

Effects of Preparation Temperature on Gelation Properties and Molecular Structure of High-Amylose Maize Starch

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ABSTRACT

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In this study, 3% aqueous high-amyllose maize starch (Hylon VII) dispersions were heated to temperatures of 140–165°C. The onset and rate of gel formation was observed using a small-strain oscillation rheometer as a function of temperature from 90 to 25°C. The gel formation clearly began earlier in high-amyllose starch paste preheated at lower temperatures, but the rate of gelation was slower and the resulting gel was weaker in comparison with starch pastes preheated at higher temperatures. In addition, the structure of the final gels was studied using large deformation compression measurements. The most rigid gel structure on the basis of small and large deformation tests was obtained for

high-amyllose starch gel preheated to 150–152°C, depending on the type of measurement. The rate of gelation was also fastest in that temperature range. High-amyllose gels heated to higher temperatures lost their rigidity. The molecular weight distribution of starch molecules was measured by size-exclusion chromatography. Heating caused extensive degradation of amylopectin, which had a great effect on amylose gel formation and the final gel properties of high-amyllose maize starch. Micrographs of Hylon VII gels showed that phase separation of starch components visible in light microscopy occurred on heating to higher temperatures.

Amylose and amylopectin are two macromolecular components of starch granules. The ratio of these components varies according to the source plant. Normal maize starch consists of ≈75% branched amylopectin; the remaining 25% is linear amylose. Since the 1950s, it has been possible to alter the ratio of amylose to amylopectin by genetic hybridization. Clear changes have been detected in granule architecture when the amylose content of starch is increased. Dry granules with a high amylose content possessed an amylopectin center surrounded by amylose, which in turn was encapsulated by an amylopectin surface (Atkin et al 1999). Today, high-amyllose starches are commonly used in the confectionery industry because they have excellent gelling and film-forming properties (Jane 1997; Case et al 1998). Starch components are dissolved by heat and shear from granules, forming a molecular dispersion of amylose and amylopectin. The gelatinization temperatures of high-amyllose starches are higher than those of normal and waxy cornstarches (Jane 1997; Case et al 1998; Edwards et al 1998; Shi et al 1998; Boltz and Thompson 1999). According to the manufacturer (National Starch and Chemical), the temperature typically used for heating Hylon VII high-amyllose maize starches is 154–171°C, depending on the soluble solids level of the desired formulation.

Different properties can be obtained for the starch gels depending on the amount and type of material solubilized during gelatinization (Ring et al 1987; Gidley 1989; Leloup et al 1992; Durrani and Donald 1995). The ratio of amylose to amylopectin can influence the formation of a network system (Doublier and Llamas 1993; Miles et al 1985; Leloup et al 1991; Klucinec and Thompson 1999). Strong gels are formed above an amylose-to-amylopectin ratio close to 15:85. At higher ratios of amylose to amylopectin, amylose forms a continuous network structure. Using dynamic viscoelastic measurements, Parovuori et al (1997) showed that the gel formation of amylose was highly dependent on both the ratio of amylose to amylopectin and the molecular weights of amylose and amylopectin. Medium size α -dextrins substantially weakened the gel formation of amylose.

The aim of this work was to study the effects of heating temperature on the gelation properties and the consistency of high-amyllose maize starch gel. The focus was on ascertaining at which temperature high-amyllose starch adequately loses its granule structure to be able to form a firm gel. Gel formation properties and the

structure of formed gels were investigated by measuring small-strain oscillation and backward extrusion during compression. The gel microstructure and network organization were examined by light microscopy. In addition, the effect of the heating temperature on the molecular weight distribution of high-amyllose maize starch heated at various temperatures was studied by size-exclusion chromatography.

MATERIALS AND METHODS

Starch

High-amyllose maize starch (Hylon VII) from National Starch and Chemical Company was used in this study. The dry matter content of the product was 90.4%. The amylose content of Hylon VII starch was 70% as determined by the potentiometric iodine method, as well as by size-exclusion chromatography; the remaining 30% was amylopectin.

