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Humidity-induced structural transitions in amylose and amylopectin films

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Abstract

The effect of humidity on the dynamic mechanical and barrier properties and on the microstructure of amylose and amylopectin films was studied. The films were solution-gel-cast, with and without glycerol as added plasticizer, and dried to equilibrium weight under various relative humidities (RH). The network microstructure of glycerol-plasticized amylose films was shown to change from dense and homogeneous to a more open structure with fluctuations in the pore size when the surrounding RH was increased. The structural change was attributed to plasticization of the amorphous areas by increased water content, leading to higher mobility in the network and subsequent inhomogeneous swelling. This structural change had a direct influence on the oxygen permeability, which drastically increased. The onset of the increase occurred at lower surrounding RH for the films containing glycerol, and these films also had higher oxygen permeability. Glycerol-plasticized amylopectin films with gradually increased crystallinity were prepared by varying the RH during film formation. Dynamic mechanical analysis at both varied temperature and RH showed that the increased crystallinity led to higher glass transition temperature, T_g, and a smaller effect of the surrounding humidity on mechanical and barrier properties. The RH during the formation of glycerol-plasticized amylose films did not affect crystallinity or T_g. These films formed at 70% RH were shown to have a marked heterogeneous network. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Amylose and amylopectin films; Structural transition; Humidity

1. Introduction

Starch and its major components, amylose and amylopectin, are biopolymers, which are attractive raw materials for use as barriers in packaging materials. Starch is inexpensive, produced from a renewable resource and biodegradable. The films have good mechanical properties and are excellent oxygen and grease barriers (Arvanitoyannis & Blanshard, 1994; Hullinger, 1965; Mark, Roth, Mehltretter & Rist, 1966; Rankin, Wolff, Davis & Rist, 1958; Wolff, Davis, Cluskey, Gundrum & Rist, 1951). Starch can also be processed thermoplastically (Tomka, 1991), and starch-based films can even be used as an edible coating directly applied on food.

Starch-based films are sensitive to water and humidity, however, which affect the physical properties of the films during and after film formation (Rindlav-Westling, Stading, Hermasson & Gatenholm, 1998; Slade & Levine, 1993). Water is a very strong plasticizer for many biopolymers, including starch (Levine & Slade, 1988). The starch-based films usually need to be plasticized to make them more flexible, and other than water, other low molecular substances such as polyols can be added as plasticizers. Of these, glycerol is most commonly used. The water sorption in starch films has been studied by several authors and been found to depend on the addition of other plasticizers, such as glycerol (Forssell, Mikkilä, Moates & Parker, 1998; Lourdin, Coignard, Bizot & Colonna, 1997a; Sala & Tomka, 1992; Slade & Levine, 1993). Sala and Tomka presented a general theory describing the sorption behaviour of swellable materials (Sala & Tomka, 1992). The water sorption isotherms were found to be different depending on whether T_g was above or below the temperature at which the sorption isotherm was recorded. For a glassy polymer, the water content was a decreasing function of the surrounding relative humidity (RH), while the opposite was found for rubbery polymers.

Water also affects the crystallinity of starch gels and starch-based films. It has been shown that starch gels and extruded starch slowly develop a crystalline X-ray diffraction pattern with time (Arvanitoyannis & Blanshard, 1994; Katz, 1930). Starch films and glycerol-plasticized amylopectin films developed a higher degree of crystallinity when formed at high RH (Rindlav, Hulleman & Gatenholm, 1997; Rindlav-Westling et al., 1998). The amylopectin films were shown to be amorphous when they were formed at

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20% RH and had a relative crystallinity of 32% when formed at 90% RH.

Plasticized amylose films were not affected by the humidity but formed films with a relative crystallinity of ~35% for a RH between 20 and 90%. At sufficiently high concentrations amylose crystallizes from solution (Miles, Morris & Ring, 1985). Leloup and co-workers proposed a model for amylose gels where a network strand consists of associated blocks of double helices aligned along the strand, almost perpendicular to the length axis of the strand (Leloup, Colonna, Ring, Roberts & Wells, 1992). Here, loops of amorphous amylose would be found around the strands and at the cross-links, thus linking the helices. The crystallinity detected by wide angle X-ray diffraction would originate from the ordered structure in the strands. The network of amylose gels has been shown by transmission electron microscopy to be open and composed of stiff, rod-like strands 10–40 nm in diameter (Hermansson, Kidman & Svegmark, 1995). This structure was not strongly affected by the cooling rate, the presence of salts or amylose concentration in the range of 4–10% (Hermansson & Svegmark, 1996). The structure also remained constant when amylose gels were dried to films, with the only difference between gels and films being a decreased pore size in the films (Rindlav-Westling et al., 1998).

The aim of this study was first to observe the effect of relative humidity on the structure of amylose films during film formation and by direct exposure to surrounding humidity and, second, to study the influence of humidity on the mechanical and thermal properties of the already formed amylose and amylopectin films.

