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Effects of enzymically modified amylopectin on the rheological properties of amylose–amylopectin mixed gels

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Abstract

Branched α-dextrins with different molecular weights were prepared from waxy maize. A series of β-limit dextrins was prepared from α-dextrins and native amylopectin. The fine structure of the dextrin samples was investigated by debranching, and was found to be similar to the unit chain distribution of native amylopectin. The absolute molecular weights of α- and β-limit dextrins and commercial potato amylose were determined by gel-permeation chromatography (GPC) and with a dual light-scattering detector. Solubilized potato amylose and α- and β-limit dextrins were mixed at different ratios to give a total concentration of 8%. Dynamic viscoelastic measurements showed that gel formation of amylose was highly dependent both on the ratio of amylose to α-dextrin and on the molecular weight of α-dextrin. α-Dextrin caused an increase of storage modulus, G', when the amylose: α-dextrin ratio was low and the molecular weight of α-dextrin was high. The high-molecular-weight α-dextrin influenced amylose gelation in the same way as native waxy maize starch, but the medium- and low-molecular-weight α-dextrins weakened the gel formation, especially at a ratio of 25:75 (amylose: α-dextrin). When low-molecular-weight β-limit dextrins were mixed with amylose, the resulting gels were more rigid than those in which amylose was mixed with corresponding α-dextrins. When high-molecular-weight β-limit dextrins were mixed with amylose, the resulting gels were weaker.

Introduction

Starch is composed of two macromolecular components. Amylopectin is highly branched with α-(1→4) and α-(1→6) linkages and amylose is the linear component which is considered to be responsible for the gelation of aqueous starch systems. When amylopectin is hydrolysed by α-amylase, a mixture of branched oligosaccharides (α-dextrins) and short linear maltodextrins is formed. The external chains of the α-dextrins can be further hydrolysed by β-amylase, which cannot bypass inter-chain α-(1→6) linkages and therefore leaves short stubs in the remaining chains. Since isoamylase and pullulanase hydrolyse all the (1→6)-α-D-glucosidic linkages, they are used for the determination of the average chain length of α- and β-dextrins (1).

Amylose is not usually soluble in water at room temperature, but it can be dissolved at temperatures 130°C, after which gels are formed on cooling. Clark *et al.* (2) found that gelation of amyloses with a degree of polymerization >250 occurred at concentrations >1.0%. Higher concentrations resulted in more rapid gelation.

In several industrial applications, starch is dissolved by heat and shear, and molecular dispersions of amylose and amylopectin are formed. The influence of the ratio of amylose to high-molecular-weight amylopectin was studied by Leloup *et al.* (3) and Doublier and Llamas (4). Strong gels were formed above a certain amylose:amylopectin ratio. This point has been estimated as being close to an amylose–amylopectin ratio of 15:85 (4). Amylose plays a major role in gelation until the point of phase inversion is reached.

The absence of favourable interactions between amylose and amylopectin in solution leads to their tendency to separate into amylose- and amylopectin-rich phases (5). Molecular dispersion of starch obtained by mixing pure aqueous solutions of amylose and amylopectin can be used as the simplest model for the complex structures present in

starch gels. The aim of the present work was to study how amylopectins with different molecular weights and structures affect the gel formation of amylose at different amylose–amylopectin ratios.

Materials and methods

The amylose used was purchased from Sigma Chemical Co. (St Louis, MO) and the waxy maize starch (WMS) used as amylopectin was kindly donated by Raisio Chemicals. The α-amylase (*Bacillus subtilis*, liquefying type, Koch-Light) had an activity of 10.85 U/mg when measured in 0.001 mol/dm^3 sodium acetate buffer (pH 6.5) at 25°C using soluble starch (Merck) as substrate at 5 mg/ml. The isoamylase (from *Pseudomonas amyloderamosa*) and the pullulanase (from *Klebsiella pneumoniae*) were obtained from Hayashibara Shoji Inc. The β-amylase (from sweet potato) was from Boehringer Mannheim. The activities of the enzymes were 655 000, 2000 and 2500 U/ml, respectively, according to the supplier.