Preparation of Samples

Solutions of 3% (w/w, dry matter basis) Hylon VII starch were heated in a special pressure vessel (VTT Automation, Espoo, Finland) using a mixing blade under elevated pressure at 140–165°C. The detector element of the thermometer was located in the jacket of the pressure vessel. The desired temperature in the pressure vessel was reached after 30 min. It was then allowed to cool for 1 hr to 100°C. The gelatinized solution was collected in a preheated vacuum bottle before rheological measurements were taken. For compression tests, the heated solution (50 g) was poured into cylindrical cups (50 mm, i.d.) and sealed carefully; then the gels were allowed to set overnight at room temperature before measurements were taken. In addition, the resulting starch gels were freeze-dried as part of the gel permeation chromatography measurement process.

Rheological Measurements

A controlled stress rheometer (StressTech, ReoLogica Instruments AB, Lund, Sweden) was used to perform small-strain oscillatory measurements using a concentric cylinder measuring geometry (CC 25 CCE). The rheometer was preheated to 90°C. After the gelatinized solution was stabilized for 5 min in the rheometer, the oscillation measurement was started at 90°C and continued down to 25°C at a cooling rate of 1.5°C/min. The oscillation frequency was fixed at 1 Hz and the first rheological measurements were made using the smallest possible stress (0.025 Pa) to avoid any breakage of the forming network. The second and third measurements were recorded with a constant strain of 0.003. No significant differences between measurements were detected.

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Compression Measurements

Compression measurements were made using a texture analyzer (TA-HDi, Stable Micro Systems) with a back extrusion rig. The disk plunger (40 mm diameter) performed a compression test by extruding the starch gel up and around the edge of the disk. The compressing disk was set 40 mm above the bottom of the sample container and lowered at a rate of 1.5 mm/sec to compress 30 mm from the original position. The force that caused the compression was recorded.

Light Microscopy

For microscopy studies, cooled gelatinized gels were first embedded in 2% agar and then chemically fixed in 1% glutaraldehyde. Fixed samples were dehydrated and embedded in Histo-resin (Leica, Germany). Sections (4- μ m thick) were cut with a microtome (Microm HM355, Germany) and stained with iodine. Samples were examined and photographed using an Olympus BX50 microscope.

Size-Exclusion Chromatography

The molecular weight of amylose was determined by size-exclusion chromatography with postcolumn iodine addition and spectrophotometric detection as described by Suortti et al (1998).

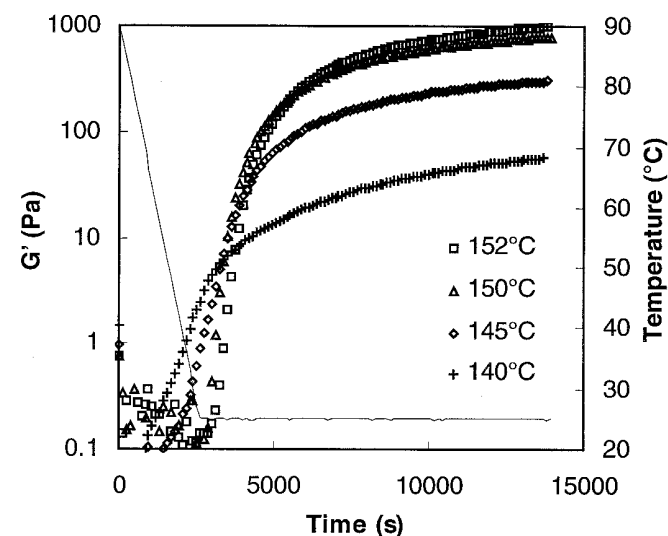


Fig. 1. Development of storage modulus (G') during cooling of 3% high-amylose starch gels heated to 140–152°C.

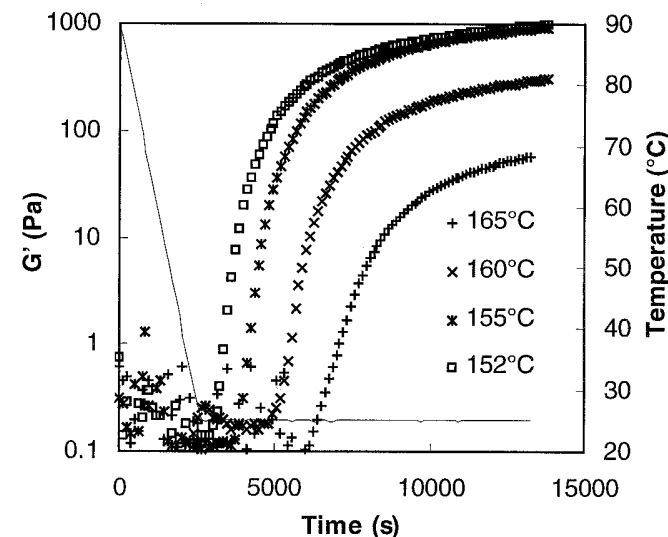


Fig. 2. Development of (G') during cooling of 3% high-amylose starch gels heated to 152–165°C.