2. Materials and methods

Amylose (104561, batch 209861511) from potato was purchased from Merck (Darmstadt, Germany). The amylopectin was kindly supplied by Lyckeby Stärkelsen (Kristianstad, Sweden) in the form of granular amylopectin potato starch. The potato used for this was developed by Lyckeby Stärkelsen and Svalöv Weibull (Svalöf, Sweden) using genetic engineering to suppress amylose synthesis (Hofvander, Persson, Tallberg & Wikström, 1992).

2.1. Film preparation

Amylose films: 5% w/w amylose was dispersed in distilled water, degassed and heated to 150°C. The amylose solution was cooled to 95°C, and glycerol (2% w/w) was added as plasticizer to some samples. The solution was poured onto a PVC dish and allowed to gel at 23°C.

Amylopectin films: 3% w/w amylopectin was dispersed in distilled water, degassed and heated during stirring to 90°C in a Brabender Amylograph and kept at 90°C for 15 min. Glycerol (1.2% w/w) was added as plasticizer to some samples. The solution was poured onto a polystyrene dish and cooled to 23°C.

The plates with amylose and amylopectin were dried at a constant relative air humidity (RH) of 20, 50, 70 or 90% at 23°C in a controlled environment chamber for three days. The films were peeled off and reconditioned for at least two days at 50% RH before measurements. The films referred to as *glycerol-plasticized* all have a glycerol/polymer ratio of 0.4. The thickness of the films was measured with a digital indicator (Mitutoyo IDC-112CB, Mitutoyo Corp, Japan) with an accuracy of ±3 μm. The films were 70–100 μm thick.

2.2. Water vapour sorption isotherm

The films were ground in liquid nitrogen in a mortal before equilibration to various water contents over saturated salt solutions. The salts used were LiCl (11% RH), $MgCl_2$ (33% RH), $Mg(NO_3)_2$ (54% RH), NaCl (75% RH) and K_2SO_4 (97% RH). The samples were equilibrated for one week before being dried at 120°C for 2 h. The water content was determined gravimetrically and calculated from $\text{weight}_{\text{water}}/\text{weight}_{\text{total}}$. All films were conditioned at 54% RH before equilibration over the saturated salt solutions. This means desorption for the two lowest humidities and sorption for the three highest. Since the samples are not at thermodynamic equilibrium, we will not use the term "water activity" but rather "surrounding relative humidity" to make clear what we mean. Slade and Levine have used the corresponding term "relative vapour pressure" (RVP) (Slade & Levine, 1991).

2.3. Oxygen permeability

The oxygen transmission was measured in accordance with ASTM D3985-81 using a Mocon Oxtran 2/90 equipment (Modern Controls Inc., Minneapolis, USA). The permeability was calculated from the transmission and the measured thickness of the films and is presented in the units $cm^3\ \mu m/(m^2\ d\ kPa)$, where $1\ d = 24\ h$. The RH during the measurements was set between 20 and 95%.

2.4. Differential scanning calorimetry

A Perkin–Elmer Pyris 1 DSC (Perkin–Elmer, Norwalk, CT, USA) was used for the differential scanning calorimetry (DSC) measurements. Scans were performed by heating from −110 to 160°C, cooling to −110°C and reheating to 160°C with heating and cooling rates of 10°C/min. The measurements were performed in triplicate in closed steel cups. All films were ground in liquid nitrogen and reconditioned in 54% RH for at least one week before measurement. The inflection point of the step in the heat flow curve in the first heating scan was taken as the glass transition temperature, T_g.

2.5. Dynamic mechanical analysis

Dynamic mechanical analysis of the films was performed in tension in a Rheometrics RSA-II (Rheometric Scientific,

