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Influence of Amylose–Amylopectin Ratio on Gel Properties

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The aim of this work was to study the structural features of aqueous starch gels as a function of their amylose:amylopectin ratio, r. Structural characteristics of amylose–amylopectin gels were studied using uniaxial compression measurements, mild acid hydrolysis, α-amylolysis, X-ray diffractometry and hot water solubility. Two families of gels were isolated according to the amylose:amylopectin ratio (r). Mixed gels had similar behaviour either to pure amylopectin gels for $r < 0{\cdot}43$ or to pure amylose gel for higher values of r. Amylopectin-rich gels were fairly well degraded both chemically (60 to 100%) and enzymically ($\sim$ 50%), but had poor mechanical properties and solubility behaviour. Amylose-rich gels however were slightly degraded ($\sim$ 20% in all cases), but exhibited good mechanical and thermal resistances. Results were explained in terms of supra-molecular organization, suggesting a phase-separated structure with a continuous matrix of one polymer embedding microdomains of the second polymer. Polymer composition of each phase was determined by the ratio r, and the particular value of $r = 0{\cdot}43$ was interpreted as the inversion point between the two types of gel.

Introduction

Understanding of starch gelation has been a topic of many scientific investigations over half a century. Opaque elastic gels are formed upon cooling of concentrated starch solutions ($\geqslant 1{\cdot}5\%$ w/w), as a consequence of a phase separation[1,2] and local crystallizations[3]. Many workers have studied starch gel characteristics[4,5], and particularly crystallization kinetics according to starch origin[6–9] or gelatinization procedure[10–12]. It was shown that amylose gelation was achieved within a few hours[1], whereas longer times were needed for amylopectin (several weeks)[13].

Rheological studies on starch gels obtained from partial dispersion of starch granules, have indicated a behaviour of composite material. Ott and Hester[6] found a linear relationship between the concentration of amylose and the strength of the starch-gels, made by dispersion of waxy starch granules in amylose solution. A minimal amylose concentration of 1·06% was necessary for gelation to occur[6]. But less amylose was required for gel formation in the presence of starch granules (0·5%), the difference being due to granule fragmentation. These results were interpreted in terms of a continuous matrix filled with a dispersed phase[14–17]. Elasticity was dependent on a number of parameters, amongst which elasticity of the continuous phase, volume fraction, shape and deformability of the dispersed phase were the main ones. The similarity between the

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reinforcement of amylose gels by enriched amylopectin granules (ghosts) and polymer gels filled with porous deformable materials was then pointed out[1,16,18]. An additional factor shown by Kalichevsky and Ring[19] was amylose–amylopectin incompatibility, resulting from unfavorable thermodynamic interactions between these two polymers.

Incompatibility between gelling polymers (A and B) is a fairly common phenomenon[20] (agar–κ–carrageenan[21], agar–gelatine[22–26], amylose–agarose[27]) and usually leads to mixed gels formed of two phases, each of these being essentially composed of a single polymer (A or B). The polymer compositions of the continuous and dispersed phases are dependent on the ratio A:B. For a particular value of the A:B ratio (called the phase inversion point), the continuous matrix becomes the discontinuous filler and vice versa. Starch gels obtained from a total dispersion of starch macromolecules can be considered as a particular case of two phase mixed gels.

Amylose content is determined genetically and varies over a limited range (16 to 28 %), although high amylose (amylomaize, barley, rice, wrinkled pea) and amylose-free genotypes may be found. In order to model the starch gelation process, it should be interesting to study the behaviour of such biphasic systems as a function of amylose:amylopectin ratio. In addition, some recent interest has been shown in preparation of noodles from gluten-free starchy materials, such as rice (*Oryza sativa*) and mungo bean (*Phaseolus aureus*) starches[28]; high amylose genotypes containing over 30 % of amylose gave the best products[29,30]. However, it was observed that gel structure was responsible for a decreased enzymic degradability of starch macromolecules, although better metabolic responses were obtained[31]. These slowly hydrolysed foods are of medical interest because they could improve diabetic control and reduce serum lipid levels[32].

