched polymer having random coil conformation is lower than 0.5. This suggests that amylopectin in DMSO exists as a flexible spherical chain with much branching in DMSO. The result support the conclusion aforementioned.

Molecular parameters from SEC-MALLS

Recently, the common size exclusion chromatography (SEC) has been further developed by the on-line detection of concentration via a refractometer, multi-angle laser light scattering (MALLS). Under ideal conditions such a setup should admit determination of the molecular weight distribution and the radii of gyration as a function of M_w . It is a convenient method for the determination of true molecular

weight and distribution without standard samples. The result obtained from the SEC-MALLS experiments were based on a specific eluted fractions of sample, which were separated by the SEC column. The molecular weight distribution chromatograms of the three samples separated by the SEC were shown in Figure 4. The values of M_w derived from the plots were in good agreement with those obtained from MALLS, just a little different in high molecular weight. In the batch mode, all the molecular species, including the small molecular species and large aggregates present in the sample, were averaged in the LS calculations.

From the SEC chromatogram detected by LLS, we can obtain M_w and $\langle S^2 \rangle^{1/2}$ value of numberless fractions, which have been estimated from many experimental points in the SEC.^{20,21} Therefore, the chain conformation of II-b was further predicted from a log-log plot of $\langle S^2 \rangle^{1/2}$ versus M_w in Figure 5. The straight lines fitting the experimental points from SEC chromatograms are represented by

$$\langle S^2 \rangle^{1/2} = 0.34M_w^{0.347}(\text{nm}) \quad (7)$$

The exponent α of the equation is in good agreement with the above result of 0.35. It is further demon-

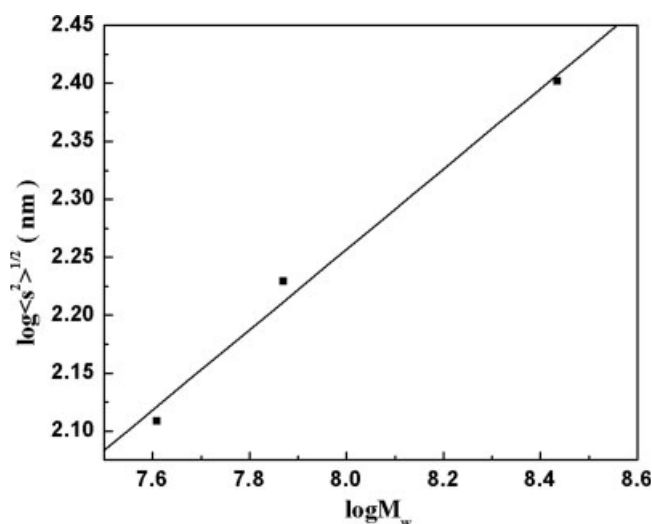


Figure 2 M_w dependence of $\langle S^2 \rangle^{1/2}$ for the amylopectin from three kinds of rice in DMSO at 25°C by LLS.

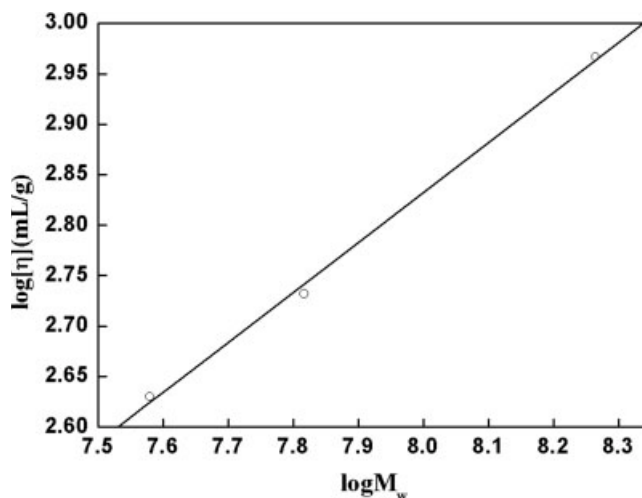


Figure 3 M_w dependence of $[\eta]$ for the amylopectin from three kinds of rice in DMSO at 25°C.

strated that amylopectins exist as compact spherical conformation in DMSO.

CONCLUSIONS

With both laser LS and SEC-LLS, we obtained the value of M_w and $(\langle S^2 \rangle)^{1/2}$ of amylopectin from rice of India, japonica, and glutinous in DMSO solution. The M_w values were determined to be 4.06×10^7 to 2.72×10^8 , and they are in good agreement between two methods. The $(\langle S^2 \rangle)^{1/2}$ values were much lower than that of normal polymers, indicating a small molecular volume of amylopectin, as a result of highly branched structure. Approximated dependences of $(\langle S^2 \rangle)^{1/2}$ and intrinsic viscosity $([\eta])$ on M_w for amylopectins in DMSO at 25°C were estimated to be $(\langle S^2 \rangle)^{1/2} = 0.30 M_w^{0.35}$ (nm) and $[\eta] = 0.331 M_w^{0.41}$ (mL g⁻¹) in the M_w range studied. Moreover, from the $(\langle S^2 \rangle)^{1/2}$ values of numberless fractions obtained from many experimental points in the SEC chromatogram, the dependence of $(\langle S^2 \rangle)^{1/2}$ on M_w for the II-b sample was estimated to be $(\langle S^2 \rangle)^{1/2} = 0.34 M_w^{0.347}$, supporting the above conclusion.