RESULTS

Small-Strain Oscillation Measurements

The effects of heating temperature on the onset and rate of gelation as well as the rigidity of gels formed were studied using small-strain oscillation measurements. During oscillation, measurements of the storage modulus (G'), the loss modulus (G''), and the phase angle (δ) were recorded. The onset of gelation can be detected by a decrease in $\tan \delta$ and an increase in the G' of high-amylose starch. The gelation of 3% high-amylose starch solutions heated at 140–165°C is illustrated in Figs. 1 and 2. The increase in G' was first observed in the starch sample heated to 140°C, while the last occurred in the sample heated to 165°C. The equilibrium point, in which both the storage and loss moduli reached a plateau, occurred for the starch sample heated to 140°C immediately at 25°C when the sample was cooled; whereas, for the starch sample heated to 165°C, >1 hr was needed to reach the constant plateau at 25°C.

Moreover, the rate of gelling and the G' of 3% Hylon VII gels depended on the heating temperature. Starch samples preheated to <152°C began to gel earlier and at a slower rate, forming less rigid gels than the sample heated to 152°C. High-amylose starch heated

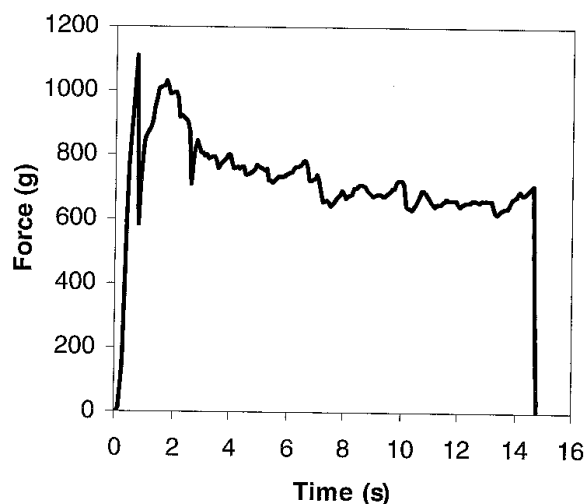


Fig. 3. Typical force-time curve for 3% high-amylose starch gels heated to 150°C.

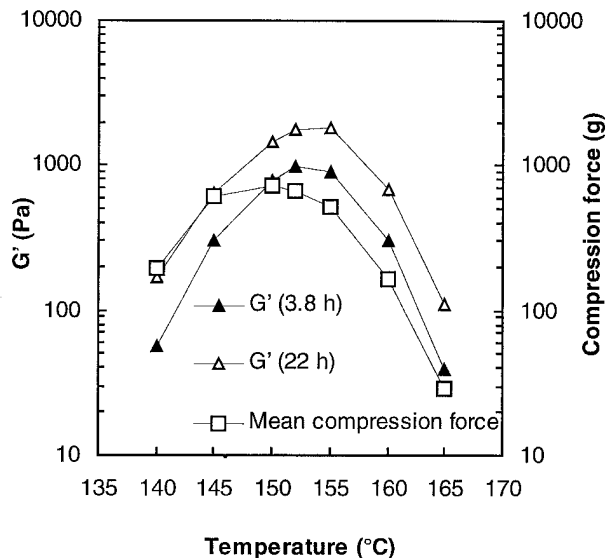


Fig. 4. Effects of heating temperature and gelling time on G' of 3% high-amylose starch gels. Mean compression force values are also represented as a function of the heating temperature.

to 140°C formed an even weaker network during the cooling process than starch heated to 145°C. The sample heated to 152°C reached the maximum G' value resulting in the most rigid structure; whereas, the high-amylose starch heated >152°C started to gel more slowly and the network became weaker with further increases in temperature.