The aim of the present work was to study the influence of the amylose:amylopectin ratio on the functional and *in vitro* nutritional properties of starch gels. A range of gels from pure amylose to pure amylopectin gels, was studied in terms of their macromolecular network structure.

Experimental

Materials

Potato amylose was purchased from Avebe (The Netherlands). Waxy maize was kindly given by Roquette Frères (Lestrem, France). Amylose and amylopectin were solubilized at room temperature in 95 % dimethylsulphoxide (DMSO) for 72 h, recovered by precipitation using pure ethanol and dried by solvent exchange (acetone, diethylether). Iodine binding capacity was determined using an amperometric method as previously described[33].

α-amylase from *Bacillus subtilis* (EC 3.2.1.1) was supplied by Koch-Light (Haverhill, U.K.).

Preparation of gels

Weighed amounts (total mass 2 g, dry basis (d.b.)) of amylose and amylopectin were dispersed in distilled water (25 ml). The suspension was heated in a boiling water bath for 10 min with N_2 bubbling. Water losses, estimated by weighing, were offset by adding corresponding amounts of warm distilled water. The 8 % concentrated pastes were then poured rapidly into cylindrical moulds (diameter 3·0 cm, height 3·0 cm) of which the top edge was raised by means of a tape of acetate film. After cooling in order to avoid diffusion phenomena, the surface of the gel was coated with light oil to prevent dehydration. Prepared gels were kept at 4 °C for 5 days before any experiment.

Enzymic hydrolysis

Mixed amylose-amylopectin gels were first of all ground with a Polytron homogenizer until a homogeneous suspension was obtained (5 min). Enzymic hydrolysis of gels by *B. subtilis* α-amylase were performed as described previously[34]. Soluble α-glucans were measured by an amyloglucosidase–glucose oxidase determination[35]. The extent of hydrolysis was expressed as the amount of soluble oligosaccharides formed as a percentage of total starch. Precision of the determination method of oligosaccharides was 5%.

Mechanical characterization

Compressional tests were carried out on gel cylinders using an Instron Testing Machine. A compression rate of 0·2 cm/min and a controlled temperature of 22 °C were chosen as experimental conditions. Gel compression ratio E was calculated from the initial slope ($F/\Delta h$) as follows:

$$E = \frac{F}{A}\frac{h}{\Delta h} = \frac{F}{\Delta h}\frac{h_0}{A}$$

with F being the total compressive forced applied to the sample, h_0 the initial gel height, A the initial gel cross-section.

Physicochemical characterizations

Gels were freeze-dried after immersion in liquid nitrogen, ground and rehydrated in a saturated water atmosphere for 24 h. X-ray diffraction patterns were then recorded from 4 to 30° (diffraction angle, 2θ) as previously described by Buléon *et al.*[36].

Mild acid hydrolysis was performed at 35 °C for 5 days on samples (1·5 g, d.b.) of mixed gel immersed in 100 ml of HCl (0·05 to 0·25 M). Percentages of soluble oligosaccharides present in the supernatant was measured by an amyloglucosidase-glucose oxidase test as described elsewhere[35].

Water solubility

Gels were cut into ribbons (2·5 × 0·2 × 0·2 cm³), and air-dried at 40 °C for 5 days. Samples (5 g, d.b.) of gel ribbons were cooked under reflux in boiling distilled water (150 ml) for 10 min. Samples were drained for 5 min, and rapidly weighed (W_1). Cooked products were predried in an infra-red oven and then placed in an oven at 130 °C to constant weight (W_2). Cooking water dry weight (W_3) was determined by the same procedure. Total cooking loss (TCL, %) was calculated using the following equation (DW, dry weight proportion of crude samples):

$$TCL = \frac{W_3}{5DW}100$$

Swelling index (SI, %) after cooking was calculated by the equation:

$$SI = \frac{W_1 - W_2}{W_2}100$$

Results

Preliminary results

Potato amylose and amylopectin complexed respectively 19·5 and 0·2 mg iodine per 100 mg of α-glucans.