Preparation of α-dextrins

Waxy maize starch was defatted with hot aqueous (85%) methanol using a Soxhlet apparatus and dried with acetone. The starch (70 g) was gelatinized in deionized water (2.193 l) for 90 min. α-Amylase (140 ml, 0.6 U/ml) in 0.2 mol/dm^3 sodium acetate (pH 6.5) was added at 25°C. After 15 min, the hydrolysis was stopped with 5 mol/dm^3 KOH (58 ml) and the pH was adjusted to 11.0 with 2 mol/dm^3 HCl.

The mixture of dextrins in the hydrolysate was fractionated with different ratios of methanol–water at room temperature, as outlined in the scheme in Figure 1. Methanol was carefully added to a ratio of 0.25:1 and after 20 h the precipitate (fraction 1) was separated from the supernatant by decanting. Fraction 1 was composed of a heavier precipitate (fraction 1.1) that was firmly packed at the bottom and a lighter precipitate (fraction 1.2) that settled over fraction 1.1 as a viscous layer. Fraction 1.2 was separated from fraction 1.1 by decanting and careful rinsing. The methanol–water ratio in the supernatant was increased to 0.5:1 and the precipitate formed after 24 h was collected by centrifugation in a Sorvall RC2-B centrifuge (700 *g*, 10 min) and washed with 50% and then 100% methanol (fraction 2).

The large dextrins obtained in fraction 1.1 were dissolved in deionized water to a concentration of 10 mg/ml and purified by repeated precipitation with methanol–water 0.25:1 and centrifugation (200 *g*, 10 min) at room temperature until no more intermediate-sized dextrins could be removed. The precipitate was finally dissolved in deionized water and freeze–dried (sample 1.1.1). Dextrins of intermediate sizes were precipitated from the combined supernatants with methanol–water 0.5:1, centrifuged and freeze–dried (sample 1.1.2). Fraction 1.2 was treated similarly and fractionated into samples 1.2.1 and 1.2.2.

Fraction 2 was also treated like fraction 1.1, and when no more larger dextrins could be removed, the remaining small dextrins in the supernatants were precipitated with methanol–water 0.5:1 at 4°C. The precipitates were centrifuged at –20°C (7500 *g*, 40 min) combined, dissolved in deionized water and freeze–dried (sample 2.1).

Preparation of β-limit dextrins

The isolated α-dextrins were dissolved in hot deionized water (11.1 mg/ml) overnight under gentle stirring. Sodium acetate