It appeared occasionally that starch samples heated at >155°C started to form a network as early as the starch samples heated to 140 or 145°C and they had several small onsets of gelation during the cooling process. The final G' remained the same for several onsets as it was for that of the delayed onset of gelation. On the basis of repeated measurements, it could be concluded that the forming network was too weak to be measured and was usually destroyed during measurement, even though the applied stress was as low as possible. After all, it was demonstrated that the onset of gelation of Hylon VII starch was delayed with increasing temperature. Each gel was held on the rheometer overnight at 25°C and gelling then followed. The next morning, after 22 hr, the storage and loss moduli were measured as a function of frequency. In all gels, $G' > G''$ and both moduli of each gel were independent of frequency, indicating a strong gel.

Determination of Compression Hardness

High-amylose maize starch gels heated at various temperatures were subjected to backward extrusion during compression measurements. Gels heated to different temperatures were allowed to set at room temperature for 23 hr before measurements were taken. A typical force-time curve for a high-amylose maize starch gel is shown in Fig. 3. The magnitude of the first force peak in the curve, the mean force needed for compression, and the total area in the force-time curve of each gel were recorded. Average values

and the standard deviation of three to nine compression tests were calculated (Table I). The first force peak of each gel was naturally higher than the mean force needed for the deformation. The mean force that caused the compression was highest for high-amylose starch heated to 150°C, indicating the strongest structure of all gels measured. Gels heated to $\pm 150^\circ\text{C}$ were softer and deformed during compression with a lower force. Values of storage moduli after 3.8 and 22 hr for high-amylose starch gels heated to various temperatures are shown in Fig. 4. G' values are higher after 22 hr than after 3.8 hr, as expected. However, it was unexpected that G' of high-amylose starch gels increased so clearly during overnight measurement at 25°C.

The correlation between large-strain compression testing and small-strain oscillation measurement is not easy to interpret, even though the concentrations of measured gels were the same in both measurements. The mean compression force values for 3% Hylon VII gel as a function of the heating temperature are shown in Fig. 4. The shape of the curve of the mean compression force is similar to the curve of the storage moduli of high-amylose gels, except that the slopes are different at the area of low temperature. The correlation coefficients (R^2) were 0.46 and 0.62, respectively, for the results of first force peak to G' and for mean compression force and force-time area. If only the three last temperatures (155, 160, and 165°C) were included in the calculation, $R^2 = 0.99$ for each measurement.

Microscopic Examination

Micrographs of iodine-stained embedded sections of 3% high-amylose starch Hylon VII gels heated to various temperatures are shown in Fig. 5. At 140 and 145°C, remnants of granules and dispersed amylopectin embedded with a continuous blue-stained