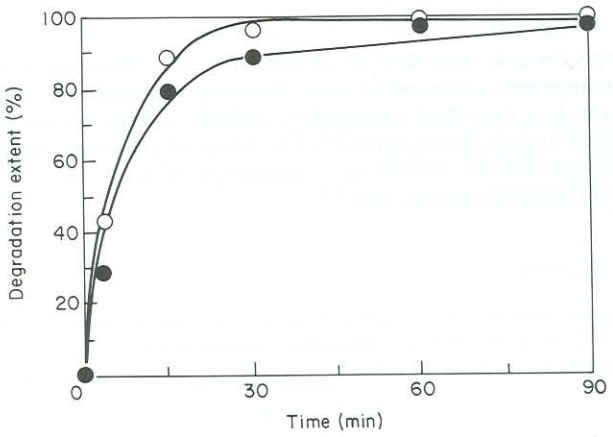

FIGURE 1. α-amylolysis kinetics of amylose —○—, and amylopectin —●—, solutions (2·5% w/w).

α-Amylase activity was determined by studying the first 15 min during the hydrolysis of an amylopectin solution (10 mg/ml). Its specific activity at 35 °C and pH 6·9 was 575 ± 19 nKat (μmol of glucose equivalent/s) per mg of protein. Maltase activity was not detected in this preparation. Kinetic parameters K_M was V_{max} were determined at the initial stage of hydrolysis on both amylose and amylopectin solutions (0·2–1·7 mg/ml). For amylose and amylopectin, the K_M was 8·20 and 3·44 mg/ml respectively and V_{max} was 0·347 and 0·0816 mg of glucose equivalent/min respectively. α-amylase had a higher specificity for amylose ($K_{cat}/K_M = 5·07$) than for amylopectin ($K_{cat}/K_m = 2·84$).

Enzymic degradation

Amylose and amylopectin solutions were degraded enzymically by *B. subtilis* α-amylase using 10·1 nKat of enzyme/mg of α-glucan. Figure 1, showing the hydrolysis extent versus time, indicates no significant difference between these two polymers. These are both completely hydrolysed into small oligosaccharides within 2 h.

Enzymic hydrolysis of gel particle suspensions was studied in the same way. Figure 2 presents hydrolysis kinetics for different amylose:amylopectin ratios (r). The curves were well accounted for a two steps process: (1) a rapid initial hydrolysis (15–20 min) within which 15–40% of the α-glucan macromolecules were solubilized; and (2) a slow final hydrolysis within which α-amylase did not produce complete solubilization for any amylose:amylopectin ratio. The final hydrolysis rate was linear and almost identical for all gels (5–10%/h). Extrapolation to zero time of the slow hydrolysis step allowed one to determine the easily degradable fraction. A least square analysis was achieved and correlation coefficient $> 0·92$ was in each cases found. Figure 3 shows this fraction F as a function of amylose:amylopectin ratio r. A rapid transition occurs for amylose:amylopectin ratios ranging from 0·35 to 1·0, with a change in curvature for a ratio $r \sim 0·43$. Two families of gels may thus be distinguished according to this ratio. For

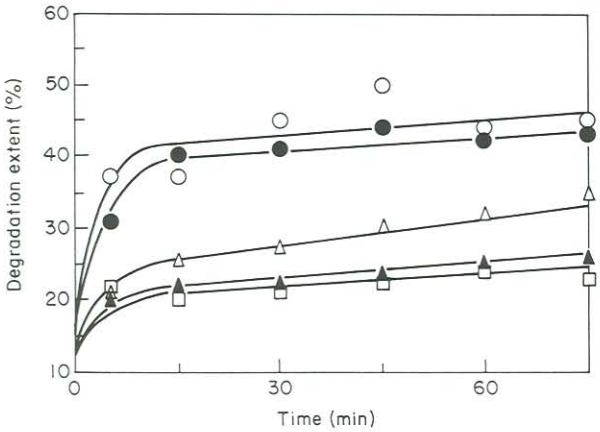

FIGURE 2. α-amylolysis kinetics of amylose–amylopectin mixed gels as a function of amylose:amylopectin ratio, r: ○, amylopectin; —●—, $r = 0{\cdot}43$; —△—, $r = 0{\cdot}66$; —▲—, $r = 4$; —□—, amylose.