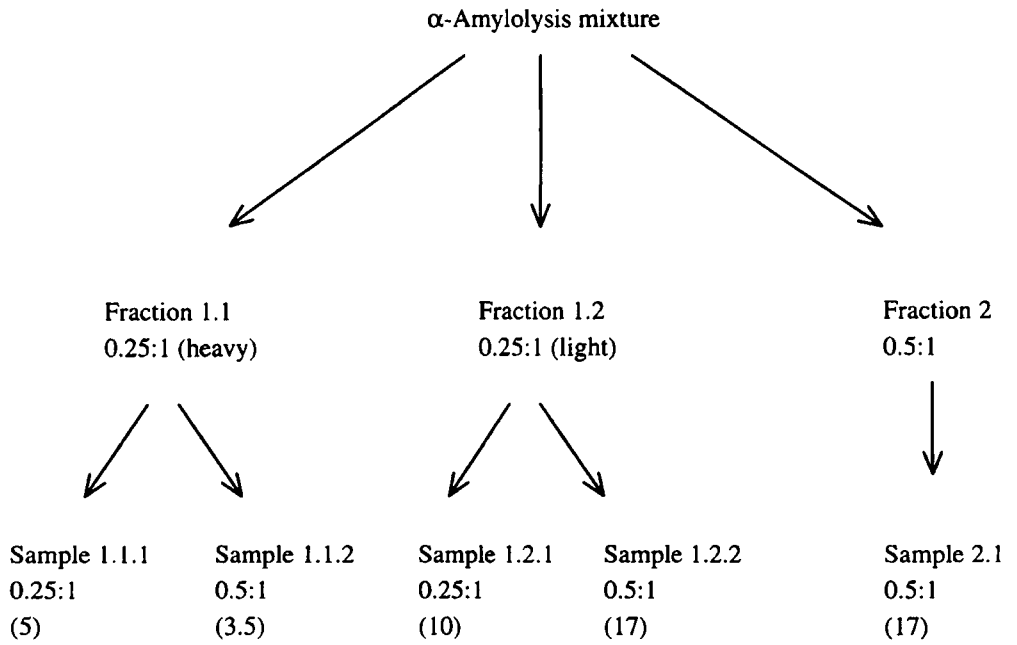

Figure 1 Scheme of the fractional precipitation of α-dextrins obtained from waxy maize starch. The fractions were obtained with the methanol–water ratios shown, and the percentage yields are shown in parentheses.

buffer (0.1 mol/dm^3, pH 4.8) was added together with β-amylase to give a final concentration of 500 U/g dextrins and 10 mg dextrins/ml in 0.01 mol/dm^3 buffer. The mixture was incubated overnight at room temperature and the β-amylolysis limit (β-limit %) was determined by calculating the percentage of maltose formed from the gel-permeation chromatograms obtained directly after β-amylolysis. The average β-limit for the samples was 54%. The β-limit dextrins were precipitated with 0.5 vol of methanol at 4°C, dissolved, re-precipitated and freeze–dried.

Characterization of the dextrins

The α-dextrins were dissolved in hot water and the β-limit dextrins in hot 90% DMSO (50 mg/ml), and diluted with water to stock solutions of 10 mg/ml.

The stock solutions of α-dextrins (0.7 ml) were treated with 0.1 mol/dm^3 sodium acetate buffer (0.24 ml, pH 3.5) and freshly diluted (×10) isoamylase (2 μl) at room temperature. The mixtures were boiled after 5 h and water (0.3 ml) and 5 mol/dm^3 KOH (12 μl) were added to 0.3 ml aliquots of the boiled hydrolysate before analysis on a Superdex 75 column (1.0 × 80 cm). Fractions (0.5 ml) were collected and the carbohydrate contents in the fractions were analysed using the phenol–sulphuric acid method (6). The column was calibrated using dextrins with known degrees of polymerization (7,8).

The stock solutions of β-limit dextrins (0.51 ml) were treated with 0.1 mol/dm^3 sodium acetate buffer (0.09 ml, pH 5.5) and undiluted pullulanase (15 μl). The mixtures were incubated overnight at room temperature, boiled for 5 min and finally analysed on the Superdex 75 column.

The pH in the remaining aliquots was adjusted to 4.8 with either 0.005 mol/dm^3 sodium acetate or 0.005 mol/dm^3 acetic acid and the volume was adjusted to 0.59 ml with 0.1 mol/dm^3 sodium acetate buffer (pH 4.8) before the addition of β-amylase (7 μl). The samples were incubated overnight, then treated with 5 mol/dm^3 KOH (12 μl) and finally applied to the Superdex 75 column. All samples were hydrolysed into >98% maltose in these control experiments, which confirmed that the debranchings were complete.

Preparation of mixed gels

Amylose solutions (8%) were prepared by heating amylose in deionized water at 160°C for 2 h in a bomb calorimeter. A correction was later made for any loss due to evaporation. Amylopectin (WMS) suspension (8%) was prepared in deionized water by heating in a boiling water bath for 10 min after gelatinization. The solutions were mixed hot to give amylose/amylopectin ratios of 50:50, 30:70, 25:75, 20:80 and 15:85. Mixed samples were shaken thoroughly and held at 95°C for 5 min before transferring to a rheometer. Fractions of WMS with large, medium and small molecular weight distributions (samples 1.1.1, 1.2.2 and 2.1 respectively) were dissolved in deionized water at 95°C and mixed with amylose solution to give amylose–amylopectin ratios of 50:50 and 25:75.