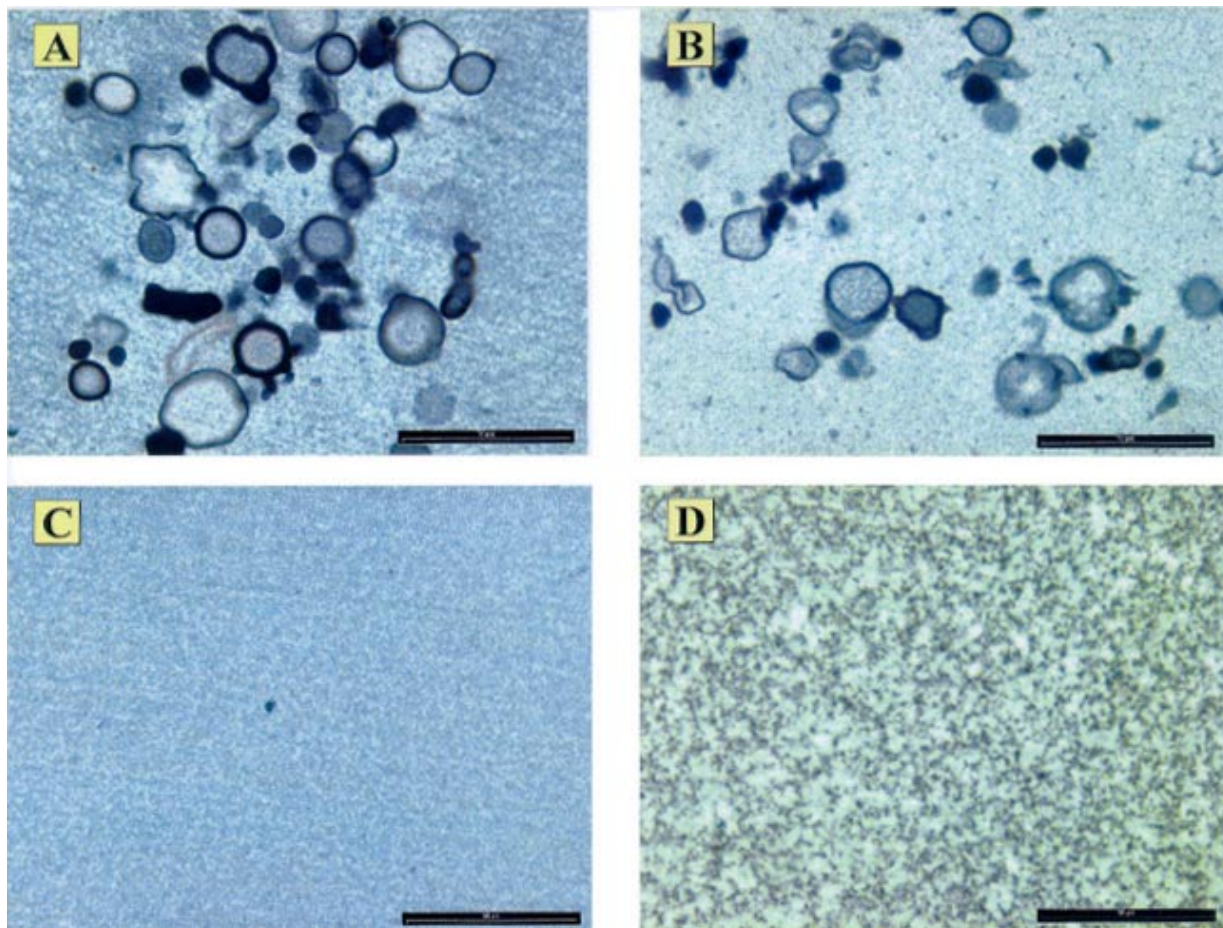


Fig. 5. Effect of heating temperature on microstructure of high-amylose starch gels. Micrographs of iodine-stained, embedded sections of 3% high-amylose starch gels heated to 140, 145, 150, and 160°C (A–D, respectively). Bar = 50 μm .

amylose matrix were observed. Amylose and amylopectin in the continuous phase were phase-separated. With a further increase in temperature to 150°C, all the granules disappeared and amylose and amylopectin formed the most homogeneous texture of all Hylon VII gels measured in the present study. At 160°C, amylose and amylopectin were once again phase-separated, with the area of the individual components being larger (Fig. 5D) than in gels heated to 140 or 145°C (Fig. 5A and B).

Molecular Weight Measurements

Size-exclusion chromatograms of 3% Hylon VII starch heated to different temperatures are shown in Fig. 6. In the chromatograms, the distribution of the amylose fraction moves toward the direction of low molecular weight, indicating that the molecular weight of amylose decreases by $\approx 15\%$ with increased heating temperature. Also, the shoulder peak at 33 min decreases with increased heating temperature, indicating degradation of amylopectin. Table II shows average molecular weight of Hylon VII. The results indicate that a very dramatic decrease in the molecular weight of amylopectin occurred during the heating process because the molecular weight of Hylon VII starch heated to 165°C decreased ≈ 3.5 -fold compared to that of the starch sample heated to 140°C.

DISCUSSION

Gel properties of high-amylose maize starch were very sensitive to the temperature of preparation. Different heating temperatures have an obvious effect on the rate of gelling as well as on the final gel properties of high-amylose starch. Gel formation and the final gel properties also seemed to be dependent on the starch concentration and the amount of solution heated in the pressure vessel. Therefore, all starch dispersions needed for various measurements in this study were exposed to the same conditions during the heating process (3%, 200 mL) to get equally gelatinized dispersions at a given temperature.

The results of the present study are in good agreement with the results of Edwards et al (1998) concerning the increase in gel hardness with increasing heating temperature to 150°C as well as increased storage time of the final gels. The results of the delayed onset and the faster rate of gelation of high-amylose gels heated to higher temperatures are the same as for Case et al (1998). However, based on the results of this study, it can be concluded that there are other factors besides the solubilization of polymers affecting the consistency of high-amylose starch gels. Amylose

and amylopectin fractions in high-amylose starches have different solubilities and, therefore, they dissolve and behave differently at different temperatures during the heating process.