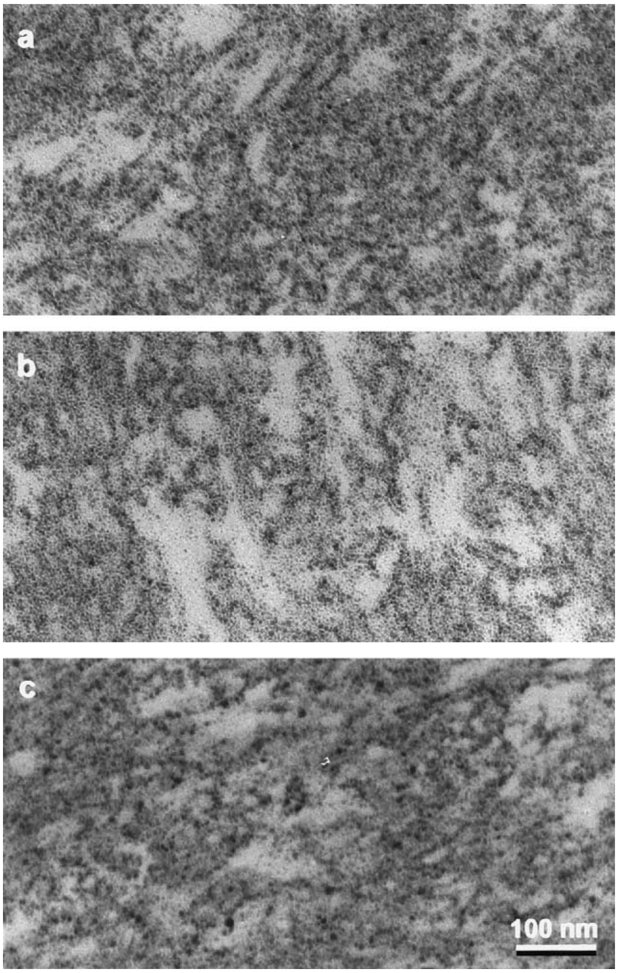

Fig. 1. Transmission electron micrographs of glycerol-plasticized amylose films at a surrounding relative humidity of (a) 35%; (b) 75%. (c) Films exposed to 35% RH were then kept at 75% RH for 4 days and then again exposed to 34% RH.

Piscataway, NJ, USA). A rectangular film strip 4 mm × 30 mm was clamped in the instrument, and a sinusoidal deformation was applied on top of a static deformation, the sum of these being non-destructive. A recording was made of the resulting force and phase shift, δ, to the applied deformation, and the complex tensile modulus, E^*, was calculated. E^* can be divided into the storage modulus, E', and loss modulus, E'', using the relations $E^* = E' + jE''$, $\tan \delta = E''/E'$, where $j^2 = -1$.

The sample was tested in an air-convection oven with circulating dry air. The instrument was also equipped with a humidity control system designed in-house (Stading, 1998). The system consists of a RH sensor in the oven which sends a signal to a PID regulator. The regulator controls two electric valves that mix a dry and a humid air stream to the set RH.

The films were stored in 50% RH, transferred to the instrument and again conditioned at 50% RH before measurement. The *humidity scans* were then performed by changing the RH in steps of 10% from 90 to 10% RH at a constant temperature of 25°C. The *temperature scans* were performed after the initial conditioning at 50% RH by first covering the film with hydrophobic grease to prevent evaporation (Stading, 1998). The grease must be applied to the film to avoid moisture loss, since the air-convection oven works with a dry airflow that would otherwise rapidly decrease the moisture content of the film. Despite the grease, the films starts to lose water at temperatures higher than 60°C, which sets the upper temperature limit. The crystallization of the grease sets the lower temperature limit at about −30°C (Stading, 1998). The instrument was set to temperature mode, and the temperature was directly lowered to −80°C and then raised from −80 to 60°C at 1°C/min. The applied static force and dynamic strain were automatically controlled during the measurement within the intervals (0.15, 1.5 N) and (0.01, 0.1), respectively, to apply as low a deformation as possible without falling below the resolution of 0.1 N. The applied strain was well within the linear region. The glass transition temperature, T_g, was taken as the peak in the phase angle.

2.6. Microscopy

The microstructure of the amylose films was studied with transmission electron microscopy (TEM) on a JEOL microscope (STEM-JEOL 100 CX-II). Small pieces from the centre of the films were prepared by fixation in 2% glutaraldehyde and stepwise dehydration in an ethanol series. The ethanol was then replaced in steps, first by propylene oxide and then by a cured epoxy resin (Polybed, Polyscience Inc.). The embedded samples were sectioned in ~70 nm thick slices using a diamond knife in a Reichert-Jung Ultracut E. The sections were transferred onto Formvar supported gold grits and stained with periodic acid, thiosemicarbazide and silver-proteinate (PA-TSC-SP) by the method described by Thiéry (1967).

3. Results and discussion

The films were formed under controlled environmental conditions at 23°C and a set RH and thereafter always reconditioned at 23°C and 50% RH. The RH in the formation of standard films was 50%. The properties of these films will be described first and the effect of varying the set RH during film formation later.

3.1. Effect of surrounding humidity on film properties and microstructure

The microstructure of amylose films containing glycerol formed at 50% RH is shown in Fig. 1 in TEM micrographs. The images show thin sections of film where the amylose is stained black and the pores are bright. The amylose solutions immediately formed gels on cooling and the network of the resulting films was composed of stiff strands. The network resembled that of the original gel but was denser

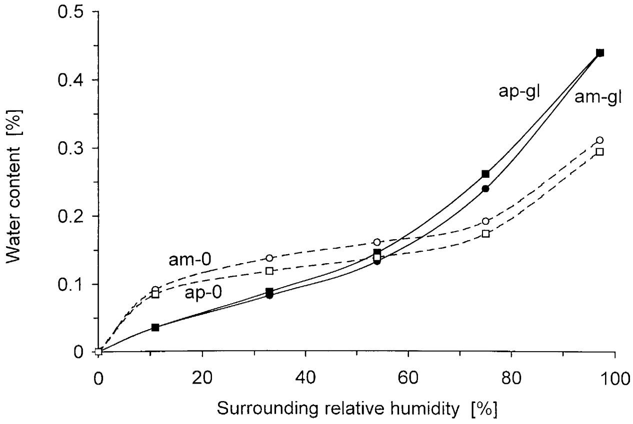

Fig. 2. Water vapour sorption isotherms at 20°C for films formed at 50% RH with and without glycerol as plasticizer. Amylose without glycerol (am-0 ○), glycerol-plasticized amylose (am-gl ●), amylopectin without glycerol (ap-0 □) and glycerol-plasticized amylopectin (ap-gl ■).