Rheological measurements

Rheological measurements were performed with a Bohlin VOR rheometer (Bohlin Reologi Ab, Lund, Sweden) in the oscillatory mode, with a strain of 0.003 and a frequency of 1 Hz. At this strain, all the samples were within the linear range. Samples were loaded hot to the rheometer, where they were cooled from 90 to 25°C at a rate of 1.5°C/min. A thin coat of silicone oil was used to prevent drying of the sample.

Molecular weight distribution

For the gel-permeation chromatography (GPC) analysis, a 200 mg starch sample was moistened for 1 h with 5 ml of water and then 35 ml of 1 mol/dm^3 sodium hydroxide solution was added. The samples were stirred overnight and diluted 2:5 with 1 mol/dm^3 sodium hydroxide solution.

The HPLC-GPC instrument consisted of an M-490 pump, M-715 automatic injector and μHydrogel 250, 500 and 2000 columns at 70°C. The eluent was 50 mol/dm^3 sodium hydroxide fed at a flow rate of 0.5 ml/min. A refractive index detector containing a laser light-scattering detector (PDI 2000, Precision Detectors, Amherst, MA) measuring at 15 and 90° was used for the measurement of absolute molar masses. Post-column on-line iodine colourization with spectroscopic detection in the wavelength range of 540–800 nm was used for structure elucidation. The system was controlled and the data handled by a Millennium workstation. All the equipment with the exception of the laser light-scattering detector was from Waters (Millford, MA).

Results

Molecular weight distribution

As can be seen in the GPC chromatograms of Figure 2, limited hydrolysis of amylopectin and subsequent fractional precipitation with methanol produced different fractions

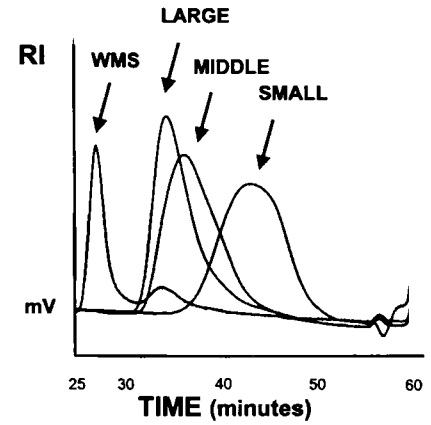

Figure 2 Molecular weight distributions of native waxy maize starch and three amylopectin fractions.

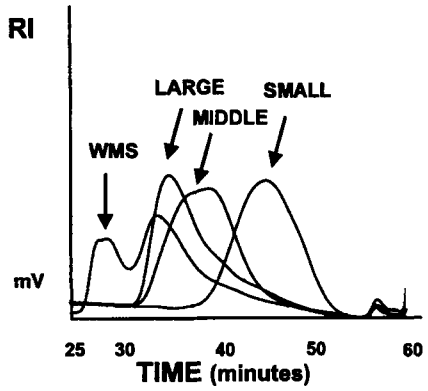

Figure 3 Molecular weight distributions of β-limit dextrins from native waxy maize starch and three amylopectin fractions.

Table 1 Molecular weights of amylopectin fractions and amylose

	M_w (× 10^6)	
		β-limit dextrins
Native waxy maize starch	100	80
Large fraction	18	10.2
Medium fraction	8.4	3.8
Small fraction	0.82	0.37
Amylose	0.55	–
After dissolving	0.025	–

of α-dextrins. Further depolymerization with β-amylase resulted in fractions with lower molecular weights (Fig. 3). The average molecular weights of the fractions are given in Table 1. Dissolving amylose in a pressurised vessel decreased its molecular weight considerably. The reduction in M_w strongly suggests that the amylose was extensively depolymerized. This molecular weight reduction was reproducible and the storage modulus values obtained for pure amylose gels agreed well with those of Svegmark *et al.* (9). The same commercial amylose preparation and an almost identical heat treatment were used as in their study. The shear modulus of monodispersed amylose has been shown to be strongly dependent on the concentration and on the chain length (2). The purity of the amylose was ~95%. The branched portion of the amylose sample was detected both with post-column iodine colourization and laser light-scattering detection. The small leading edge presenting the branched molecules was then quantified from a refractive index chromatogram.