Micrographs showed the different microstructures of high-amylose starch gels heated to various temperatures. The leaching of amylose and amylopectin in high-amylose starch paste heated to lower temperatures leads to the early onset of network formation and lower gel rigidity of high-amylose starch. With further increases in temperature, the amount of granules and dispersed amylopectin decreased and disappeared, finally resulting in a gel with a continuous starch matrix and with good mechanical and cohesive properties. On the other hand, the rate of gelling became faster and the rigidity of gels increased on heating due to the increased amount of solubilized amylose in high-amylose starch pastes.

However, gel strength and rigidity started to decrease past a certain temperature. Both rheometer and compression measurements indicated the existence of this point. According to the small-strain oscillation measurements, this clear change in gel rigidity occurs at 152°C and, in compression measurement, a few degrees earlier. Size-exclusion chromatograms showed that the amount of the high-molecular weight fraction in gelatinized high-amylose starch paste decreases with increasing temperature in the 140–165°C range. At the same time, the distribution of the amylose fraction in size-exclusion chromatograms moves in the direction of lower molecular weight. An interpretation of these results could be that amylopectin and also, to a minor extent, amylose molecules degrade during the increase in temperature needed for dissolution of amylose. After a certain temperature, degradation of amylopectin changes the ratio of the high-molecular weight fraction to linear polymers, causing a delayed network formation, decreased gel rigidity, and disaggregated structure of the final gel. In iodine-stained micrographs, this change can be seen as phase-separation. Interphase of microdomains of different components increases and the gel structure loses cohesiveness.

In their study of various high-amylose starches, Case et al (1998) found that the differences in the physical properties between different high-amylose starch hybrids were clearly influenced by the amylose-to-amylopectin ratio. A decrease in amylopectin content enhanced phase-separation and aggregation of the amylose in solution, and the increase in modulus and fracture strength appeared to be related to the increase in the concentration of amylose. They also observed that the temperature of preparation of high-amylose starches had an effect on the gelation behavior as well as on the final properties of the starch gels, and it was believed that higher preparation temperatures resulted in better solubilization of polymers,

TABLE I
Results of Compression Measurements for 3% High-Amylose Maize Starch Gels Heated at 140–165°C

Temp (°C)	First Force Peak (g)	Mean Force (g)	Force-Time Area (g × sec)
140	259 ± 10	194 ± 5	2,844 ± 121
145	1,001 ± 3	607 ± 16	8,931 ± 318
150	1,055 ± 37	721 ± 10	10,654 ± 207
152	905 ± 21	667 ± 12	9,804 ± 281
155	632 ± 48	510 ± 40	7,458 ± 601
160	208 ± 13	166 ± 7	2,450 ± 73
165	35 ± 2	29 ± 1	422 ± 15

TABLE II
Molecular Weight-Average (M_w) and Number-Average (M_n) of 3% High-Amylose Maize Starch Heated at Different Temperatures

Temp (°C)	$M_w \times 10^{-6}$	$M_n \times 10^{-6}$	Polydispersity
140	2.05	0.66	3.11
145	1.74	0.51	3.41
150	1.15	0.29	3.97
155	1.45	0.41	3.54
160	1.16	0.31	3.74
165	0.61	0.16	3.81

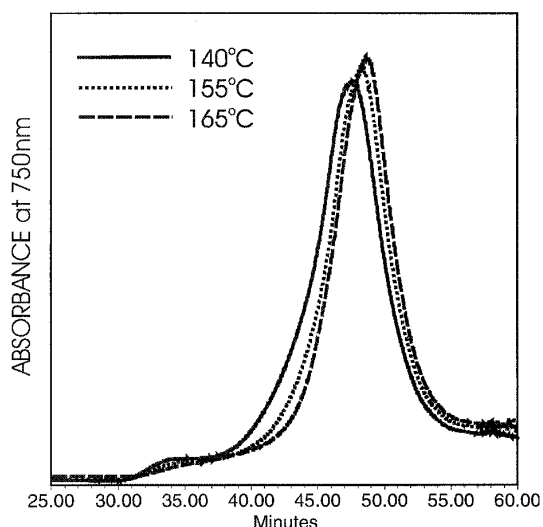


Fig. 6. Size-exclusion chromatograms of high-amylose starch heated to various temperatures.

which in turn led to the delayed initiation of phase-separation and network development. The effects of composition, temperature, and storage time on the texture of high-amylose Hylon VII starch gel systems using textural profile analysis tests were studied by Edwards et al (1998). They found that gel hardness increased as the treatment temperature increased from 110 to 150°C, and the microscopy samples showed that more starch samples were gelatinized at higher temperatures. Also, gel hardness increased at all compositions with storage time.