(Hermansson et al., 1995; Rindlav-Westling et al., 1998). The pores of the glycerol-plasticized films are assumed to contain water and glycerol. There is probably also some remaining amylose since a pure liquid phase would be entropically unfavourable. Leloup and co-workers proposed that the network strands of an amylose gel are linked with amorphous amylose (Leloup et al., 1992). It is probable that the amylose in the pores is also amorphous. The films shown in Fig. 1 were first formed and reconditioned at 50% RH and then subjected to a low surrounding RH of 35% (Fig. 1a), moved to high surrounding RH of 75% (Fig. 1b) and then back again to a low surrounding humidity of 35% RH (Fig. 1c). The network at low RH is dense and homogeneous (Fig. 1a and c). The maximum pore size at 35 and 50% RH was about 40 nm. The network becomes more heterogeneous when it is subjected to 75% RH (Fig. 1b), with large pores in the order of 100 nm and dense areas. The change was reversible when the surrounding RH was lowered to 35%, as shown in Fig. 1c.

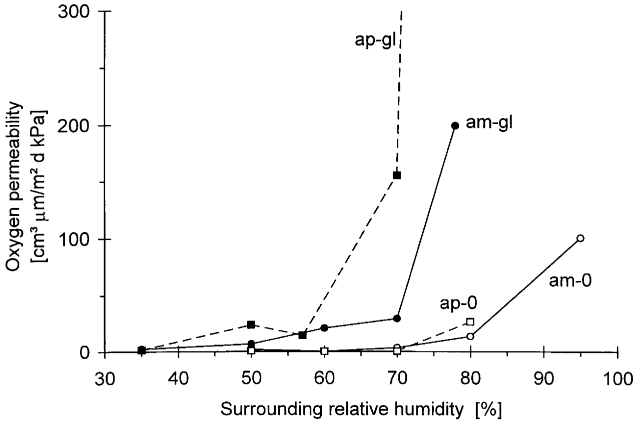

Fig. 3. Oxygen permeability at increasing surrounding relative humidity for films formed at 50% RH. Amylose without glycerol (am-0 ○), glycerol-plasticized amylose (am-gl ●), amylopectin without glycerol (ap-0 □) and glycerol-plasticized amylopectin (ap-gl ■).

The mechanism is proposed to be the following: When the surrounding RH rises the film takes up water, which then plasticizes the amorphous areas of the amylose network. The strands are highly crystalline whereas the amorphous areas are found mainly at the cross-links. The plasticization leads to a more flexible network and allows possibilities for rearrangements in the network structure. On a further increase in the surrounding RH, the network will take up more water and swell. The elasticity of the strands is not completely homogeneous throughout the network and thus the structure is heterogeneous due to the swelling pressure. Open areas with large pores and denser areas with smaller pores are formed.

The proposed mechanism is supported by the water vapour sorption isotherm in Fig. 2, which shows the water sorption isotherms for films formed at 50% RH. The shapes of the curves were different and the glycerol-plasticized films had a larger increase in water content with RH than the films without glycerol. The water content of amylose films plasticized with glycerol increased considerably, from 8.2 to 23%, when the surrounding RH was increased from 35 to 75%. The films that were not plasticized with glycerol did not show as great a change in water content in this RH interval. For films without glycerol, amylose showed a slightly higher absorption of water, which was also found by Bizot and co-workers (Bizot, Le Bail, Leroux, Davy, Roger & Buleon, 1997). For the films plasticized with glycerol, however, amylopectin contained slightly more water than the amylose films did. The hysteresis of water vapour sorption for starch materials is a well known phenomenon (Slade & Levine, 1991). The water content during desorption can also be a few percent higher than during resorption for starch with water (Chinachoti & Steinberg, 1986). The hysteresis was limited and the film taken from 35 → 75 → 35% (Fig. 1c) had a water content of 8.4% as compared with 8.2% in the film in Fig. 1a. The hysteresis was found to disappear when glycerol was introduced into the material as was also observed by Sala and Tomka (1992).

The swelling of the films and increase in water content by an increase in the surrounding RH had a drastic influence on the barrier properties of the films, as shown in Fig. 3. All films had low permeability up to moderate surrounding RH. For comparison, EVOH, which is used in packaging applications because of its low oxygen permeability (OP), has an OP of 0.1–12 cm^3 μm/m^2 d kPa (McHugh & Krochta, 1994). The amylose and amylopectin films had an OP in the same range, although the amylose films had a lower OP than the amylopectin films. The OP increased sharply, with the actual onset depending on plasticizer content and type. The onset occurred at lower surrounding RH for amylopectin films than for amylose films. The onset also occurred earlier for the films containing glycerol, which can be explained in terms of increasing water content.