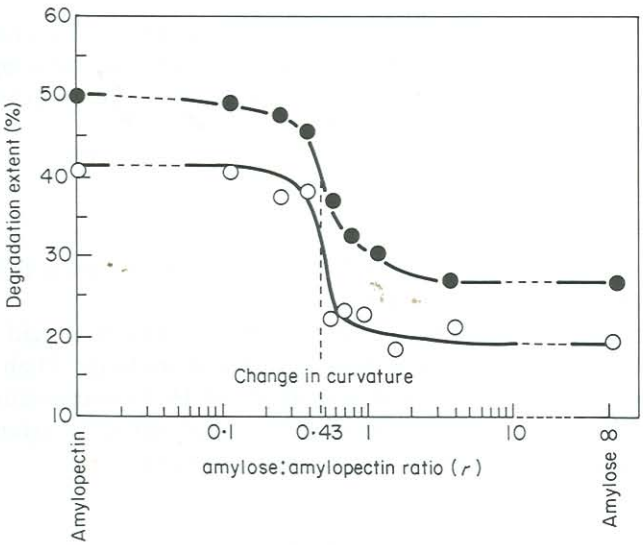

FIGURE 3. Easily degradable fraction, F ○; and extent of degradation after 24 h —●—, of amylose–amylopectin gels as a function of amylose:amylopectin ratio.

values of r lower than 0·43 (gel rich in amylopectin), about 40% of the gels were easily solubilized. For r higher than 0·43 (gel rich in amylose), the amount of hydrolysed gel was decreased to 20%.

In a second experiment α-amylolysis was carried out for 24 h as a function of enzyme concentration (7–138 nKat/mg of α-glucan). Figure 4 presents the degradation extent (%) versus enzyme activity per mg of substrate. Limits of solubilization were independent

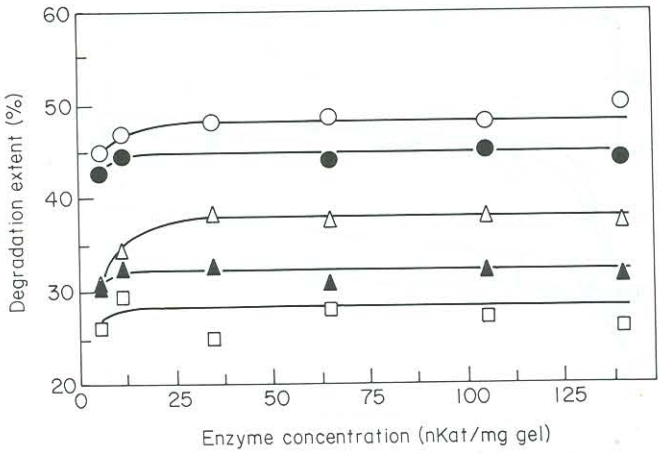

FIGURE 4. Extent of degradation of amylose–amylopectin gels as a function of α-amylase concentration for different amylose:amylopectin ratios (r): —○—, amylopectin; —●—, $r = 0{\cdot}43$; —△—, $r = 0{\cdot}66$; —▲—, $r = 1$; —□—, amylose.

of α-amylase concentration above 69 nKat/mg of α-glucan. The total degradation extent varied as a function of amylose:amylopectin ratio from 50·0 (pure amylopectin gel) to 27·5% (pure amylose gel). The same marked decrease in degradation extent was found for an amylose:amylopectin ratio around 0·43 (Fig. 3).

Physicochemical degradation

The X-ray diffraction patterns of all the gels studied exhibited typical B-type peaks at Bragg angles 5·6, 15·4, 17·0, 21·9 and 24·3° (Fig. 5).

Figure 6 shows the amount of polysaccharides solubilized by mild acid hydrolysis as a function of the amylose:amylopectin ratio and of acid molarity. High amylopectin gels ($r \leqslant 0{\cdot}43$) were easily and totally degraded at all HCl concentrations used. In contrast, high amylose gels ($r > 0{\cdot}43$) were slightly hydrolysed to an extent lower than 40% and a small dependence on HCl molarity was observed.