The gel permeation chromatograms in Figure 4 show a representative example of the debranched α-dextrins. All samples possessed similar unit chain profiles that were typical for WMS (8,10–12). The average chain lengths (CLs) were calculated from the curves and are presented in Table 2. The CLs of the α-dextrins were similar to or only slightly smaller than the value found for the original amylopectin. In a β-limit dextrin the external chains were reduced to two glucosyl residues on average (13). Thus, after debranching,

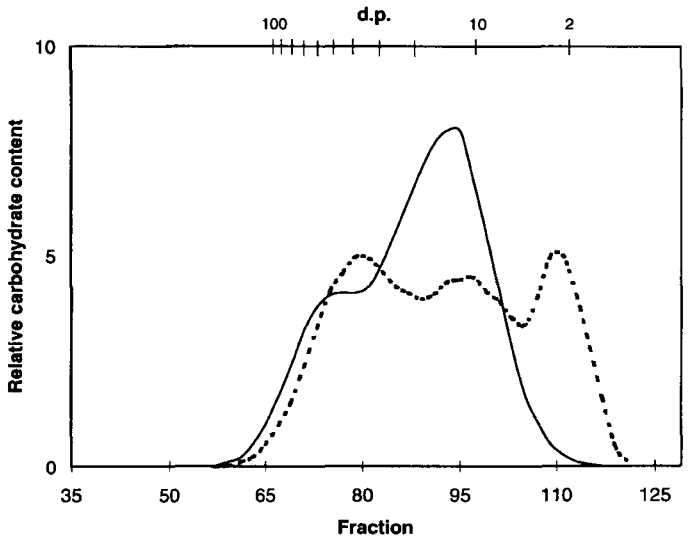

Figure 4 Molecular weight distribution on Superdex 75 after debranching of α-dextrins (——) and β-limit dextrins (- - - -) of sample 2.1.

Table 2 Characterization of the dextrin fractions obtained from amylopectin

Sample	Amylopectin	Large	Medium	Small
CL of α-dextrins	19.8	20.3	18.5	19.7
CL of β-limit dextrins	10.3	9.3	8.6	8.9

CL, chain length.

the internal structure of the dextrins is revealed. All samples were similar and the CLs of the β-limit dextrins were approximately half the original values, which agreed with the β-limit values obtained. The data thus confirmed that the fine structures of the dextrins obtained by limited α-amylolysis were almost identical to that of the native amylopectin and the isolated fractions could therefore be considered as low-molecular-weight amylopectins.

Mixed gels

Pure amylose formed very firm gels in the concentration range of 4–8% and the *G* values agreed well with those of Svegmark *et al.* (9). When high-molecular-weight amylopectin was present, it weakened or strengthened the gel-forming property of amylose depending on the ratio of amylose to amylopectin (Fig. 5). Amylopectin caused a decrease of *G* when the amylose–amylopectin ratio was 50:50 and an increase of *G* at a lower ratio (25:75). This result is in agreement with that of Leloup *et al.* (3). High-molecular-weight amylopectin probably acts as a gel reinforcer by increasing the effective concentration of amylose. The time of gelation was always shorter in mixed gels than in pure amylose gels.