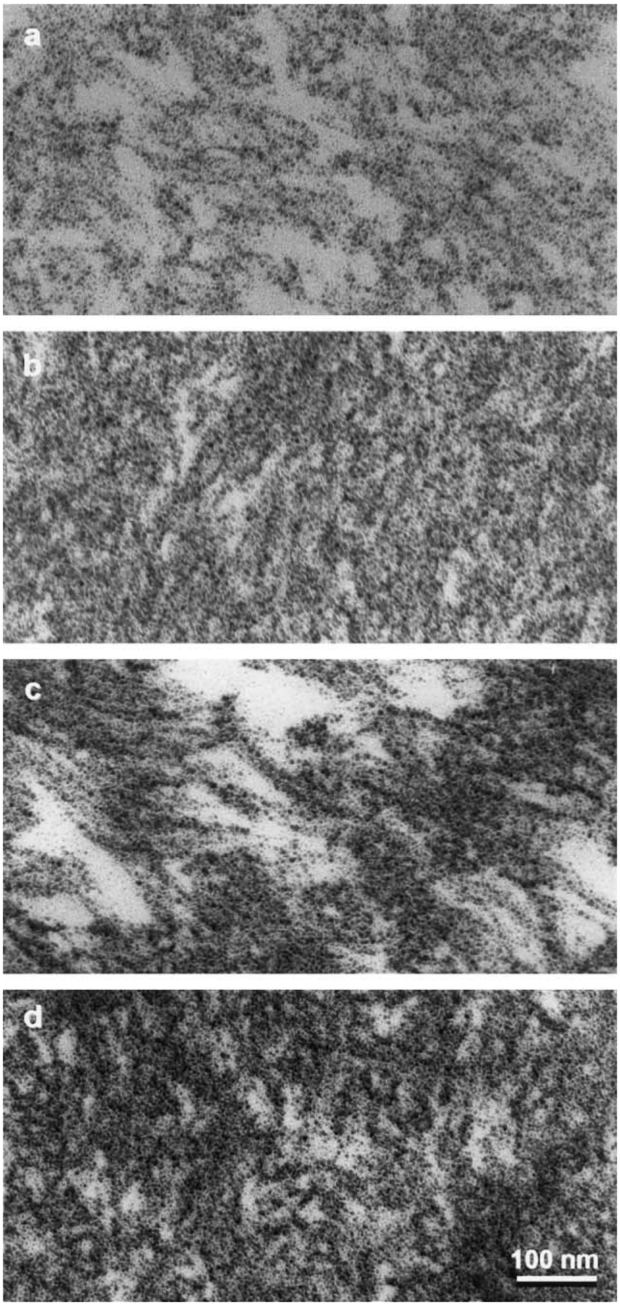

Fig. 4. Transmission electron micrographs of glycerol-plasticized amylose films formed at (a) 20%; (b) 50%; (c) 70%; and (d) 90% RH and reconditioned to 50% RH.

Fig. 2 shows that the increase in water content with increasing surrounding RH is faster for the films containing glycerol. The increase in OP coincided with the onset of the swelling of the network, showing that the water content is important for the OP. The micrograph of the network structure of the amylose film shown in Fig. 1b was taken after the film had been subjected to 75% RH. The network had had time to swell, and the resulting large, water-filled pores consequently gave a high OP. At low surrounding RH, the network structure was dense, with smaller pores (Fig. 1a and c), and the OP was much lower. The diffusion path through the strands by plasticization is a much slower process than permeation parallel to the strands through the pores. It is therefore also plausible that a more open network structure with larger pores would have higher permeability.

The onset of increased oxygen permeability also correlated well with the plasticization of the sample through T_g, which is further discussed below.

3.2. *Effect of humidity during film formation*

The films were prepared by casting water solutions. The amylose solutions formed gels immediately on cooling, whereas the amylopectin solutions remained viscous solutions. Films were formed by drying at a controlled air humidity of 20, 50, 70 or 90% RH and thereafter reconditioned to 50% RH.

We recently showed that the film formation at increasing levels of relative humidity increased the relative crystallinity of glycerol-plasticized amylopectin films, whereas glycerol-plasticized amylose films remained at a constant relative crystallinity of ~34% regardless of the conditions during film formation (Rindlav-Westling et al., 1998). However, the RH during film formation did not seem to have a significant influence on the water content of the films after reconditioning to 50% RH. On the other hand, starch films without glycerol have been shown to have an increasing water content with increased crystallinity, probably owing to the higher water content in the crystalline phase in comparison with the amorphous phase (Rindlav et al., 1997). The crystallinity did not increase the water content in the case of glycerol-plasticized amylopectin, possibly due to the high amount of glycerol present.