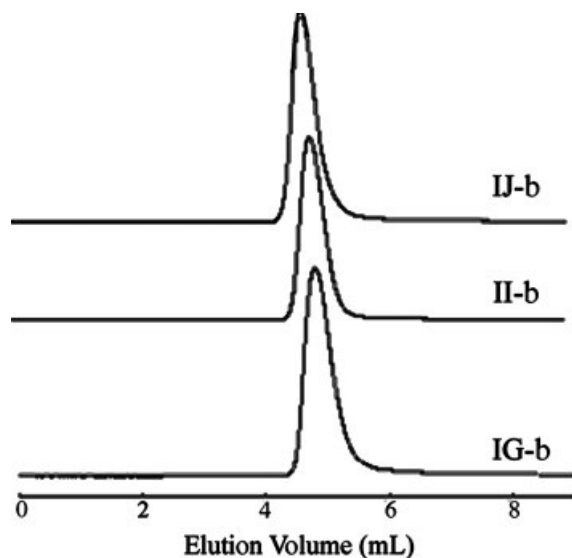


Figure 4 Light scattering intensity (Detector: 11) depends on elution volume time of the samples IG-b, II-b, and IJ-b in DMSO at 25°C.

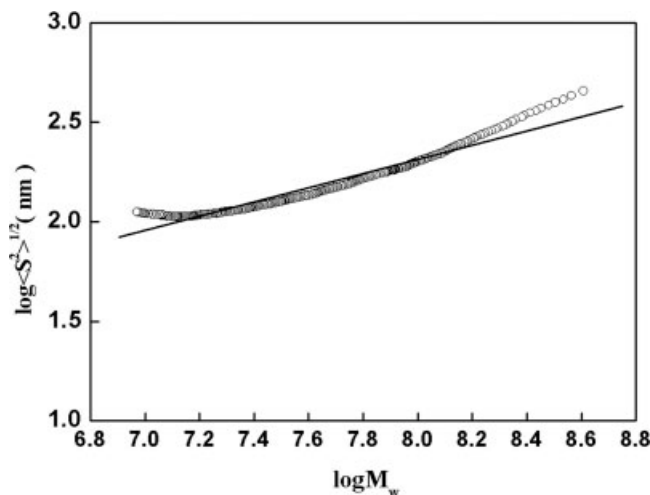


Figure 5 M_w dependence of the $(\langle S^2 \rangle)^{1/2}$ of the II-b sample, using SEC-MALLS technique.

REFERENCES

- Hizukuri, S.; Maehara, Y. *Carbohydr Res* 1990, 206, 145.
- Suzuki, A.; Kaneyama, M.; Shibamura, K.; Takeda, Y.; Abe, J.; Hizukuri, S. *Cereal Chem* 1992, 69, 309.
- Takeda, Y.; Preiss, J. *Carbohydr Res* 1993, 240, 165.
- Joseph, C. *J Agric Food Chem* 1992, 40, 1683.
- Jideani, I.; Takeda, Y.; Hizukuri, S. *Cereal Chem* 1996, 73, 677.
- Bello-Perez, L. A.; Oaredes-Lopez, O.; Roger, P.; Colonna, P. *Cereal Chem* 1996, 73, 12.
- Aberle, T.; Burchard, W.; Vorwery, W.; Radosta, S. *Starch/Stärke* 1994, 46, 329.
- Lelievre, J.; Lewis, J. A.; Marsden, K. *Carbohydr Res* 1986, 153, 195.
- Stacy, C. J.; Foster, J. F. *J Polym Sci* 1956, 20, 57.
- Banks, W.; Geddes, R.; Greenwood, C. T.; Jones, I. *Starch/Stärke* 1972, 24, 245.
- Callaghan, P. T.; Lelievre, J. *Biopolymers* 1985, 24, 441.
- Stacy, C. J.; Foster, J. F. *J Polym Sci* 1957, 25, 39.
- Callaghan, P. T.; Lelievre, J. *Anal Chim Acta* 1986, 189, 145.
- Berry, G. C. *J Chem Phys* 1966, 44, 4550.
- Hanselman, R.; Rhrat, M.; Widmer, H. M. *Starch/Stärke* 1995, 46, 345.
- Yoo, S.-H.; Jane, J.-L. *Carbohydr Polym* 2002, 49, 307.
- Galinsky, G.; Burchard, W. *Macromolecules* 1997, 30, 4445.
- Wyatt, P. J. *Anal Chim Acta* 1993, 272, 1.
- Galinsky, G.; Burchard, W. *Macromolecules* 1995, 28, 2363.
- Mendichi, R.; Giammonab, G.; Cavallarob, G.; Giacometti Schieronia, A. *Polymer* 2000, 41, 8649.
- Peng, Y.; Zhang, L. *J Biochem Biophys Methods* 2003, 56, 243.