During cooling of amylose–amylopectin mixed gels, there

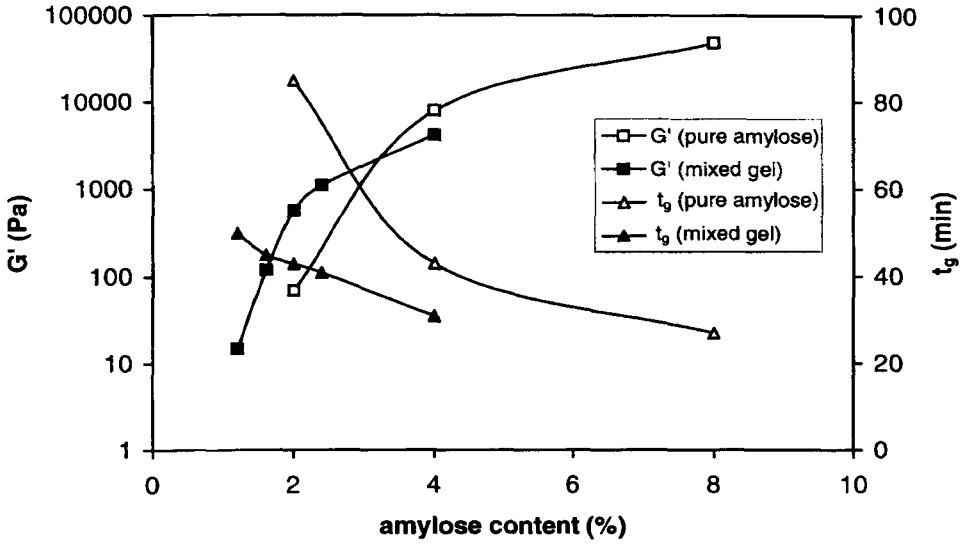

Figure 5 Storage moduli (G') and times of gelation (t_g) of amylose (open symbols) and amylose–amylopectin (filled symbols) gels as a function of amylose content.

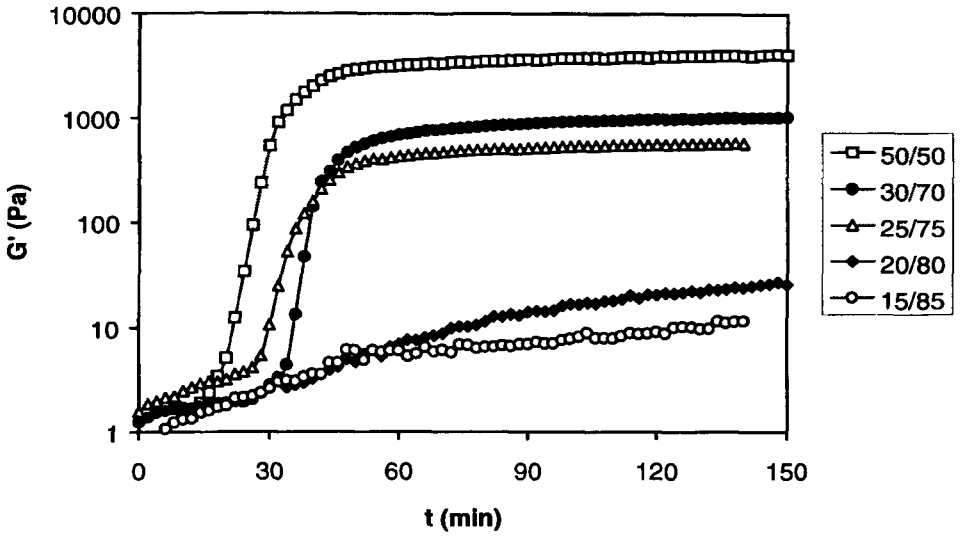

Figure 6 Gelation of amylose–amylopectin mixtures during cooling.

was a sharp increase in storage modulus 20 (50:50 gel) or 30 (30:70 and 25:75 gels) min after the cooling sequence started and the final plateau value of G' was reached soon thereafter. In the gels that contained only 15 or 20% amylose, there was no sharp increase in G', but the storage modulus increased slowly and continuously (Fig. 6).

High-molecular-weight α-dextrin affected the gelation of amylose in a similar way to native WMS at mixing ratios of 25:75 and 50:50. α-Dextrin caused a decrease of G' when the amylose–amylopectin ratio was 50:50 and an increase of G' at a lower ratio. When medium- or low-molecular-weight α-dextrins were mixed with amylose, the resulting gel was much weaker than with large dextrins, especially at a ratio of 25:75, at which medium-size dextrin did not form a gel with amylose at all.