3.2.1. *Microstructure of glycerol-plasticized amylose films*

Fig. 4 shows the TEM micrographs of sections of the glycerol-plasticized amylose films formed at various RH. The RH during film formation affected both the pore size distribution and the homogeneity of the amylose network. A dense and homogeneous network with a maximum pore size of about 40 nm was found in the films formed at the standard conditions of 23°C and 50% RH, as shown in Fig. 4b. At 70% RH (Fig. 4c), large fluctuations in pore size were observed and the network consisted of dense areas, and of large open areas with pore sizes of up to 200 nm. The network was again more homogeneous when formed at 90% RH (Fig. 4d) and similar to the structure formed at 50% RH. The network structure formed at 20% RH differed slightly from the higher humidities but was more homogeneous than that formed at 70% RH. The film formed at 50% RH shown in Fig. 4b was kept at 50% RH unlike the film shown in Fig. 1a which was formed at 50% RH and then moved to 35% RH. The resulting networks are still very similar despite the different treatments, and the swelling observed in Fig. 1b can therefore be considered to occur at a RH > 50%.

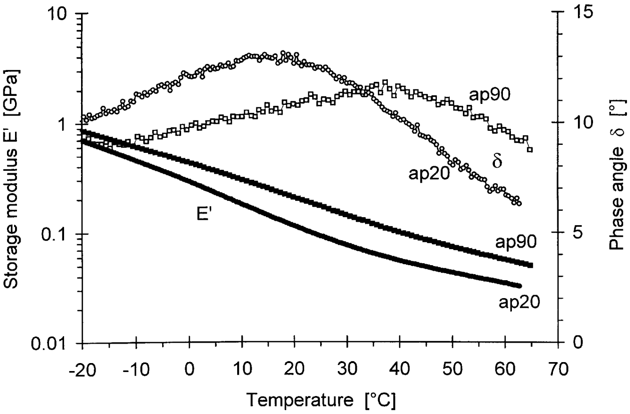

Fig. 5. Storage modulus E' (filled) and phase angle δ (open) as a function of temperature for glycerol-plasticized amylopectin films formed at 20% RH (■), and 90% RH (●).

Glycerol-plasticized amylose films were shown to remain at a constant relative crystallinity of ~34% regardless of the RH during film formation, but the fracture properties had a maximum in elongation and a minimum in film strength when the films were formed at 70% RH (Rindlav-Westling et al., 1998). This correlates well with the inhomogeneous microstructure formed at 70% RH shown in Fig. 4c. The exact mechanism is not known, but we assume that the less dense areas are responsible for the fracture properties in a way similar to the fracture of a soft matrix with a stiff filler which fractures in the matrix. The dense areas of the inhomogeneous network structure may be regarded as the stiff filler and the open areas as the softer matrix. The open areas are probably more flexible and can be extended further than the denser areas of a corresponding homogeneous network leading to a higher elongation.

3.2.2. Dynamic mechanical properties of glycerol-plasticized amylopectin films formed at various RH

The glycerol-plasticized amylopectin films formed at increasing RH had an increasing relative crystallinity that affected the mechanical properties. Fig. 5 shows the temperature scans by dynamic mechanical analysis (DMA) of glycerol-plasticized amylopectin films formed at 20 and 90% RH. The T_g was 15°C for the films formed at 20% RH and 37°C for the films formed at 90% RH. The films formed at 50 and 70% RH are not shown, but both had a T_g around 25°C. The δ-peaks were broad, but there was a clear transition within the experimental temperature window. Fig. 5 shows that T_g increases with increasing crystallinity, with $T_g = 37$°C for the most crystalline amylopectin films and $T_g = 15$°C for the amorphous films. Similar observations of increased T_g with increasing crystallinity have been made for starch where a pre-gelatinized, less crystalline wheat starch gave a lower T_g than native wheat starch (Kalichevsky, Jaroskiewicz, Ablett, Blanshard & Lillford, 1992). T_g has also been observed to increase with increasing degrees of crystallinity for linear synthetic polymers with anhydrous crystalline regions (Jin, Ellis & Karasz, 1984). The effect has been explained by a stiffening effect of dispersed microcrystalline cross-links, which leads to less mobility of the chain segments in the connecting amorphous regions (Gaeta, Apicella & Hopfenberg, 1982; Slade & Levine, 1991). The increase in T_g for increasing crystallinity in the amylopectin films also means in practice that, the more crystalline the films were, the less sensitive they were to the surrounding RH, i.e. less sensitive to water. The T_g for glycerol-plasticized amylopectin films is comparable to published T_gs for starch films. Lourdin and co-workers presented T_g from DMA measurements of potato starch films with a 40% glycerol/polymer ratio containing 15.4% water (Lourdin, Bizot & Colonna, 1997b). They defined T_g as the midpoint between the onset of the drop in E' and the δ-peak and found it to be around 20°C. Forssell and co-workers studied glycerol-plasticized barley starch films and found $T_g = 21$°C for 20% glycerol/polymer ratio at a surrounding 54% RH (Forssell et al., 1998). They could not detect a T_g in that range at higher glycerol contents, but it should decrease further because of the plasticization.