Mechanical properties

At the concentration studied (amylose + amylopectin = 8% w/w), amylose-amylopectin mixtures started to form gels, rather than viscous opalescent solutions, for r higher than 0·25. The load-deformation curves obtained for mixed gels with $r \geqslant 0{\cdot}25$ were similar in shape and linear after adjustment for the sample height. Figure 7 presents elastic modulus measurements versus amylose:amylopectin ratios. Gels' elastic modulus showed an evident seventh-power dependence on polymer concentration. The most outstanding difference observed was a sharp increase of apparent modulus E from 27 ± 2 to 3354 ± 190 N/m^2 when ratio r increased from 0·43 to 2·33. For r values higher than

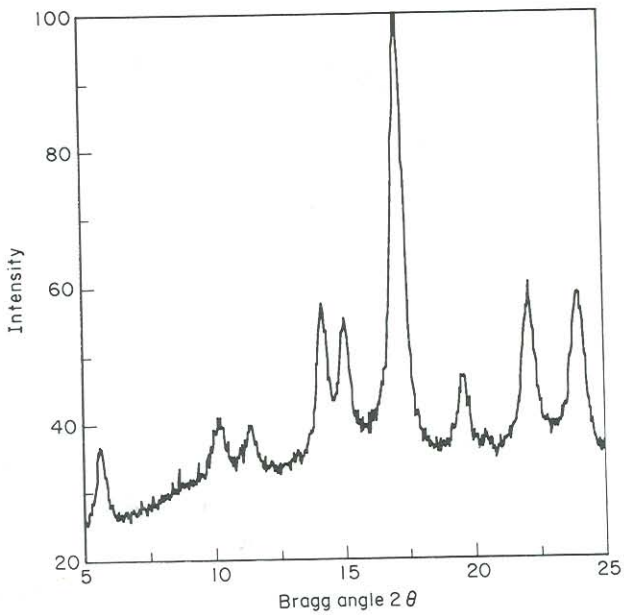

FIGURE 5. X-ray diffraction patterns of a 50:50 (w/w) amylose–amylopectin gel ($r = 1{\cdot}0$).

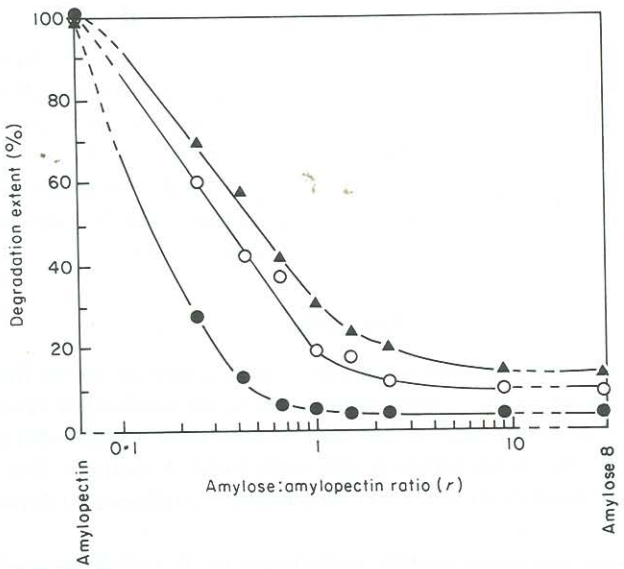

FIGURE 6. Extent of degradation of amylose–amylopectin gels as a function of HCl molarity. —●—, 0·05 M; ○, 0·15 M; —▲—, 0·25 M.

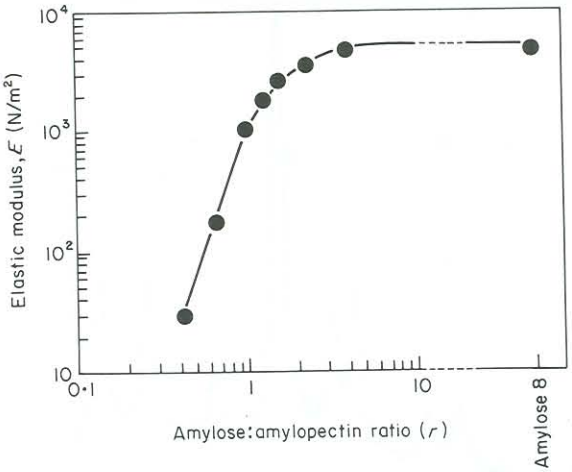

FIGURE 7. Elastic moduli (E) of amylose–amylopectin gels as a function of amylose:amylopectin ratios.