When the dextrins had a high molecular weight (native and large dextrins), the mixed amylose–β-limit dextrin gels had a lower storage modulus than the corresponding mixed gels containing α-dextrins, except that the native mixed gel containing β-limit dextrins had a similar modulus to native WMS at a ratio of 50:50 (Fig. 7). At a ratio of 25:75, however, the high-molecular-weight β-limit dextrins improved the gel formation of pure amylose, whereas at a ratio of 50:50 the opposite effect was observed. On the other hand, when the molecular weight of dextrins was low (medium-size and small dextrins), the mixed gels composed of β-limit

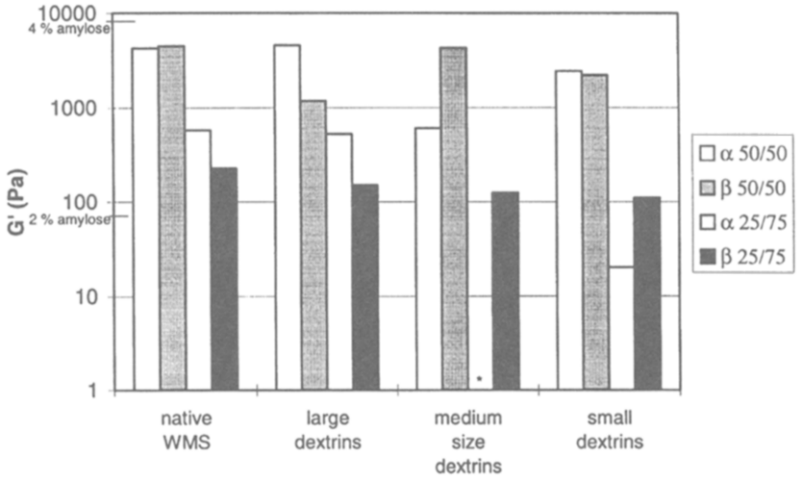

Figure 7 Effect of amylopectin molecular size on the storage modulus of mixed gels of amylose and α-dextrins or β-limit dextrins at mixing ratios of 50:50 and 25:75 (* not gelled).

dextrins were mainly more rigid than those composed of α-dextrins. At a ratio of 25:75, low-molecular-weight β-limit dextrins caused an increase in G' compared with that of pure amylose. The variability of the observations was ~10% or less.

Discussion

The ratio of amylose to amylopectin had a marked effect on the rheological properties of mixed gels. At a ratio of 50:50, the mixed gels were less rigid than pure amylose gels at the same concentration, regardless of the molecular weight or chemical structure of amylopectin. The gel formation of mixed gels was greatly dependent on the molecular weight and chemical structure of amylopectin at the low amylose–amylopectin ratios (25:75) commonly existing in native starches. Depending on the structure of amylopectin, amylose together with amylopectin formed more or less rigid gels than amylose alone.

The molecular weight of α-dextrins is thought to affect the miscibility of amylose and dextrin. It is well known that high-molecular-weight amylopectin and amylose are not miscible, and tend to separate into amylose- and amylopectin-rich domains enriched to ~70–80% of each component (5). The rest of amylose and amylopectin was co-crystallized. At a low amylose:amylopectin ratio (25:75), amylopectin probably acts as a gel reinforcer by increasing the effective concentration of amylose, independently of its chemical structure. At higher ratios, part of the amylose is co-crystallized with amylopectin at the interphase of the microdomains and this amylose is not available for the amylose network.

With both ratios, the small α-dextrins gave more rigid mixed gels with amylose than the medium-sized α-dextrins. Large branched species are particularly effective in promoting phase separation with other polymers (14). It is possible that the great difference in storage modulus values of mixed gels containing large or medium α-dextrins at low amylose–amylopectin ratios is due to a change from phase-separated to a more miscible system in which the interactions between amylose and dextrins are less favourable than in the case of amylose and small α-dextrins. Our results support the suggestion made by Schierbaum *et al.* (15), Vorweg *et al.* (16) and Biliaderis and Zawistowski (17) that molecular interaction and complex formation between amylose and small-sized α-dextrins can take place. Amylose probably associates with the linear chains in the amylopectin.

In most cases, the medium-sized and small β-limit dextrins formed more rigid mixed gels than the corresponding α-dextrins. This is due to the fact that interactions between segments of two different polymers are less favourable than those between similar chains. The important structural difference is that the α-dextrins contain external chains (CL ~ 15), whereas they have been removed from the β-limit dextrins. In fact, the external chains in amylopectin interfere with gelation, whereas the internal chains stabilize the amylose network.

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