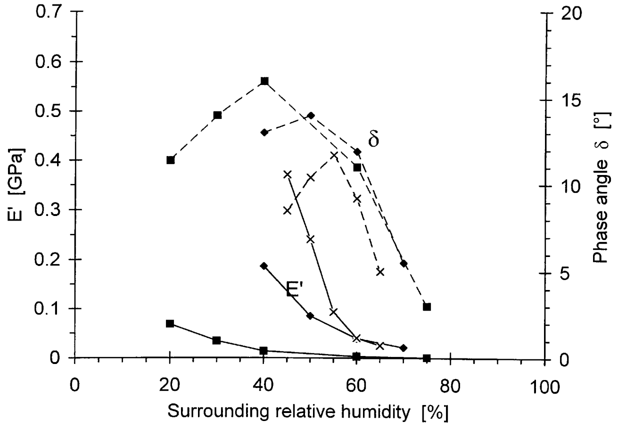

Fig. 6. Storage modulus E' (—) and phase angle δ (- - - -) as a function of surrounding relative humidity for glycerol-plasticized amylopectin films formed at 20% RH (■), 50% RH (◆) and 90% RH (×).

The dynamic mechanical properties of the films were also tested at a constant temperature of 25°C but varying surrounding relative humidities. The RH was changed in steps of 10%, and the thin films responded quickly, reaching equilibrium in the modulus within an order of minutes at the higher RH and in the order of 30 min at the lowest RH. The equilibrium values of the storage modulus, E', and phase angle, δ, are plotted against the surrounding RH for the glycerol-plasticized amylopectin films in Fig. 6. The change in the surrounding RH affected the water content of the film. An increase in water content caused plasticization of the amorphous parts, thus decreasing the modulus by decreasing T_g of the film. When the water content was adjusted to the level at which $T_g = 25$°C, i.e. the temperature of the measurement, the phase angle, δ, equalled the peak value at the glass transition. The phase angle decreased on either side of the humidity scale corresponding to the δ-peak at the

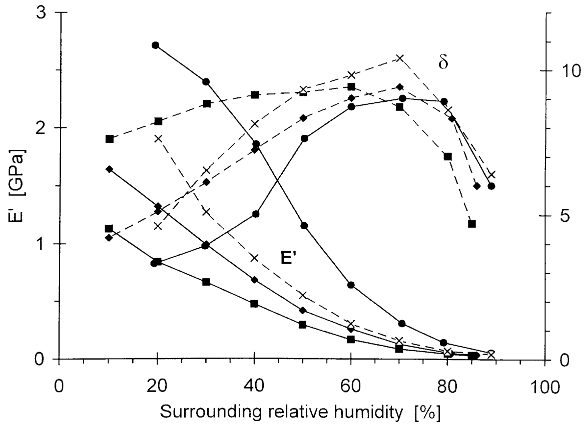

Fig. 7. Storage modulus E' (—) and phase angle δ (- - - -) as a function of surrounding relative humidity for glycerol-plasticized amylose films formed at 20% RH (■), 50% RH (◆), 70% RH (●) and 90% RH (×).

glass transition. The water content at which T_g is equal to the temperature of the measurement has been labelled W_g (Slade & Levine, 1991). While W_g occurred at 40% surrounding RH for the amorphous films formed at 20% RH, it occurred at 55% surrounding RH for the most crystalline films formed at 90% RH. The films formed at 50% RH had a W_g at 50% surrounding RH, which corresponds well with the measured T_g of 25°C. The results in Fig. 6 correspond to Fig. 5 in the respect that the films formed at 90% RH had a T_g above 25°C and thus had to be plasticized in order to decrease T_g to 25°C, i.e. W_g occurred at RH > 50% RH corresponding to a plasticization. The opposite applied for the films formed at 20% RH. They had $T_g = 15°C$ and had to lose water in order for T_g to increase to 25°C, i.e. W_g occurred at RH < 50% RH.

3.2.3. Dynamic mechanical properties of glycerol-plasticized amylose films formed at various RH

An increasing surrounding humidity during measurement affected the glycerol-plasticized amylose films in the same way as for the amylopectin films but overall E' was higher for the glycerol-plasticized amylose films than for the corresponding amylopectin films, compare Figs. 6 and 7. The W_g occurred at a higher RH for the amylose films than the amylopectin films. The plasticized amylose films all showed a similar W_g irrespective of the RH during film formation, see Fig. 7. The glycerol-plasticized amylose films were reported in a previous article to have a constant crystallinity regardless of the RH during film formation, which can explain the constant W_g (Rindlav-Westling et al., 1998). The W_g at 70% surrounding RH corresponds well with the inhomogeneous network structure in the films formed at 70% RH shown in Fig. 4c. The mobility is high at W_g, and film formation at that water content means that it is possible for the films to form an inhomogeneous structure.

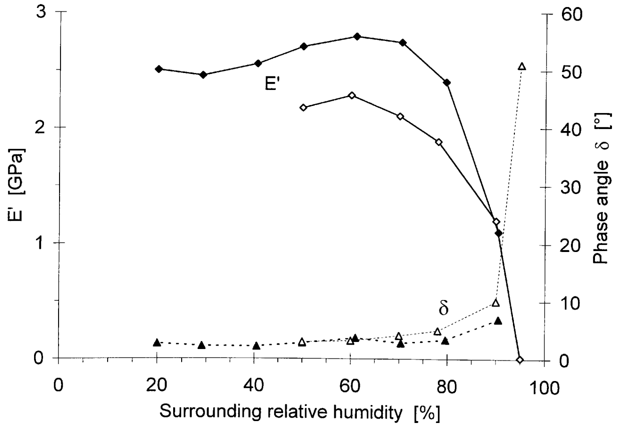

Fig. 8. Storage modulus E' (—) and phase angle δ (- - - -) as a function of surrounding relative humidity for films of amylose (filled symbols) and amylopectin (open symbols) formed at 50% RH without glycerol.