2·33 gel firmness increased at a lower rate, reaching a limit value of 4464 ± 180 N/m^2. The experimental reproducibility was fairly good, better than $\pm 7\%$ from batch to batch.

Water solubility

Gel ribbons, in which the amylose:amylopectin ratio was higher than 0·25, neither solubilize nor swell in water at room temperature. Figure 8 shows swelling indices and solubility looses observed after cooking in boiling water for the different amylose:amylopectin ratios studied. High amylose gels ($r \geqslant 2{\cdot}33$) presented both low swelling indices (1·5 g water/g dry gel) and cooking solubility losses (4·7%). These two parameters decreased with the amylose:amylopectin ratio, and fragmentation occurred representing losses of 18% for the gels with a r value of 0·43.

Discussion

The gels studied were obtained after complete solubilization of starch macromolecules whereas previous investigations[1,12] were mostly based on solubilized (leached) amylose and 'ghosts' made of amylopectin. Furthermore, in this work, the gels did not reach their equilibrium gel point. Although amylose gels were formed within a few hours, longer storage at 4 °C would have been necessary to complete amylopectin crystallization (45 days at 4 °C)[17].

In solution, amylose was more rapidly hydrolysed by *B. subtilis* α-amylase than was amylopectin. However both macromolecules were totally degraded into oligosaccharides, predominantly G_2, G_3, G_4, G_5, G_6 and G_7[37]. Differences arose mainly from the branched and linear molecular structures of amylopectin and amylose respectively[38]. The α-amylolysis method used in this study, measured the formation of soluble

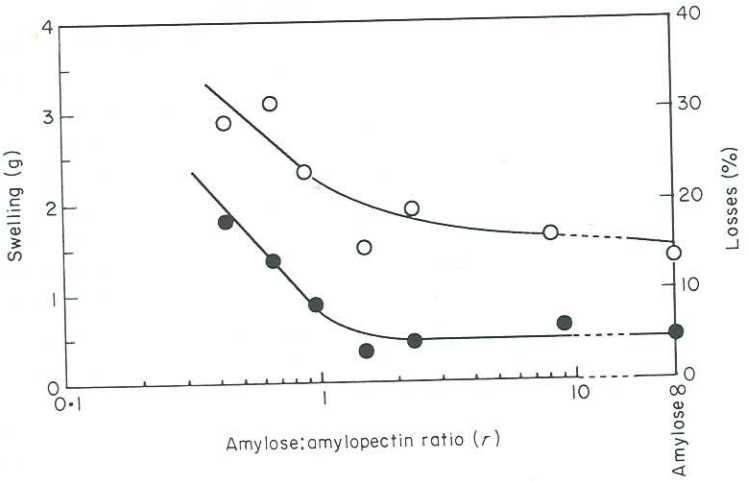

FIGURE 8. Swelling indices, ○; and hot water solubilities, ● losses of amylose–amylopectin gels as a function of amylose:amylopectin ratio (r).

oligosaccharides in 80% ethanol (G_n, with $n < 8$) rather than the effective number of glycosidic bonds hydrolysed. This procedure was well suited to the problem of hydrolysis in a heterogeneous system (solid substrate), as the amount of solubilized products is essentially the only measurable parameter in that case. It was also sufficient because it quantified all the hydrolysis products.