The results of this study confirm that solubilized amylopectin acts as a filler in the continuous amylose matrix and the differences in physical properties between different starch gels are influenced by the amylose-to-amylopectin ratio. In addition, components of high-amylose starch have different solubility and degradation behavior on heating and their ratio and their molecular structure can be influenced by preparation temperature.

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LITERATURE CITED

Atkin, N. J., Cheng, S. L., Abeysekera, R. M., and Robards, A. W. 1999. Localisation of amylose and amylopectin in starch granules using enzyme-gold labelling. *Starch* 5:163-172.

Boltz, K. W., and Thompson, D. B. 1999. Initial heating temperature and native lipid affects ordering of amylose during cooling of high-amylose starches. *Cereal Chem.* 76:204-212.

Case, S. E., Capitani, T., Whaley, J. K., Shi, Y. C., Trzasko, P., Jeffcoat, R., and Goldfarb, H. B. 1998. Physical properties and gelation behavior of a low-amylopectin maize starch and other high-amylose maize starches. *J. Cereal Sci.* 27:301-314.

Doublier, J.-L., and Llamas, G. 1993. A rheological description of amylose-amylopectin mixtures. Pages 138-146 in: *Food Colloids and Polymers: Stability and Mechanical Properties*. E. Dickenson and P. Walstra, eds. Royal Society of Chemistry: Cambridge.

Durrani, C. M., and Donald, A. M. 1995. Physical characterisation of amylopectin gels. *Polym. Gels Networks* 3:1-27.

Edwards, R. H., Berrios, J. D., Mossman, A. P., Takeoka, G. R., Wood, D. F., and Mackey, B. E. 1998. Texture of jet cooked, high amylose corn starch-sucrose gels. *Lebensm. Wiss. Technol.* 31:432-438.

Gidley, M. J. 1989. Molecular mechanisms underlying amylose aggregation and gelation. *Macromolecules* 22:351-358.

Jane, J.-I. 1997. Starch functionality in food processing. Pages 26-35 in: *Starch: Structure and Functionality*. P. J. Frazier, A. M. Donald, and P. Richmond, eds. Royal Society of Chemistry: Cambridge.

Klucinec, J. D., and Thompson, D. B. 1999. Amylose and amylopectin interact in retrogradation of dispersed high-amylose starches. *Cereal Chem.* 76:282-291.

Leloup, V. M., Colonna, P., and Buleon, A. 1991. Influence of amylose-amylopectin ratio on gel properties. *J. Cereal Sci.* 13:1-13.

Leloup, V. M., Colonna, P., Ring, S. G., Roberts, K., and Wells, B. 1992. Microstructure of amylose gels. *Carbohydr. Res.* 18:189-197.

Miles, M. J., Morris, V. J., Orford, P. D., and Ring, S. G. 1985. The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydr. Res.* 135:271-281.

Parovuori, P., Manelius, R., Suortti, T., Bertoft, E., and Autio, K. 1997. Effects of enzymatically modified amylopectin on the rheological properties of amylose-amylopectin mixed gels. *Food Hydrocoll.* 11:471-477.

Ring, S. G., Colonna, P., I'Anson, K. J., Kalichevsky, M. T., Miles, M. J., Morris, V. J., and Orford, P. D. 1987. The gelation and crystallisation of amylopectin. *Carbohydr. Res.* 162:277-293.

Shi, Y. C., Capitani, T., Trzasko, P., and Jeffcoat, R. 1998. Molecular structure of a low-amylopectin starch and other high-amylose maize starches. *J. Cereal Sci.* 27:289-299.

Suortti, T., Gorenstein, M. V., and Roger, P. 1998. Determination of the molecular mass of amylose. *J. Chromatogr.* 828:515-521.

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