3.2.4. Dynamic mechanical properties of amylose and amylopectin films without glycerol formed at various RH

The amylose and amylopectin films without glycerol all showed a similar dependence on the surrounding RH regardless of the RH during film formation. Fig. 8 thus show only the amylose and amylopectin films formed at 50% RH. All films without glycerol had a fairly constant E' at low RH, which started dropping at around 80%. This would mean that these films were in the glassy state for all measured surrounding RH values up to at least 80% RH. It can therefore be concluded that, at 25°C and 50% RH, the films without glycerol were still in the glassy state, which was also obvious by their being very brittle and difficult to handle. The glassy amylose films without glycerol had an E' five times higher than the glycerol-plasticized amylose films at 50% RH, and the corresponding amylopectin films without glycerol had a storage modulus three times higher than the glycerol-plasticized amylopectin films, compare Figs. 6–8. Neither of the films without glycerol showed a W_g within the measured RH range.

3.3. General discussion

The observed W_gs shown in Figs. 6–8 correlate well with the behaviour of the oxygen permeability shown in Fig. 3. The films retain a low OP at surrounding RH up to a water content equal to W_g, where the plasticization of the amorphous parts facilitates swelling of the films. The film takes up more water during swelling, which causes an inhomogeneous pore size distribution with large pores, as shown in Fig. 1. The films without glycerol had a lower OP and an onset of increased OP at a higher RH suggesting higher T_gs than for the glycerol-plasticized films. Glass transitions at about 90°C were observed by DSC for both amylose and amylopectin films without added glycerol. T_gs in this temperature range have also been observed for starch without added glycerol (Lourdin et al., 1997b; Rindlav et al., 1997). A difference in RH during film formation did not influence the observed transitions for films without glycerol.

The sorption isotherms for amylose and amylopectin plasticized with glycerol shown in Fig. 2 are typical according to Sala and Tomka for materials at a temperature above T_g (Sala & Tomka, 1992). The sorption isotherms were recorded at 23°C, which corresponded with the $T_g = 25°C$ for the glycerol-plasticized amylopectin films being close to the temperature of the sorption isotherm. The situation was not as clear for the glycerol-plasticized amylose films. The sorption isotherm was a decreasing function of surrounding relative humidity, suggesting a T_g at or below room temperature, which was not evident in our measurements. It has been suggested that phase separation may occur in glycerol-plasticized starch films at glycerol/starch > 0.3, leading to two glass transitions, one at low temperature for the glycerol-rich phase and one at a higher temperature for the amylose-rich phase (Kalichevsky, Jaroszkiewicz & Blanshard, 1993; Lourdin et al., 1997a). If phase separation also occurred in the glycerol-plasticized amylose films, the two glass transition temperatures could have been pushed outside the temperature window of the DMA measurements. We observed a transition using DSC at about $-65°C$ for both the glycerol-plasticized amylose and amylopectin films. The film formation conditions and the varying crystallinity of amylopectin did not influence this transition. The observed transition may be the T_g of a glycerol-rich phase. Pure glycerol has a T_g of $-78°C$, water has $T_g = -135°C$ and pure, dry amylose has an estimated $T_g = 230°C$ (Orford, Parker, Ring & Smith, 1989). A mixture of amylose, glycerol and water would have a T_g somewhere between these, and a glycerol-rich phase could very well have a T_g close to the observed transition at $-65°C$. The presence of a glycerol-rich phase has also been suggested by Forssell and co-workers for mixtures of barley starch with water and glycerol (Forssell et al., 1998). A low temperature transition at $-59°C$ has also been observed for amylose films with a low water content and without glycerol (Scandola, Ceccorulli & Pizzoli, 1991). The transition was attributed to local motions of the polysaccharide backbone. Lourdin and co-workers also observed low temperature transitions with DMA in the same range for both glycerol-plasticized and unplasticized starch films (Lourdin et al., 1997b). The nature of the transitions in glycerol-plasticized amylose is consequently not clear and needs to be further studied.

4. Conclusions

- An increased relative humidity surrounding the films leads to plasticization. The increased mobility in the network allows swelling with a resulting heterogeneous network structure and a sharply increased oxygen permeability.
- In glycerol-plasticized amylose films, the maximum heterogeneity occurred at 70% RH, explaining the maximum in fracture strain and minimum in fracture stress previously observed for these films.
- Dynamic mechanical analysis at various surrounding relative humidities at constant temperature is a useful alternative to thermal DMA scans for thin biopolymer films which rapidly change their properties when exposed to different humidities. The glass transition observed in humidity scans corresponds well with that observed with temperature scans.

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