When present in the gel state, none of the gels studied was completely degraded. However, two distinct families of gels were observed. Gels belonging to the first class, in which the amylose:amylopectin ratio was lower than 0·43, behaved as pure amylopectin gels with degradation extent after 24 h ranging from 45 to 50%. The second class of gels ($r > 0{\cdot}43$) behaved similarly to a pure amylose gel. Their enzymic susceptibilities were much lower and important residues representing from 62 to 74% of the initial gel were obtained. The amylose:amylopectin ratio value of 0·43 appears to be a critical value. Experiments carried out with different enzyme:substrate ratios gave the same values for the extent of degradation after 24 h. Therefore, neither α-amylase concentration nor gel particle granularity were the limiting parameters of hydrolysis of mixed gels. The partial resistance to enzymic hydrolysis of mixed amylose–amylopectin gels must arise from the gel molecular structure. The mixed amylose–amylopectin gels are semi-crystalline structures, as shown by X-ray diffraction, made of an amorphous fraction and a more organized fraction. The amorphous fraction forms the easily degradable fraction of the gel (F), whereas in the semi-crystalline fraction, segments of macromolecules are involved in B-type crystallites responsible for their enzymic resistance[28]. The molecular structure of B-type crystallites is now well known[39] although the packing polarity of the helical regions have still to be determined unambiguously[40]. The length of α-glucan chains implied in the crystallites is dependent on molecular structure. The linear amylose tends to form crystallites in which the degree of polymerization ($\overline{DP}$) is between 40 and 60[41], longer than the branched amylopectin, for

which only short chains ($\overline{DP} = 15$) participate in the crystalline structures[17,26,42]. Differences in hydrolytic susceptibilities of the two classes of gels could then be linked to the constitutive crystallite size. However this assumption is unable to explain the non-additive contribution of amylose and amylopectin to the gel structure.

The same trend was observed when using H_3O^+ as a catalyst for hydrolysis. Amylopectin-rich gels were highly, or even completely degraded, whereas amylose-rich gels were only slightly degraded ($< 25\%$). A transition in gel susceptibility to acid hydrolysis was observed at an amylose:amylopectin ratio around 0·43. The better acid degradation of amylopectin rich gels compared to enzymic hydrolysis, may be explained by the size differences of α-amylase and H_3O^+. The smaller size of H_3O^+ allows an easier diffusion throughout the gel, improving the solubilization efficiency. α-amylase diffusion (diameter ~ 6 nm) slows down or even precludes hydrolysis because of the steric hindrance of the gel structure[43,44].

Rheological studies have shown that no rigid gel was formed within 5 days for an amylose:amylopectin ratio lower than 0·25. This result was in agreement with previous works showing that minimum concentrations required for gelation instead of aggregation was 1·5% for amylose[15,16,41] and 10% for amylopectin[13]. Above the critical ratio of 0·25, gel rigidity, E, increased with amylose content according to C^7 (C being the amylose concentration of the gel) in agreement with the previous work of Ellis and Ring[16]. For high amylose:amylopectin ratios, gel rigidity may be ascribed to amylose gelation. Amylopectin, which makes little or no contribution to rigidity, behaves as a filler even though it represents a large volume fraction.

A minimum amylose:amylopectin ratio of 0·43 was needed to maintain the gel network during heating in water. This agrees with thermal stability of amylose and amylopectin networks[13,26,41], which melt at very different temperatures ($T_m \sim 125$ and 45 °C, respectively). The differences in melting temperatures is mainly ascribed to differences in the degree of polymerization of the segments involved in the crystallites. At low amylose content ($r < 0{\cdot}43$), the structure is totally disrupted and solubilized as a result of amylopectin gel melting. High amylose gels ($r \geqslant 0{\cdot}43$) underwent swelling upon heating, due to water absorption by the amorphous regions of the gel network. Under swelling forces, the amylose network was partially disrupted allowing the solubilization of macromolecules. These molecules, accounting for the cooking losses, diffused through imperfections in the gel network. Gel swelling and cooking losses reduced as the amylose content was increased ($> 1{\cdot}5\%$), which seems to indicate that amylose is essential for the gel cohesiveness. Studying porosity[44] of amylose gels for polymer concentration ranging from 4 to 10%, it has been established that diffusion of molecules whose hydrodynamic radii were greater than 20–200 nm (i.e. amylopectin) was prevented. For sufficiently high amylose content, amylose molecules achieved a network around the amylopectin droplets stabilizing the structure in that condition. It appears that gel functionality is partly dependent on crystallite structure, but also that the packing of the amorphous phase is important to explain the gels' characteristics.

Kalichevsky and Ring[19] have shown that amylose–amylopectin mixtures are phase-separated systems, resulting in an amylose-rich phase and an amylopectin-rich phase. However these authors did not give any spatial organization of the two phases. Three cases are possible: amylose–amylopectin mixed gels could be made: (1) of two

discontinuous phases; (2) of two continuous interpenetrated networks; or (3) of one continuous network entrapping a discontinuous phase. According to the preceeding results, the two first suggestions are very unlikely here, as they are unable to explain the sharp transition obtained in all our experiments for $r \sim 0{\cdot}25$–$0{\cdot}43$. On the contrary, these drastic changes in the gel behaviour could be well explained by an inversion between the continuous and discontinuous phases in the third model. Studying gels of branched maltodextrins, Reuther *et al.*[45] observed heterogeneities within the gel, corresponding to molecular aggregates of gyration radius around 90 nm. A simple approach with respect to the mixed amylose–amylopectin gels used in our experiments would be to consider that a continuous matrix of one polymer entraps microdomains made of the other polymer. At equilibrium, the preferred packing arrangement of spherical microdomains will correspond to the lowest free energy. The amylose:amylopectin ratio corresponding to the phase inversion was calculated from conditions for closest and uniform packing of spherical and monodisperse particles. The two high density packings are possible either in an hexagonal cell or a cubic cell[46]. In either condition, the inversion ratio (matrix:particle) is equal to 0·35[46]. In the present case of amylose–amylopectin gels, the inversion ratio should also be influenced by two parameters. Firstly, polydispersity and irregular packing of particles are likely to occur, which would lead to values of the inversion ratio higher than 0·35. Secondly, Kalichevsky and Ring[19] suggested that amylose has a better affinity for water than does amylopectin for water, which would be responsible for differences in phase volumes. In this particular case, the inversion ratio should be shifted towards the low values of the amylose:amylopectin ratio. Although it is difficult to know the respective effects of these two factors, it is reasonable to assume that the transition in the gels behaviour, observed for an amylose:amylopectin ratio of 0·43, corresponds to the inversion in the polymeric composition of the continuous and discontinuous phases. Consequently, it was concluded that mixed amylose–amylopectin gels have a continuous amylopectin matrix for $r \leqslant 0{\cdot}43$ but a continuous amylose matrix for $r \geqslant 0{\cdot}43$. The gels' properties are determined mainly by the polymeric nature of its matrix. For ratios of $r \geqslant 0{\cdot}43$, amylose macromolecules entrap amylopectin droplets in a sort of 'fish net'[44]. Pure amylose gels also present better rigidity, thermostability and longer crystallites than pure amylopectin gels[1,13]. All these characteristics are responsible for increased cohesiveness, cooking stability and resistance to hydrolysis of amylose-rich mixed gels.

However, the mixed gel structure is not as simple as it appears in the phase inversion region ($0{\cdot}35 < r < 1{\cdot}0$). Amylopectin content and the extent of degradation by acid hydrolysis differ largely (about 20%), suggesting that a fraction of amylopectin has become resistant to hydrolysis. A local co-crystallization of amylose and amylopectin macromolecules at the interface of microdomains may be proposed in that case. This would be all the more important under conditions in which the interface was developed (inversion region). This assumption is in agreement with Kalichevsky and Ring[19] who observed the presence in each phase of 20 to 30% of the other polymer. Further work using small angle X-ray scattering and electron microscopy, should be carried out to analyse the supramolecular structure of these gels.

Conclusion

The aim of this work was to understand the influence of different proportions of amylose and amylopectin on the characteristics of gels containing mixtures of these macromolecules. Mixed gels are made of a three-dimensional network mainly composed of one polymer entrapping microdomains of the other polymer. The importance of amylose content for good functional properties and low degradability of starch gels was shown. As found naturally, most starches have amylose:amylopectin ratios around 0·35 and exhibit poor gelling properties in the absence of a network of gluten. As only few natural sources have high-amylose contents (amylomaize, rice, wrinkled pea), more interest could be given to the selection of genotypes producing high-amylose starches ($r \geqslant 1{\cdot}5$) in order to produce food products (e.g. gluten-free noodles) with good functional properties and controlled release of oligosaccharides.

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